Neuronal control of energy balance and modulation of muscle aging by the transcriptional coactivator PGC-1α

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Jonathan François Gill

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Prof. Dr. Christoph Handschin und Prof. Dr. Markus A. Rüegg

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Prof. Dr. Jörg Schibler,

Dekan

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Summary

Cellular metabolic adaptations play a central role in the body's response to environmental changes and external stimuli and allow the maintenance of a proper energy balance. Transcriptional activators enable the integration of incoming signals and sensing of altered energy levels. Dysregulation of such metabolic pathways is a common mechanism of various tissue dysfunctions contributing to different diseases. A key player in cellular metabolism is the transcriptional coregulator Peroxisome proliferator-activated receptor coactivator 1 alpha (PGC-1α). PGC-1α is expressed in tissues with high basal oxidative capacity such as brain and muscle and regulates the expression of a plethora of genes in response to various external cues, including exercise and fasting. This makes PGC-1α an extremely powerful metabolic sensor and a potential therapeutic target in metabolic diseases. Similarly, its downregulation during muscle aging and its central function in the control of mitochondrial gene expression suggests its crucial role in the development of age-related muscle disorders. However, to establish the true therapeutic potential of PGC-1α, it is important to evaluate its impact on metabolic sensing in those tissues and the consequences of its modulation in such pathological conditions.

To determine the role of PGC-1α in central metabolic sensing and brain regulation of energy balance, we deleted the coactivator specifically in murine AgRP and POMC neurons located in the arcuate nucleus. The ablation of PGC-1α in POMC neurons did not reveal any major phenotype. Conversely, absence of PGC-1α in AgRP cells drove an increase in fat mass coupled with a reduction in locomotor activity and body temperature. Mice lacking the transcriptional coactivator in AgRP neurons exhibited a blunted leptin response and reduced food intake in fed and fasted conditions. Mechanistically, we demonstrated that fasting-induced AgRP expression is blunted in those mice and in an immortalized AgRP cell line, thereby leading to reduced feeding response upon fasting. Collectively, our results highlight a novel role for PGC-1α in neuronal regulation of energy homeostasis, which could be of therapeutic interest for the treatment of obesity and diabetes.

To determine the role of PGC-1α in muscle aging, we used muscle-specific PGC-1α deletion and overexpression mouse models. We confirmed that PGC-1α preserves mitochondrial function and protein expression, as well as muscle endurance during aging. More importantly, we identified a novel function for PGC-1α in muscle, involving the regulation of calcium homeostasis through the control of genes responsible for mitochondrial calcium uptake and mitochondrial association with the sarcoplasmic reticulum. We suspect that PGC-1α-mediated amelioration of calcium metabolism protected the old muscle against ER stress and prevented tubular aggregate formation, which all contributed to reducing muscle apoptosis. Finally, we used muscle cells treated with ceramide or thapsigargin to demonstrate that PGC-1α inhibited apoptosis initiated by mitochondrial or calcium homeostasis impairments. This strongly confirms the potential therapeutic usage of PGC-1α to reduce age-related muscle disorders but also other diseases involving calcium dysregulation.

In a final study, we used the same animal models that either received late-life endurance training or stayed untrained to determine the role of PGC-1α in exercise-mediated muscle improvements during aging. We demonstrated that PGC-1α could not only ameliorate muscle endurance but also age-associated motor dysfunctions. We also showed that PGC-1α muscle deletion led to pre-mature sarcopenia. Finally, we revealed that PGC-1α modulates many beneficial outcomes of exercise in the old muscle. PGC-1α muscle overexpression was sufficient to mimic or even override exercise beneficial effect on muscle aging. This further illustrates the importance of the coactivator in muscle aging and to maximize exercise positive outcomes in the old muscle

In conclusion, the work undertaken in this thesis delineates new facets of PGC-1α-controlled metabolism. We described a role for PGC-1α as metabolic sensor in the brain and presented novel aspects of muscle metabolism regulated by the coactivator. We showed that it is protective in several ways against age-related muscle disorders and that it promotes exercise effects in this context. This work therefore improves our understanding of the biological processes regulated by PGC-1α and established the transcriptional regulator as a promising target for therapeutic approaches in metabolic and age-associated muscle diseases.

Abbreviations

AgRP	Agouti-related protein
AMPK	AMP-activated protein kinase
ANS	Autonomic nervous system
ATF2	Activating transcription factor 2
BAIBA	β-aminoisobutyric acid
BAP1	BRCA1 associated protein-1
BAT	Brown adipose tissue
Bax	BCL2 Associated X
BCL2	B-Cell CLL/Lymphoma 2
BDNF	Brain derived neurotrophic factor
BNIP3	BCL2/Adenovirus E1B 19kDa interacting protein 3
BSA	Bovine serum albumin
CaMKIIα	Calmodulin-dependent protein kinase IIα
CaMKIV	Ca2+/calmodulin-dependent protein kinase IV
CART	Cocaine- and amphetamine-regulated transcript
CD36	Fatty acid translocase/CD36
CPT1	Carnitine O-palmitoyltransferase 1
CREB	cAMP response element-binding protein
CSQ1	Caslequestrin 1
CSQ2	Caslequestrin 2
CYPD	Cyclophilin D
DHRP	Dihydropyridine receptor
DMEM	Dulbecco's modified Eagle's medium
Drp1	Dynamin-related protein 1
ERRs	Estrogen-related receptors
ERRα	Estrogen related receptor α
ER stress	Endoplasmic reticulum stress
ERα	Estrogen receptor-α
FABP4	Fatty acid binding protein aP2
FAO	Fatty acid oxidation
Fis1	Fission 1
FNDC5	Fibronectin type III domain-containing protein 5
FOXO1	Forkhead box O1
FOXO3	Forkhead-Box-Protein O3
FXR	Farnesyl X receptor
GCN5	General Control Of Amino Acid Synthesis Protein 5-Like 2
GFP	Green fluorescent protein

GLUT4	Glucose transporter 4
Gpr17	G Protein-Coupled Receptor 17
GPx	Glutathione peroxidase
GR	Glucocorticoid receptor
HAT	Histone acetyltransferase
HFD	High fat diet
HFN4	Hepatic nuclear factor-4
ICV	Intracerebroventricular
IGFBP5	Insulin like growth factor binding protein 5
IL-6	Interleukin 6
IP3R	Inositol 1,4,5-trisphosphate receptor type
KO	Knock out
LDH	Lactate dehydrogenase
Letm1	lLucine zipper and EF-hand containing transmembrane protein 1
LH	Lateral hypothalamus
LXR	Liver X receptor
MAM	Mitochondria-associated-membranes
MC3R	Melanocortin-3
MC4R	Melanocortin -4 receptors
MCAD	Medium-chain acyl-coenzyme A dehydrogenase
MEF-2	Myocyte enhancer factor-2
Metrnl	Meteorin-like
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
mPTP	Mitochondrial permeability transition pore opening
mtDNA	Mitochondrial DNA
myoD	Myogenic differentiation
NAD	Nicotinamide adenine dinucleotide
NF-kB	Nuclear factor kappa B
NMJ	Neuromuscular junction
NPY	Neuropeptide Y
OGT	O-GlcNAc transferase
Opa1	Optic atrophies 1
ORA1	ORAI Calcium Release-Activated Calcium Modulator 1
OXPHOS	Oxidative phosphorylation
p38 MAPK	P38 mitogen-activated protein kinase
PDK4	Pyruvate dehydrogenase kinase 4
PGC-1α/β	Peroxisome proliferator-activated receptor γ coactivator 1α/β
pH2AX	Phospho-H2A Histone Family Member X
PKA	Protein kinase A
POMC	Proopiomelanocortin

PPARα	Peroxisome proliferator-activated receptor
PPARγ	Peroxisome proliferator-activated receptor gamma
ppRB	Phosphor-prepro-retinoblastoma-associated protein
PVN	Paraventricular nucleus
PXR	Pregnane X receptor
RER	Respiratory exchange ratio
RNF34	Ring-finger-containing protein
ROS	Reactive oxygen species
RYR	Ryanodine receptor
S6K1	S6 kinase 1
SAR	Serine/arginine-rich motifs
SERCA	Sarco/endoplasmic reticulum Ca2+-ATPase
SHP	Small heterodimer partner
shRNA	Specific short hairpin RNA
SIRT1	Sirtuin 1
SIRT3	Sirtuin 3
SOD2	Superoxide dismutase 2
SR	Sarcoplasmic reticulum
SREBP-1	Sterol regulatory element-binding protein 1
STAT3	Signal transducer and activator of transcription 3
STIM1	Stromal Interaction Molecule 1
SWI/SNF	Switch/sucrose nonfermentable
TA	Tibialis anterior
TBP	TATA binding protein
Tfam	Transcription factor A
TNF	Tumor necrosis factor
TWEAK–Fn14	TNF-related weak inducer of apoptosis
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
UPR	Unfolded protein response
VDAC	Voltage-dependent anion channel
VEGF	Vascular endothelial growth factor
VLH	Ventrolateral hypothalamus
VMH	Ventromedial hypothalamus
WAT	White adipose tissue
WT	Wild type
XIAP	X-linked inhibitor of apoptosis protein
ZNF746	Zinc Finger Protein 746
α-MSH	α-melanocyte-stimulating hormone
β3-AR	β3-adrenergic receptor

I. Introduction

A. PGC-1α: a versatile coactivator of transcription

Gene expression regulation plays a central role in cellular metabolism. For this reason, coregulators of transcription factors controlling the expression of various metabolic pathways are critical for cellular energetic adaptation to environmental changes and external stimuli. Accumulated lines of evidence firmly established the PGC-1 superfamily as particularly relevant coactivators of transcription for the control of energy metabolism. Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1 alpha (PGC-1α), the founding member of the superfamily, was originally discovered in a yeast two hybrid assay that aimed at identifying PPARγ binding proteins which determines the brown adipose tissue (BAT) and white adipose tissue (WAT) transcriptional specificity of PPARγ **(Puigserver, Wu et al. 1998)**. PGC-1β is the closest homologue of PGC-1α with 40% of identity in the N-terminal domain, 35% in the central domain and 48% at the C-terminal site **(Lin, Handschin et al. 2005)**. The PGC-1 superfamily is well conserved among various species indicating the key role in metabolic regulation **(Lin, Handschin et al. 2005, LeMoine, Lougheed et al. 2010)**. PGC-1α does not bind to DNA and regulate transcription through chromatin remodeling **(Puigserver, Adelmant et al. 1999)**, co-activation of specific transcription factors **(Puigserver, Wu et al. 1998)** and control of mRNA splicing **(Monsalve, Wu et al. 2000)**. PGC-1α is highly responsive to different environmental stimuli and acts as a molecular switch for many metabolic pathways leading to energetic adaptation such as adaptive thermogenesis or mitochondrial biogenesis **(Puigserver, Wu et al. 1998, Lin, Wu et al. 2002)**. Its versatile role is conferred by its interaction with numerous transcription factors in a tissue-specific manner **(Villena 2015)**. Conversely to most ubiquitously expressed transcriptional coregulators, PGC-1α is only expressed in highly basal oxidative tissues including brain, skeletal muscle, heart, BAT and kidney **(Esterbauer, Oberkofler et al. 1999, Wu, Puigserver et al. 1999, Knutti, Kaul et al. 2000)**. In addition, in organs where PGC-1α is expressed at low levels such as liver and WAT **(Puigserver, Wu et al. 1998, Liang and Ward 2006)**, its expression can be induced by fasting and cold exposure respectively **(Handschin and Spiegelman 2006, Barbatelli, Murano et al. 2010)**. PGC-1α expression is also induced in

hypothalamus upon fasting **(Ma, Li et al. 2010)** and in skeletal muscle after exercise **(Baar, Wende et al. 2002, Pilegaard, Saltin et al. 2003)**. The role of the coactivator in the latter two organs is discussed in more detail in this work.

1. PGC-1α structure and molecular mechanism of action

The PGC-1α gene is located on chromosome 5 in mice and 4 in humans, and encodes a nuclear protein of 91kDa that regulates transcription by binding transcription factors that will recognize specific promoter sequences on target genes. In addition, recruitment of the coactivator is also dependent on the promoter targeted by the transcription factor. Thus, in BAT, PGC-1α co-activates PPARγ to induce the expression of the uncoupling protein 1 (UCP1) but does not promote the PPARγ target fatty acid binding protein aP2 (FABP4) expression **(Puigserver, Wu et al. 1998)**. Besides PPARγ, PGC-1α can interact with various other nuclear receptors (Fig. 1) including thyroid hormone receptor **(Puigserver, Wu et al. 1998)**, retinoid receptors **(Puigserver, Wu et al. 1998)**, glucocorticoid receptor (GR) **(Knutti, Kaul et al. 2000)**, estrogen receptor-α (ERα) **(Tcherepanova, Puigserver et al. 2000)**, PPARα **(Vega, Huss et al. 2000)**, PPARβ **(Wang, Lee et al. 2003)**, RXRα **(Delerive, Wu et al. 2002)**, farnesyl X receptor (FXR) **(Zhang, Castellani et al. 2004)**, pregnane X receptor (PXR) **(Bhalla, Ozalp et al. 2004)**, hepatic nuclear factor-4 (HFN4) **(Rhee, Inoue et al. 2003)**, liver X receptor (LXR) **(Lin, Yang et al. 2005)** and the estrogen-related receptors (ERRs) **(Huss, Kopp et al. 2002, Schreiber, Emter et al. 2004)**. Non-nuclear transcription factors have been identified as PGC-1α partners such as myocyte enhancer factor-2 (MEF-2) **(Michael, Wu et al. 2001)**, forkhead box O1 (FOXO1) **(Puigserver, Rhee et al. 2003)**, or sterol regulatory element-binding protein 1 (SREBP-1) **(Lin, Yang et al. 2005)** (Fig. 1). This extensive but non-exhaustive list of PGC-1α partners indicates the ability of the coactivator to regulate many different metabolic pathways. PGC-1α interacts with its different transcription factors through multiple protein domains. Most of the nuclear receptors bind to a LXXLL motif at the N-terminal site of PGC-1α (Fig. 1), which is the case for PPARα **(Vega, Huss et al. 2000)** and ERα **(Tcherepanova, Puigserver et al. 2000)**. 3 LXXLL motifs are identified **(Knutti, Kressler et al. 2001)**. In most cases the link through the second LXXLL motif relies on the fixation of a ligand by the receptor, but not for hepatic nuclear factor-4 for

example **(Yoon, Puigserver et al. 2001)**. When the binding depends on the second LXXLL motif, PGC-1α generally interacts with the activation function 2 region of the receptor within the domain bound to the ligand, located in the C-terminus of the receptor. If this motif is not involved, the interaction with the receptor is mainly done at the central or N-terminal part of the receptor. Some transcription factors interact with other regions of PGC-1α. As follows, MEF-2 binds to PGC-1α in the region located between the amino acid 400 and 550 (Fig. 1) **(Michael, Wu et al. 2001)**, whereas PPARγ can bind to both the second LXXLL motif and in another region between amino acid 200 and 400 **(Wallberg, Yamamura et al. 2003)**.

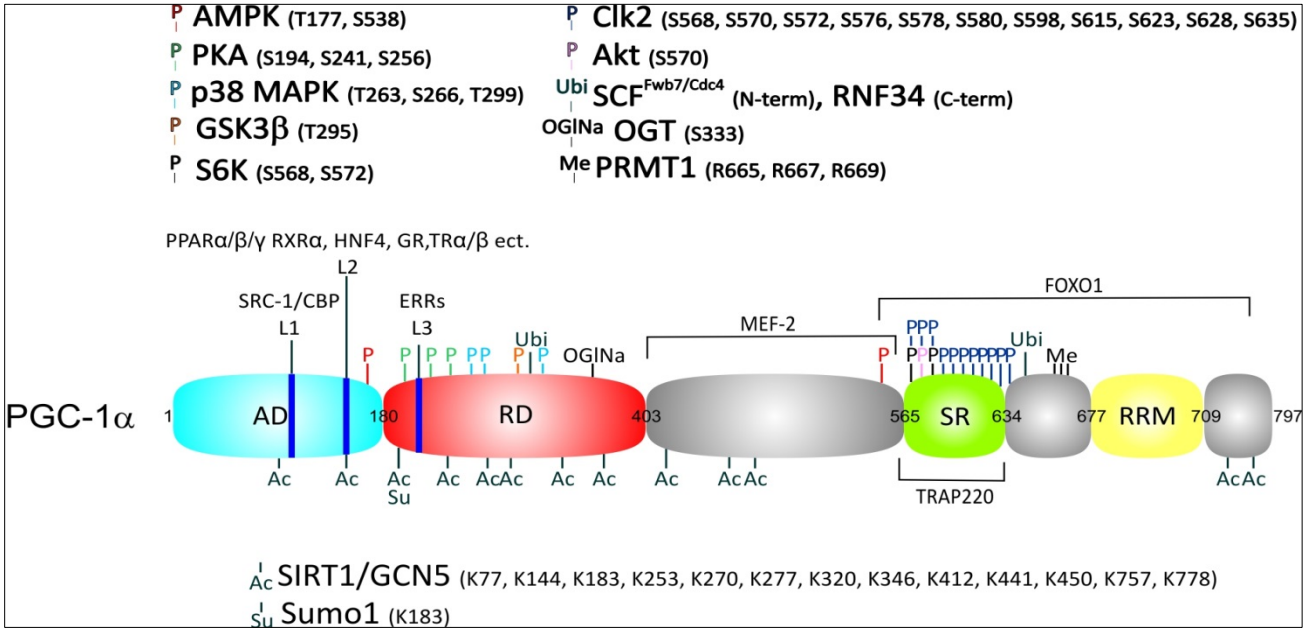

Figure 1: Structure, interaction sites and post-translational modifications sites of the PGC-1α transcriptional coactivator. adapted from **(Kupr and Handschin 2015)**

Conversely to other coactivators such as cAMP response element-binding protein (CREB) binding protein (CBP/p300) and members of the steroid receptor coactivator (p160/SRC) family, PGC-1α does not have an intrinsic histone acetyltransferase (HAT). Therefore, the strong transcriptional activation domain at the N-terminal site of PGC-1α is in charge to bind coactivators with a HAT domain to remodel chromatin structure into a state that allows the transcriptional machinery to access the targeted genes **(Puigserver, Adelmant et al. 1999)**. At the C-terminal extremity, PGC-1α associates with the RNA polymerase II and the TRAP 220 sub-unit of the complex TRAP/mediator (Fig. 1) (thyroid hormone receptor-associated proteins), which facilitates interactions with the transcription initiation machinery **(Wallberg, Yamamura et al. 2003)**. This extremity also recruits the switch/sucrose nonfermentable (SWI/SNF) chromatin-remodeling complex through its interaction with BAF60a **(Li, Liu et al. 2008)**. On top of that, PGC-1α carboxyterminal region contains a RNA recognizing motif involved in RNA and single-strand DNA binding and short serine/arginine-rich motifs (SAR) (Fig. 1), which have been demonstrated to couple pre-mRNA splicing and transcription **(Monsalve, Wu et al. 2000)**. In this fashion, after docking to transcription factors, the first mechanism by which PGC-1α activates gene expression is the recruitments of coactivators remodeling chromatin and the transcriptional machinery. However, PGC-1α transcriptional activator complexes are also able to remove repressors of transcription such as histone deacetylases or other inhibitors of transcription. For example, interaction of PGC-1α and the glucocorticoid receptor prevents the inhibiting binding of the corepressor small heterodimer partner (SHP) to the transcription factor, which further enhances gluconeogenic gene expression **(Borgius, Steffensen et al. 2002)**. Similarly, the corepressor NCor1 and PGC-1α regulate oxidative metabolism in an antagonistic manner through the modulation of ERRα transcriptional activity in skeletal muscle **(Perez-Schindler, Summermatter et al. 2012)**. This demonstrates that gene expression regulation is dictated by a balance of co-repression and co-activation.

2. PGC-1α: a nodal regulator of cellular energy metabolism

PGC-1α regulation as a metabolic sensor

PGC-1α expression regulation under different stimulus is the first mechanism by which PGC-1α is responding to energetic changes. PGC-1α level increase can be triggered by a plethora of stimulus in a tissue-specific manner (Fig. 2). In muscle, PGC-1α expression is induced after exercise through different mechanisms (Fig. 2). First, exercise has been shown to induce an increase of PGC-1α through the activation of the p38 mitogen-activated protein kinase (p38 MAPK) pathway in an activating transcription factor 2 (ATF2) dependent manner **(Akimoto, Pohnert et al. 2005)** (Fig. 2). Secondly, activation of Ca2+/calmodulin-dependent protein kinase IV (CaMKIV) and calcineurin A upon nerve stimulation interact and activate the transcription factor MEF2, which in turn induces PGC-1α transcription **(Handschin, Rhee et al. 2003)** (Fig. 2). Interestingly, MEF2 is also activated by p38 MAPK and might also mediate PGC-1α level increases though this pathway. Alternatively, CaMKIV also phosphorylates and activates the transcription factor CREB, which ultimately binds to the PGC-1α gene and activates its transcription **(Wu, Kanatous et al. 2002, Handschin, Rhee et al. 2003)** (Fig. 2). In a third pathway, Sirtuin 1 (SIRT1) expression and activity are stimulated upon nicotinamide adenine dinucleotide (NAD)(+)/NADH concentration ratio changes upon exercise **(White and Schenk 2012)** and stimulate PGC-1α promoter activity **(Amat, Planavila et al. 2009)** (Fig. 2). Remarkably, in the presence of myogenic differentiation (myoD) and SIRT1, PGC-1α transcription induction further promotes its own promoter activity in a positive auto regulatory feedback loop **(Handschin, Rhee et al. 2003, Amat, Planavila et al. 2009)**. Finally, AMP-activated protein kinase (AMPK) is stimulated by a high AMP/ATP ratio reflecting defects in energy production or increased energy consumption and therefore is activated by exercise **(Jorgensen, Wojtaszewski et al. 2005)**. Activation of AMKP leads to increase of PGC-1α gene expression **(Suwa, Nakano et al. 2003, Jorgensen, Wojtaszewski et al. 2005)** (Fig. 2). Contrary to exercise, insulin down-regulates PGC-1α mRNA expression by Akt phosphorylation and nuclear exclusion of FoxO1, a protein known to activate PGC-1α promoter (Fig. 2). In BAT, cold exposure is sensed by the sympathetic nervous system via the β3-adrenergic receptor (β3-AR) **(Puigserver, Wu et al. 1998,**

Boss, Bachman et al. 1999) that will activate cAMP signaling and Protein kinase A (PKA). cAMP/PKA signaling stimulation will increase PGC-1α transcription through both CREB **(Handschin, Rhee et al. 2003)** and the p38 MAPK-ATF2 axis **(Cao, Daniel et al. 2004)** (Fig. 2). Alternatively, cold exposure induced PGC-1α mRNA expression in BAT is mediated by nitric oxide-cGMP pathway **(Nisoli, Clementi et al. 2003)**. In the liver, similar to cold exposure in BAT, glucagon stimulates the cAMP/PKA pathway that will induce CREB and the p38 MAPK-ATF2 axis, ultimately resulting in PGC-1α gene expression elevation **(Herzig, Long et al. 2001, Cao, Collins et al. 2005)** (Fig. 2). Heart, BAT, and liver thyroid hormones also increase PGC-1α mRNA levels indicating that PGC-1α expression additionally responds to hormonal changes **(Puigserver, Wu et al. 1998, Weitzel, Radtke et al. 2001, Goldenthal, Weiss et al. 2004)** (Fig. 2). Fascinatingly, PGC-1α is able to potentiate its own transcription by interacting with transcription factors that promote its expression such as myoD/SIRT1 as depicted above and MEF2 in muscle **(Handschin, Rhee et al. 2003)**, FOXO1 in the liver **(Daitoku, Yamagata et al. 2003)**, PPARγ in WAT **(Hondares, Mora et al. 2006)** and ERRγ in BAT **(Wang, Liu et al. 2005)** (Fig. 2). Those specific positive auto-regulatory feedback loops dependent on the presence of particular transcription factors further increase the specificity and the fine-tuning of the PGC-1α responses to different stimulus in different tissues.

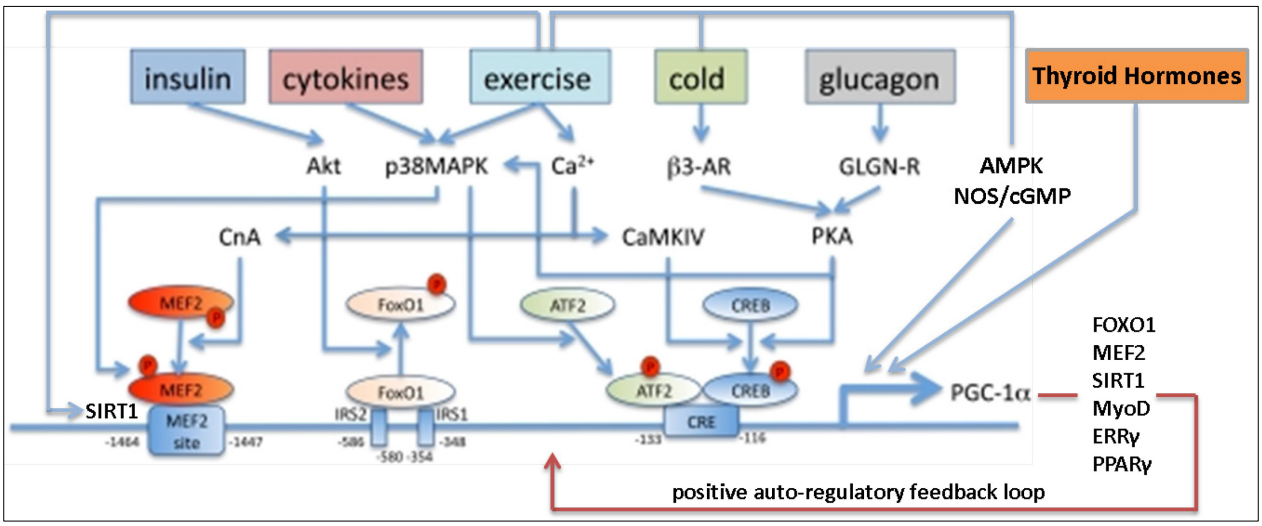

Figure 2: Regulation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) transcription. adapted from **(Fernandez-Marcos and Auwerx 2011)**

Several post-translational modifications of PGC-1α are equally important to regulate PGC-1α function according to different environmental changes. In addition to increasing its expression upon exercise **(Akimoto, Pohnert et al. 2005)**, p38 MAPK phosphorylates PGC-1α at threonine-262, serine-265, and threonine-298 in response to cytokines **(Puigserver, Rhee et al. 2001)** (Fig. 1). PGC-1α phosphorylation by p38 MAPK releases the co-repressor p160MBP from PGC-1α, which increases its co-transcriptional activity **(Fan, Rhee et al. 2004)** and enhances its stability **(Puigserver, Rhee et al. 2001)**. The latest stabilization mechanism is important for PGC-1α activity as its normal half-life lasts between 2 and 3 hours **(Puigserver, Rhee et al. 2001, Sano, Tokudome et al. 2007)**. AMPK, induced by exercise in skeletal muscle **(Jorgensen, Wojtaszewski et al. 2005)**, also phosphorylates PGC-1α at two other threonine-177 and serine-538 sites to enhance PGC-1α co-transcriptional activity **(Jager, Handschin et al. 2007)** (Fig. 1). In an opposite manner, in addition to repress its gene expression **(Southgate, Bruce et al. 2005)**, insulin stimulates hepatic Akt inhibition of PGC-1α activity through phosphorylation of its serine-570 **(Li, Monks et al. 2007)** (Fig. 1). A second post-translational modification that dictates PGC-1α activity is its acetylation status. PGC-1α is deacetylated by SIRT1 thereby promoting its co-transcriptional action, whereas it is heavily acetylated relocated within the nucleus by General Control Of Amino Acid Synthesis Protein 5-Like 2 (GCN5) which inhibits its activity **(Lerin, Rodgers et al. 2006, Gerhart-Hines, Rodgers et al. 2007)** (Fig. 1). As mentioned above, Sirt1 activity generally increases during energetic states such as fasting, exercise, or oxidative stress in order to promote cellular energy loads to overcome these situations **(Houtkooper, Canto et al. 2010)**. Likewise GCN5 is sensitive to different energetic status **(Dominy, Lee et al. 2010)**. As follows, in a coordinated manner, GCN5 and SIRT1 amounts are respectively increased and reduced upon caloric excess, lowering PGC-1α co-transcriptional function **(Dominy, Lee et al. 2010)**. Consistently, GCN5 and SIRT1 levels are respectively reduced and elevated after caloric restriction, promoting PGC-1α action **(Dominy, Lee et al. 2010)**. These data indicate that the SIRT1/GCN5-PGC-1α pathway acts as a powerful and flexible metabolic sensor of the cellular energetic level. Similarly, methylations of arginine-665, -667, and -669 by the protein arginine methyltransferase 1 have been shown to improve PGC-1α mediated transcriptional activation **(Teyssier, Ma et al. 2005)** (Fig. 1). On the contrary, PGC-1α SUMOylation in conserved lysine-183 reduces its activity (Fig. 1). Lastly, while ubiquitination does not affect PGC-1α function, it

regulates its stability and therefore its cellular concentration. For example, the Ring-finger-containing protein (RNF34) interacts with the C-terminal site of PGC-1α in BAT and drives its degradation via its specific E3 ubiquitin ligase activity **(Wei, Pan et al. 2012)**. Interestingly, RNF34 is suppressed upon cold exposure and β3-adrenergic receptor signaling in BAT, suggesting that ubiquitination at least contributes to the PGC-1α expression pattern and thermogenesis action in this tissue **(Wei, Pan et al. 2012)**. Plus, while both the E3 ubiquitin ligase SCF ubiquitin ligase complex subunit CDC4 and F-box, WD repeat domain containing 7 trigger ubiquitin-proteasome degradation of PGC-1α, Necdin stabilizes PGC-1α through inhibition of its ubiquitin-mediated proteolysis.

Counter to post-translational modification, transcription of different isoforms upon environmental changes alters the structure and therefore the different domains of PGC-1α **(Martinez-Redondo, Pettersson et al. 2015)**, which ultimately also changes PGC-1α activity and regulation. For example, a shift of promoter activity towards an upstream transcription start site drives the expression of alternative PGC-1α transcripts, named PGC-1alpha-b and PGC-1alpha-c, upon exercise and β-adrenergic stimulation in skeletal muscle **(Miura, Kai et al. 2008, Chinsomboon, Ruas et al. 2009)**. Captivatingly, fasting promotes the expression of the regular PGC-1alpha-a transcript from the proximal promoter in liver, but not the PGC-1alpha-b and PGC-1alpha-c variants **(Miura, Kai et al. 2008)**. This further suggests that specific isoform expression could be induced to drive the expression of precise targets and prevent the loss of energy in the activation of non-required metabolic pathways. Accordingly, different PGC-1α variants can also be regulated and activated in a specific manner. Thus, even if NT-PGC-1α proteins that lack the C-terminal domain can still co-activate PPARα and PPARγ, this interaction is strictly ligand dependent **(Zhang, Huypens et al. 2009)**. Moreover the lack of the C-terminal site makes NT-PGC-1α resistant to Twist-1-mediated inhibition in BAT and for this reason NT-PGC-1α cannot be repressed by the negative feedback loop engaging Twist-1 **(Jun, Gettys et al. 2012)**. To date, more than ten PGC-1α isoforms have been identified with variations in their tissue expression, regulation and function **(Martinez-Redondo, Pettersson et al. 2015)** which gives another dimension to the complexity and the versatility in the response that PGC-1α can bring to different stimuli.

PGC-1α: a central metabolism molecular switch

Through its regulation and activation, PGC-1α will integrate environmental stimuli as well as the current cellular energetic status and regulate the transcription of its target genes to adapt cellular and tissue metabolism accordingly (Fig. 3). As mentioned before, PGC-1α can be induced in tissues where its expression is basally low and is highly expressed in organs requiring elevated oxidative metabolism. Its expression in multiple tissues implies that it regulates various metabolic functions. Skeletal muscle is one of the tissues where PGC-1α has been the most extensively studied. The different processes that PGC-1α control in the skeletal muscle includes mitochondrial biogenesis and function **(Wu, Puigserver et al. 1999, Lin, Wu et al. 2002)**, conversion of muscle type II glycolytic fibers to type IIa and I oxidative fibers **(Lin, Wu et al. 2002)** and fatty acid oxidation (FAO) **(Olesen, Kiilerich et al. 2010)** (Fig. 3). The role of PGC-1α in skeletal muscle is discussed more extensively in section I.C.2.

Comparable to skeletal muscle, cellular and animal models depicted PGC-1α as a strong inducer of mitochondrial biogenesis in hearts **(Lehman, Barger et al. 2000, Russell, Mansfield et al. 2004)** (Fig. 3). Global loss of PGC-1α leads to 30–50% reduction in oxidative gene expression and slight reductions in mitochondrial activity in the heart that results in mild contractile alterations on basal conditions **(Arany, He et al. 2005, Lehman, Boudina et al. 2008)**. However, those moderate deficiencies become detrimental under stress conditions. As a result, PGC-1α knockout mice display major heart malfunctions leading to heart failure when exposed to transverse aortic constriction **(Arany, Novikov et al. 2006)** (Fig. 3). In addition, these mice display lower heart contraction force and beat rate, as well as ventricular dysfunctions following extreme exercise or dobutamine treatments **(Arany, He et al. 2005, Leone, Lehman et al. 2005)**. These dysfunctions could be imputed to insufficient ATP and fatty acid generation by cardiac cell of knock out animals (Fig. 3) that would not be able to sustain an increased contraction required in these situations **(Leone, Lehman et al. 2005)**. Therefore, if basal heart functions are not deteriorated by the absence of PGC-1α, its role as a molecular switch is particularly important when the heart is intensely stimulated. Prenatal development is another condition that requires high energy production through elevated mitochondrial biogenesis and during which the heart

undergoes a switch in energy substrate preference from glucose in the fetal period to fatty acids **(Lehman and Kelly 2002)**. Intriguingly, while double deletion of PGC-1α and PGC-1β does not severely affect the myocardial function if induced in the adult stage, the same gene ablations triggered perinatal death with reduced mitochondrial biogenesis and absence of arrest of fetal gene expression when induced before birth **(Lai, Leone et al. 2008, Martin, Lai et al. 2014)** (Fig. 3). These data further show that albeit dispensable for basal metabolism, the PGC-1 coactivators are critical in the process of metabolic adaptation to changes in energetic requirements.

Furthermore, in BAT, while PGC-1α global knockout mice are cold intolerant due to failure in the upregulation of the thermogenic program through UCP1 and deiodinase-2 (Dio2) transcription, its ablation does not alter their expression upon normal temperatures **(Lin, Wu et al. 2004)**. Increased transcription of the thermogenic and mitochondrial program upon PGC-1α upregulation in human **(Tiraby, Tavernier et al. 2003)** and mouse **(Puigserver, Wu et al. 1998)** white adipocytes further strengthen the importance of PGC-1α for the control of thermogenesis (Fig. 3). In WAT, scattered brown-like adipocytes can differentiate to beige adipocytes after stimulation with cold and FGF21 **(Fisher, Kleiner et al. 2012)**. In line with its role in BAT, PGC-1α plays a role in this differentiation process (Fig. 3), notably by promoting the expression of mitochondrial and thermogenic genes **(Fisher, Kleiner et al. 2012)**.

In liver, PGC-1α is induced and activated by fasting and will accordingly regulate a broad metabolic program including gluconeogenesis, fatty-acid β-oxidation, ketogenesis, heme biosynthesis, and bile acid homeostasis to adapt liver metabolism to the energetic crisis triggered by starvation **(Lin, Handschin et al. 2005)** (Fig. 3). This is done through the co-activation of numerous hepatic transcription factors such as HNF4α, PPARα, GR or FOXO1 **(Lin, Handschin et al. 2005)** (Fig. 3). Following these observations, mice lacking PGC-1α expression or with specific liver PGC-1α knock-down, fail to regulate hepatic gluconeogenic gene expression and hepatic glucose production **(Lin, Wu et al. 2004, Handschin, Lin et al. 2005)** (Fig. 3) and display insulin resistance **(Koo, Satoh et al. 2004)**, hypoglycemia and hepatic steatosis upon fasting **(Leone, Lehman et al. 2005)**. Interestingly, SITR1 deacetylation and S6 kinase 1 (S6K1)

phosphorylation of hepatic PGC-1α respectively potentiates and represses the regulation of gluconeogenic genes by PGC-1α without altering its capacity to induce the transcription of genes related to oxidative metabolism **(Rodgers, Lerin et al. 2005, Lustig, Ruas et al. 2011)**. This further demonstrates the fantastic ability of PGC-1α to induce specific gene networks to propose the appropriate answer to certain energetic and metabolic demands.

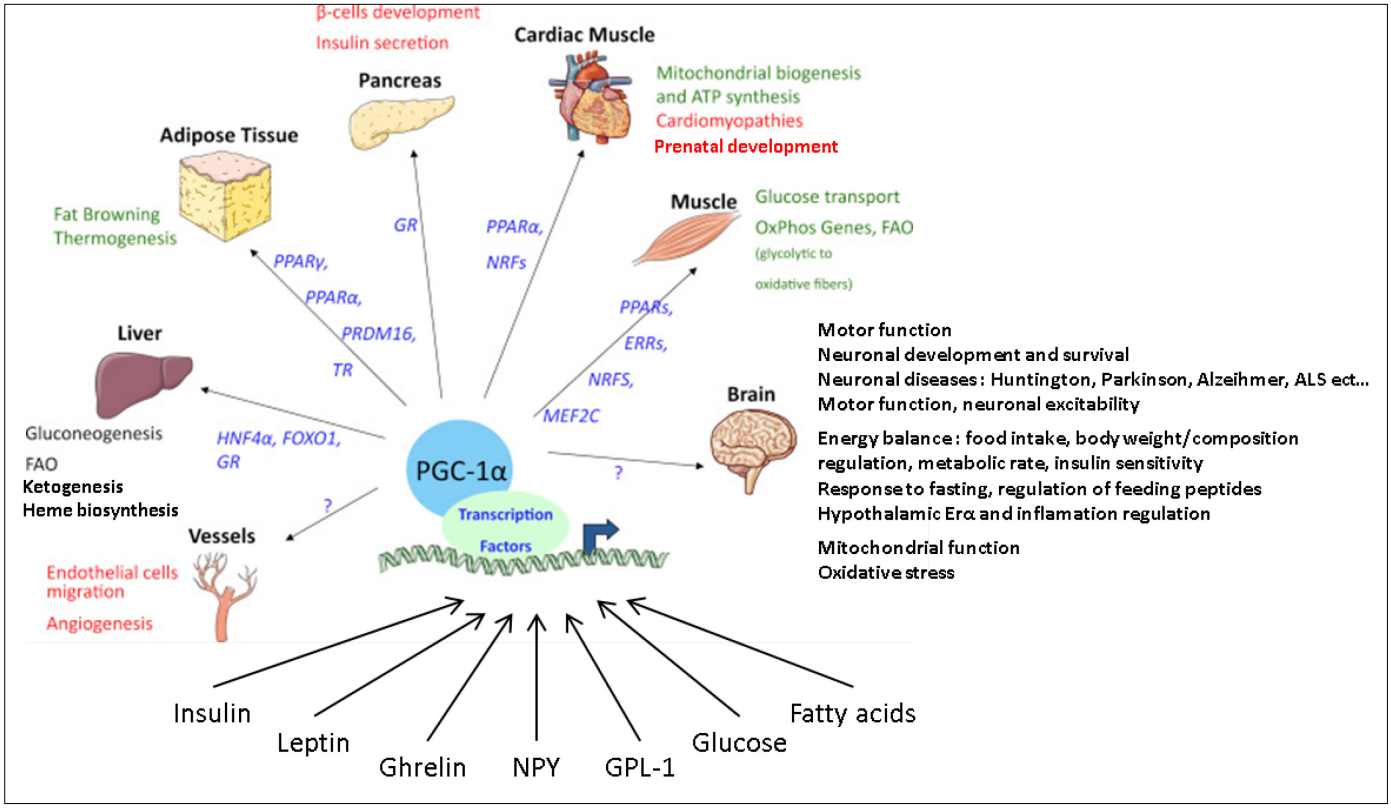

Figure 3: PGC-1α functions in various tissues, as well as its partners and metabolic regulators. adapted from **(Besseiche, Riveline et al. 2015)**

B. PGC-1α and the brain in the regulation of energy homeostasis

For survival, a living organism has to conserve a tight energetic balance, which is determined by energy intake and expenditure. Body energy homeostasis is achieved through fast metabolic adaptation to various situations and environmental changes such as physical activity, fasting or temperature alteration. Those metabolic changes are mainly mediated by a

transcriptional mechanism controlled by transcription factors and their coactivators **(Francis, Fayard et al. 2003)** that are sensitive to the cellular energetic status and that consequently regulate gene expression.

1. Brain control of whole body energy homeostasis

The ability to maintain energy homeostasis is a fundamental prerequisite for the proper of any living being. The body needs to store excess of calories when available to face periods of energetic deficit and ensure basal physiologic functions as well as additional critical energy demanding activities such as flight from predators or food foraging. This can occur over different amounts of time, from the simple sleeping overnight fast to longer periods of hibernation for example. Body weight and temperature maintenance are two examples perfectly illustrating how well healthy organisms are able to preserve stable energetic states. Accumulated lines of evidence implicates the central nervous system as a primary regulatory system of energy balance for the body **(Morton, Meek et al. 2014)**. Indeed, as PGC-1α can sense signals of the energetic status in the cell and adapt cellular metabolism accordingly, the brain is able to monitor and integrate multiple metabolic inputs from peripheral organs to enact appropriate and consequential metabolic and behavioral responses (Fig. 4). Thus, the central nervous system balances mechanisms of food intake with processes regulating energy expenditure, including thermoregulation, basal metabolism, and physical activity to meet changes in the body energy state **(Sandoval, Cota et al. 2008)**. Hypothalamus has been shown to control a broad range of function essential for the regulation of energy homeostasis such as sleep and arousal, hunger and thirst (Fig. 4) **(Waterson and Horvath 2015)**.

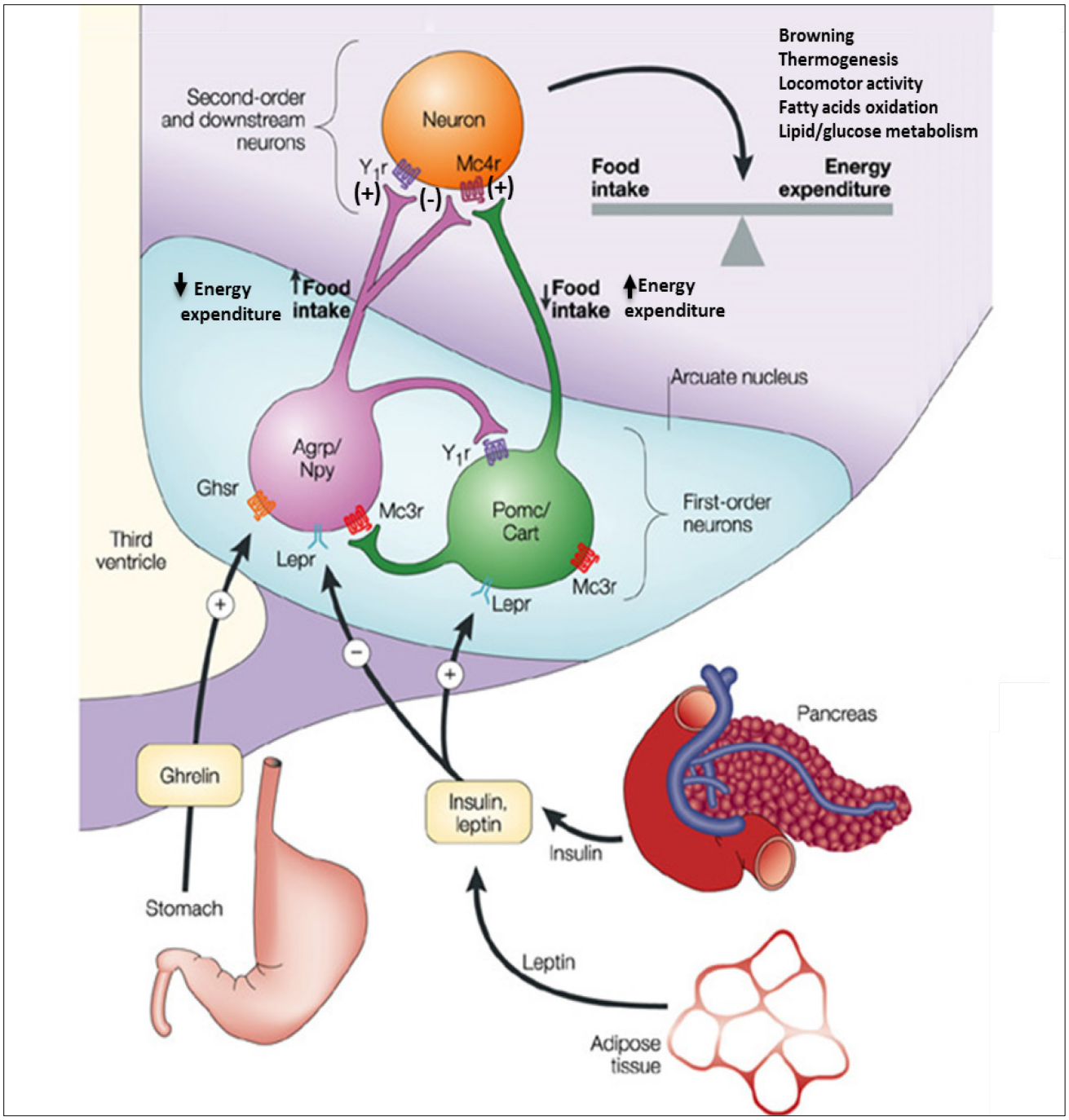

Figure 4: Control of energy homeostasis by arcuate nucleus neurons. adapted from **(Barsh and Schwartz 2002)**

Hypothalamic control of food intake and body weight

The role of the hypothalamus as a central regulator of feeding has been established in a study done in 1951 where the authors showed that ventromedial hypothalamus (VMH) and ventrolateral hypothalamus (VLH) lesions respectively trigger over- and under-feeding in the rat **(Anand and Brobeck 1951, Anand and Brobeck 1951)**. It is now established that the arcuate nucleus, located in the hypothalamus, is a key element of the regulation of energy balance. It is adjacent to the median eminence and surrounds the third cerebroventricle, which facilitates the entry of hormones and nutrients from the systemic circulation and cerebrospinal fluid **(Xu, Kaelin et al. 2005)**. Two main types of neurons expressing either neuropeptide Y (NPY) and agouti-related protein (AgRP) or proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) populate the arcuate nucleus (Fig. 4). The ablation of NPY and AgRP neurons leads to food intake and body weight reduction **(Gropp, Shanabrough et al. 2005, Luquet, Perez et al. 2005)** whereas the loss of POMC and CART neurons promotes an opposite effect **(Xu, Kaelin et al. 2005)**, firmly demonstrating the importance of these two kinds of neurons in the control of energy homeostasis (Fig. 4). Furthermore, AgRP and NPY expressions are increased during food deprivation while POMC and CART expression levels are decreased **(Ziotopoulou, Erani et al. 2000, Savontaus, Conwell et al. 2002)**, illustrating their role for the integration of energy deficit signals. Neurons of the arcuate nucleus project and act on a second order of neurons (Fig. 4) in different other nuclei of the hypothalamus such as the paraventricular nucleus (PVN), VMH and lateral hypothalamus (LH), as well as on autonomic preganglionic neurons in the brain stem and spinal cord to regulate energy balance **(Bouret, Draper et al. 2004)**. α-melanocyte-stimulating hormone (α-MSH), a product of posttranscriptional processing of POMC, is released from the synaptic terminal of POMC neurons and binds to melanocortin-3 (MC3R) and melanocortin-4 receptors (MC4R) on second-order neurons to reduce food intake and increase energy expenditure **(Millington 2007)**. Consistently, MC4R deletion leads to hyperphagia and obesity with reduced energy expenditure **(Huszar, Lynch et al. 1997)**. AgRP is released from AgRP neurons and competes with α-MSH for MC3Rs and MC4Rs to antagonize its effects **(Ollmann, Wilson et al. 1997)** while NPY induces food intake via Y1 or Y5 receptors **(Yulyaningsih, Zhang et al. 2011)**. NPY triggers rapid feeding

stimulation whereas AgRP action is promoting food intake for a longer period **(Bingham, Anderson et al. 2008)**.

Hypothalamic control of energy expenditure and metabolism

Opposite to energy intake that creates a positive energy homeostasis, energy expenditure promotes a negative energy balance. Besides regulating feeding, the hypothalamus and the arcuate nucleus can alter various mechanisms resulting in energy expenditure to maintain an appropriate energetic state including locomotor activity, FAO and thermogenesis (Fig. 4) **(Spiegelman and Flier 2001)**. For example, leptin administration in the arcuate nucleus stimulates sympathetic BAT innervation **(Rahmouni and Morgan 2007)** whereas suppression of the leptinergic pathway prevents sympathetic BAT activation (Fig. 4) **(Harlan, Morgan et al. 2011)**. Consistently, alteration of the MCR4 prevents central leptin induction of UCP1 in both BAT and WAT and decreases oxygen consumption **(Ste Marie, Miura et al. 2000, Zhang, Kilroy et al. 2005)**. AgRP and NPY neurons naturally inhibits BAT thermogenesis but their inhibition releases this repression and activates sympathetic fibers on BAT **(Bewick, Gardiner et al. 2005, Shi, Lau et al. 2013)**, therefore contributing to the leaner phenotypes of these mice compared with controls animals. Plus, intracerebroventricular (ICV) administration of NPY reduces core body temperature **(Currie and Coscina 1995, Hwa, Witten et al. 1999)**, oxygen consumption **(Currie and Coscina 1995, Hwa, Witten et al. 1999)** and represses thermogenic activity in BAT **(Egawa, Yoshimatsu et al. 1991)**. Opposite to the AgRP/NPY-BAT inhibition, POMC neuronal activation and α-MSH central treatment promote BAT activation through the sympathetic nervous system **(Butler and Cone 2002, Yasuda, Masaki et al. 2004, Yasuda, Masaki et al. 2004)**. In line with these data, α-MSH analogue MTII central administration elevates core body temperature **(Murphy, Nunes et al. 2000)**, oxygen consumption **(Hamilton and Doods 2002)** and uncoupling proteins expression in both BAT and skeletal muscle **(Cettour-Rose and Rohner-Jeanrenaud 2002)**. In addition to BAT regulation, co-administration of leptin and insulin stimulates WAT browning and energy expenditure that protects against diet induced obesity **(Dodd, Decherf et al. 2015)**. This suggests that thermogenesis represent a substantial source of energy expenditure that can compensate an excess of calorie intake.

Several lines of evidence indicate that the arcuate nucleus also regulates locomotor activity (Fig. 4). Both intracerebroventricular administration of NPY and deletion of the MC4R reduce spontaneous locomotion **(Heilig, Vecsei et al. 1989, Ste Marie, Miura et al. 2000)** whereas intracerebroventricular infusion of CART leads to the opposite effects **(Kimmel, Gong et al. 2000)**. These results fit with the lower activity observed during energetic deficit where AgRP and NPY levels are increased and CART and POMC expression decreases **(Ziotopoulou, Erani et al. 2000, Savontaus, Conwell et al. 2002)**. Voluntary activity is therefore used as an energy-conserving mechanism during energetic crisis. Further genetic modifications point towards a role for the arcuate nucleus in the regulation of locomotor activity. For example, voluntary activity is increased in obese mice that express leptin receptor only in POMC neurons compared to mice completely lacking leptin receptors. **(Huo, Gamber et al. 2009)**. Similarly, constitutive activation of Signal transducer and activator of transcription 3 (STAT3) in AgRP cells, which mediates leptin inhibiting signaling in AgRP neurons increases spontaneous activity and partially protects from high fat diet feedings **(Mesaros, Koralov et al. 2008)**. These results demonstrate that leptin induced spontaneous locomotor activity is driven by both POMC and AgRP neurons. Finally, deletion of other elements such as PPARγ in POMC neurons and ROCK1 in AgRP neurons also alters locomotor activity **(Huang, Lee et al. 2013, Long, Toda et al. 2014)**.

The metabolic adaptation of different tissue to environmental changes also includes alterations in the control of nutrient storage, transformation and substrate utilization. Therefore, autonomic nervous system (ANS) modulation of peripheral organ activity via efferent nerves is an essential adaptive response initiated by the brain, according to the different energetic states recorded. Interestingly, both AgRP and POMC neurons project to the pre-autonomic structures such as the PVN and show direct action on peripheral tissues (Fig. 4). Thus, central MCRs inhibition triggers elevation of lipid synthesis and storage and insulin stimulated glucose uptake in the WAT, glucose metabolism decrease in BAT and muscle and triglyceride synthesis and export from the liver, all independent from food intake **(Nogueiras, Wiedmer et al. 2007)**. Conversely, central activation of MCR promotes lipid mobilization in WAT. This perfectly illustrates initiation of changes in substrate utilization and storage by the melanocortin system. Furthermore, AgRP release has been shown to be mandatory for fasting-

induced hepatic stimulation by the sympathetic nervous system resulting in liver triglycerides synthesis while central AgRP knock-down preserves animals from liver steatosis upon high fat diet feeding **(Warne, Varonin et al. 2013)**. Lastly, restauration of leptin receptors in POMC neurons of mice with global leptin receptor knock-out restored blood glucose levels **(Huo, Gamber et al. 2009)** while mice with AgRP neurons specific ablation of insulin receptors display hepatic insulin resistance **(Konner, Janoschek et al. 2007)**. Changes in arcuate leptin signaling alter hepatic glucose production via reduction of hepatic gluconeogenic gene expression and BAT glucose uptake **(German, Kim et al. 2009)**. However, leptin microinjection in the VMH increases glucose uptake in skeletal muscle, heart and BAT **(Minokoshi, Haque et al. 1999)**. Together, those data show that the arcuate nucleus is important for nutrient partitioning and for whole body glucose metabolism.

Peripheral regulators of appetite

Various circulating elements reflect fat storage and inform our brain about the energetic reserve of our body (Fig. 4 and 5). Leptin is mainly expressed in the adipose tissue **(Zhang, Proenca et al. 1994)** and its circulating levels are highly correlated to fat mass **(Maffei, Halaas et al. 1995)**, although it is suppressed by food restriction and restored by insulin administration or refeeding **(Frederich, Lollmann et al. 1995, Maffei, Halaas et al. 1995)**. Central leptin administration reduces spontaneous and fasting-mediated hyperphagia **(Davis, Mullins et al. 1998)** through repression of AgRP and NPY expression and elevation of CART/POMC levels resulting in AGRP and POMC neurons inactivation and activation respectively **(Stephens, Basinski et al. 1995, Schwartz, Baskin et al. 1996, Hahn, Breininger et al. 1998, Elias, Aschkenasi et al. 1999)**. Similar to leptin, pancreas secreted insulin reflects long-term energy homeostasis **(Woods, Decke et al. 1974)** and correlates with total adiposity but also fat distribution **(Porte, Baskin et al. 2002)**. Insulin is rapidly secreted after feeding **(Polonsky, Given et al. 1988)** and decreases food intake and body weight when injected centrally **(Air, Strowski et al. 2002)**. Intracerebroventricular insulin administration abolishes fasting-induced increase in arcuate nucleus NPY transcript levels **(Schwartz, Sipols et al. 1992)** and elevates POMC expression **(Benoit, Air et al. 2002)**.

Hormones produced by the gut provide information about energy intake. Ghrelin is secreted by the stomach in periods of fasting **(Cummings, Clement et al. 2002)** and its plasma levels inversely correlates with adiposity **(Otto, Cuntz et al. 2001)**. Central injection of ghrelin elevates food intake, body weight and represses fat utilization **(Tschop, Smiley et al. 2000, Wren, Small et al. 2001)** while central administration of anti-ghrelin antibodies diminishes fasting induced refeeding **(Nakazato, Murakami et al. 2001)**. Failure of ghrelin to induced feeding after arcuate nucleus ablation **(Tamura, Kamegai et al. 2002)** and in mice lacking AgRP/NPY signaling, **(Chen, Trumbauer et al. 2004)** demonstrates that ghrelin regulation of food intake is mediate through the arcuate nucleus. Glucagon-like peptide-1, secreted from the L cells of the small intestine **(Herrmann, Goke et al. 1995)** inversely correlates to body mass **(Holst, Schwartz et al. 1983)**, inhibits food intake **(Ranganath, Beety et al. 1996, Naslund, Barkeling et al. 1999)** and is released post-feeding **(Herrmann, Goke et al. 1995)**.

Some circulating nutrients such as glucose, fatty acids or amino acids, signal nutrients availability and the energy status to the brain that can then regulate their production and utilization (Fig. 5). Thus, glucose reflects available energy resources and hypoglycemia indicates energetic deficits **(Myers and Olson 2012)**. Consequently, central injection of glucose or fatty acids decreases food intake **(Obici, Feng et al. 2002)** while glucose anti-metabolite 2-deoxy-D-glucose promotes it **(Miselis and Epstein 1975)**. Similarly, inhibition of carnitine palmitoyltransferase-1 elevates Long-chain fatty acyl-CoA content in the hypothalamus leading to reduced feeding **(Obici, Feng et al. 2003)**. Finally, Interleukin-6, which is released by the muscle according to exercise intensity and duration, induces energy expenditure and reduces body fat upon intracerebroventricular injection **(Wallenius, Wallenius et al. 2002)**.

2. The role of PGC-1α in the brain

PGC-1α in the healthy and sick brain

Like in every other highly energy demanding tissue, PGC-1α is highly expressed in the brain **(Esterbauer, Oberkofler et al. 1999, Wu, Puigserver et al. 1999, Knutti, Kaul et al. 2000)** where it is required for GABAergic neuronal development **(Cowell, Blake et al. 2007)** (Fig. 3). The brain largely relies on mitochondria to produce ATP that will serve for ion homeostasis and neuronal firing activity. Mitochondrial dysfunction therefore represents a common mechanism for many neurological disorders **(Chaturvedi and Flint Beal 2013)**. Given the master role of PGC-1α in the regulation of the mitochondrial function **(Houten and Auwerx 2004)**, it is natural to speculate about its contribution to the neuronal function. On that account, while the role of PGC-1α in the brain has not been investigated as extensively as in peripheral tissues (e.g. skeletal muscle or BAT), several studies already provided evidence for its fundamental role in the central nervous system. For example, global deletion of PGC-1α leads to motor dysfunctions **(Lucas, Dougherty et al. 2012)**, neurological abnormalities such as myoclonus and dystonia, and signs of neurodegeneration in the cortex, thalamus, basal ganglia, substantia nigra and hippocampus **(Lin, Wu et al. 2004, Jiang, Kang et al. 2016)** (Fig. 3). Interestingly, striatal degeneration, hyperkinetic movement disorders and mitochondrial dysfunction, also displayed in PGC-1α global knockout mice **(Lin, Wu et al. 2004, Leone, Lehman et al. 2005)**, are all reminiscent symptoms of the Huntington disease (Fig. 3) **(Dayalu and Albin 2015)**. Moreover, PGC-1α expression and activity are decreased in brains of both Huntington disease patients **(McGill and Beal 2006)** and mouse models **(Cui, Jeong et al. 2006, Weydt, Pineda et al. 2006)** and PGC-1α deletion aggravates the neurodegeneration of striatal neurons and motor abnormalities of the mouse model **(Cui, Jeong et al. 2006)**. In this animal model, mutant huntingtin interacts and binds with the CREB/TAF4 complex, which impairs PGC-1α promoter activation and represses the transcription of its target genes **(Cui, Jeong et al. 2006, McGill and Beal 2006)**. Importantly, overexpression of PGC-1α in both cultured striatal neurons and in the striatum of the huntingtin disease mouse model leads to neuroprotection and enhances mitochondrial function (Fig. 3) **(Cui, Jeong et al. 2006)**. Finally, polymorphism in PGC-1α and its

target genes like nrf1 or tfam modify the age onset of the Huntington disease **(Weydt, Soyal et al. 2009, Taherzadeh-Fard, Saft et al. 2011)**, confirming its important place in the pathogenesis of this neurodegenerative disorder.

Similar to Huntington disease, a decrease of PGC-1α levels and expression of its target genes is observed in patients with Parkinson disease **(Zheng, Liao et al. 2010)** and PGC-1α global knockout results in neurodegeneration of the dopaminergic neurons in the substantia nigra which characterizes the disease **(Lin, Wu et al. 2004)** (Fig. 3). Consistently, PGC-1α global deletion leads to increased vulnerability to MPTP induced degeneration of nigral dopaminergic neurons **(St-Pierre, Drori et al. 2006)** while its overexpression or activation by resveratrol protects dopaminergic neurons of the MPTP mouse model of Parkinson disease **(Mudo, Makela et al. 2012)**. PGC-1α promoter has recently been shown to be more methylated in Parkinson disease patients, which participates to its transcription repression **(Su, Chu et al. 2015)**. In addition, a study showed that PGC-1α knock-down increases α-synuclein accumulation in human neuronal cells, which also occurs in Parkinson **(Tsunemi and La Spada 2011)** disease patients. Consistently, another work shows that PGC-1α protects from a mutant α-synuclein-mediated cell death in vitro by improving expression of oxidative phosphorylation (OXPHOS) genes **(Zheng, Liao et al. 2010)**. Correlating with lower expression of PGC-1α in Parkinson disease patients, several studies report that alterations of Parkinson disease genes expression affect PGC-1α levels. For instance, upon inactivation of parkin, the parkin interacting substrate PARIS (ZNF746) is not degraded and represses the expression of PGC-1α by binding insulin-response sequences in the PGC-1α promoter **(Shin, Ko et al. 2011)**, simultaneously leading to the selective loss of dopamine neurons in the substantia nigra. The substantia nigra neurodegeneration induced by PARIS overexpression is reversed by either PARKIN or PGC-1α co-expression **(Shin, Ko et al. 2011)**.

Other investigations indicate beneficial effects of PGC-1α in Alzheimer and amyotrophic lateral sclerosis diseases and other neuronal conditions (Fig. 3). In Alzheimer disease patients, PGC-1α levels are decreased **(Qin, Haroutunian et al. 2009)**. In a mouse model of Alzheimer disease, PGC-1α decreases the accumulation of aggregated Aβ peptide **(Katsouri, Parr et al.**

2011), promotes neuronal survival and prevents learning impairments **(Kim, Nguyen et al. 2007)**. In an amyotrophic lateral sclerosis mouse model, PGC-1α improves survival, motor function, blood glucose and motor neuron survival **(Zhao, Varghese et al. 2011)** (Fig. 3). Besides these conditions, recent investigations illustrated beneficial effect of PGC-1α after intracerebral hemorrhage **(You, Hou et al. 2016)**, traumatic spinal cord injury **(Hu, Lang et al. 2016)** and in multiple sclerosis **(Nijland, Witte et al. 2014)**. Mechanistically, PGC-1α improves neuronal mitochondrial dysfunctions and reduces oxidative stress (Fig. 3) **(Lin, Wu et al. 2004, St-Pierre, Drori et al. 2006)**, which greatly participates to neuronal protection and many disorders ameliorations mediated by PGC-1α. In addition, deletion of PGC-1α alters inhibitory synaptic transmission in the motor cortex, which could contribute to cortical hyper-excitability and motor abnormalities of various neurological conditions **(Dougherty, Bartley et al. 2014)**. Together those elements confirm the importance of PGC-1α for healthy neuronal function and suggest that modulation of PGC-1α could be an attractive target in the fight against multiple neurological diseases. However, upregulation of PGC-1α expression would need to be carefully controlled as sustained overexpression of PGC-1α in the substantia nigra impairs the function of dopaminergic neurons **(Ciron, Lengacher et al. 2012)**.

PGC-1α as a potential regulator of energy homeostasis in the arcuate nucleus

Since PGC-1α is a master inducer of mitochondrial function **(Houten and Auwerx 2004)** and drives utilization of fat as fuel **(Liang and Ward 2006)**, one would expect its global deletion to result in weight gain and elevate the susceptibility to induced obesity. Paradoxically, mice with whole body PGC-1α ablation display resistance to obesity upon high fat diet feeding **(Lin, Wu et al. 2004)**. This unexpected result is likely driven by the increased metabolic rate, insulin sensitivity and spontaneous activity of these mice. However, it is difficult to discriminate which tissue could be responsible for the altered systemic energy balance. In addition, PGC-1α controls various metabolic pathways in different tissues further modulating systemic metabolism through crosstalks and secondary effects, which makes the interpretation even more difficult. To test whether the changes in whole body energy homeostasis were due to the action of PGC-1α in the brain or in peripheral tissues, Lin and collaborators developed a PGC-1α

brain knockout mouse by crossing PGC-1α flox/flox mice with calcium/calmodulin-dependent protein kinase IIα (CaMKIIα)-Cre transgenic mice **(Ma, Li et al. 2010)**. Similar to whole body deletion of PGC-1α, its neuronal ablation results in resistance to obesity and liver steatosis, despite high fat diet feeding and hyperphagia, and leads to increased metabolic rate and insulin sensitivity suggesting a role for PGC-1α in the central control of energy balance (Fig. 3). However, the resistance to body weight gain upon a high fat diet remains less pronounced than for the PGC-1α whole body knockout mice and brain deletion of PGC-1α does not lead to hyperactivity. This suggests that CaMKIIα negative neurons or peripheral tissues might also contribute to energy balance alteration observed in PGC-1α whole body knockout mice. Alternatively, it cannot be ruled out that some CaMKIIα positive cells still lack cre expression or that PGC-1α expression is not fully abolished in a subset of CaMKIIα neurons and could still mediate its role in the regulation of energy homeostasis. Further evidence suggests that PGC-1α not only influences energy homeostasis in the brain but also directly regulates energy balance from the central hypothalamic feeding center. For example, hypothalamic PGC-1α levels are increased upon fasting **(Coppari, Ramadori et al. 2009)** (Fig. 3) and PGC-1α is present in NPY neurons **(Draper, Kirigiti et al. 2010)**. Interestingly, both its whole body **(Lin, Wu et al. 2004)** and neuronal **(Ma, Li et al. 2010)** ablation blunt the fasting-induced expression of the feeding peptides AgRP and NPY (Fig. 3). These results indicate that PGC-1α responds to fasting signals in the hypothalamus and that it is required to coordinate transcriptional changes necessary to cope with fluctuations in body energy content. Converse to fasting, high fat diet and fatty acids treatment depletes PGC-1α in the hypothalamus, which affects ERα expression (Fig. 5) and induces hypothalamic inflammation **(Morselli, Fuente-Martin et al. 2014)** (Fig. 3) supporting the role of PGC-1α in the hypothalamus for transcriptional adaptation to changes in energetic signaling. Besides inflammation, ERα downregulation in different hypothalamic neurons that alters food intake, energy expenditure, and body weight could also be regulated by PGC-1α **(Xu, Nedungadi et al. 2011)**. In addition, the disruption of the circadian rhythm by the global deletion of PGC-1α **(Liu, Li et al. 2007)** and the circadian oscillation of PGC-1α in POMC neurons **(Agapito, Zhang et al. 2014)** (Fig. 3) reinforces the idea of a major role for PGC-1α at the central level.

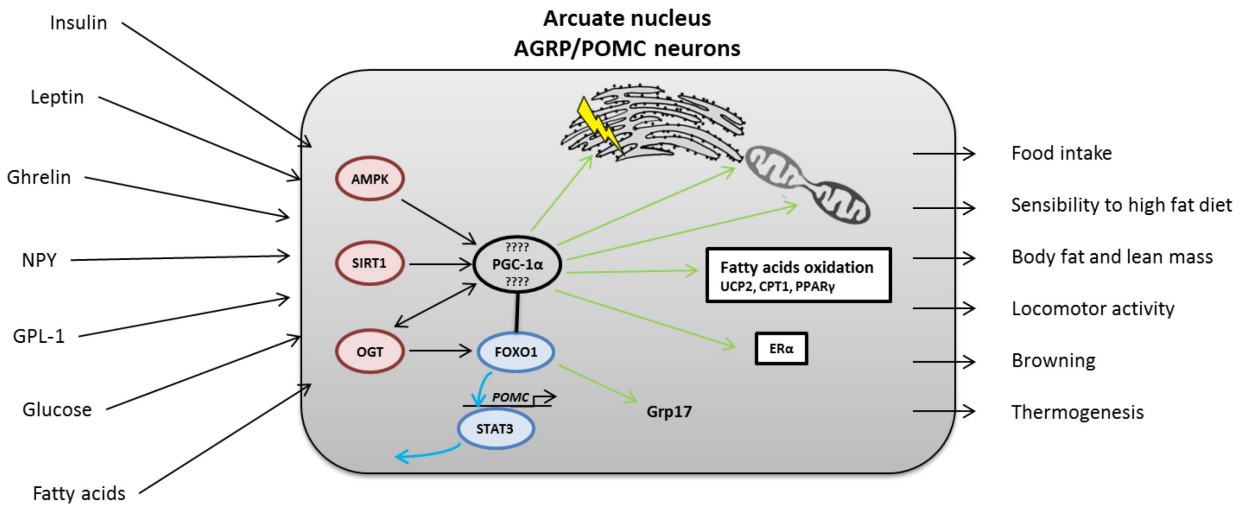

Figure 5: Signaling pathways in AgRP and POMC neurons regulating energy homeostasis.

As described above, PGC-1α is an excellent energy metabolic sensor and can integrate various signals in peripheral tissues that are also used in the arcuate nucleus to regulate whole body energy homeostasis (Fig. 3 and 5). As a result, in liver, PGC-1α responds to subcutaneous ghrelin injections to increase hepatic gluconeogenic production **(Barazzoni, Zanetti et al. 2007)** (Fig. 3). In a chronic kidney disease mouse model showing muscle mass decline, ghrelin has been shown to reduce the methylation of a cysteine residue upstream of the PGC-1α gene which improved the PGC-1α expression decrease observed in these mice **(Tamaki, Hagiwara et al. 2015)**. The ghrelin mediated increase in PGC-1α levels likely contributed to the improved mitochondrial amounts, muscle mass and exercise performance of ghrelin treated mice, indicating that PGC-1α responds to ghrelin and can in turn coordinate an appropriate metabolic adaptation. Similarly, a study indicates that PGC-1α ameliorates insulin-stimulated glucose transport in muscle and that its upregulation mediates insulin sensitivity improvements induced by exercise **(Bonen 2009)**. In line with these results, PGC-1α overexpression ameliorates disrupted insulin signaling in muscle and its deletion disturbs it **(Pagel-Langenickel, Bao et al. 2008)** (Fig. 3). Interestingly, muscle knock-down of the insulin receptor reduces PGC-1α expression and mitochondrial bioenergetics **(Pagel-Langenickel, Bao et al. 2008)**. In liver, insulin indirectly regulates PGC-1α transcriptional activity through foxo1 interaction to control the expression of the gluconeogenic program **(Puigserver, Rhee et al. 2003)**. Leptin, another

regulator of appetite, also influences muscle PGC-1α expression to regulate muscle mass (Fig. 3). Indeed leptin injections restore muscle mass and PGC-1α expression that were decreased in leptin-deficient ob/ob mice compared to control mice **(Sainz, Rodriguez et al. 2009)**. The restoration of muscle mass is likely promoted by PGC-1α mediated downregulation of atrophic genes **(Sandri, Lin et al. 2006)**. Likewise, in BAT, PGC-1α expression is reduced in both leptin-deficient ob/ob mice and leptin-unresponsive db/db mice and leptin treatments rescued PGC-1α and UCP1 expression **(Kakuma, Wang et al. 2000)** (Fig. 3). Moreover, PGC-1α cold-exposure induction was largely reduced in leptin unresponsive rats **(Kakuma, Wang et al. 2000)**. Those results indicate that PGC-1α integrates leptin signaling to promote cold exposure adaption in BAT. Finally, in liver, leptin treatment stimulates PGC-1α expression, thereby activating mitofusin 1 (Mfn1) and ultimately ameliorating hyperlipidemia **(Hsu, Lee et al. 2015)**. In liver cells, PGC-1α also responds to GLP-1 through miR-23a downregulation and mediates GLP-1 protection against apoptosis **(Wang, Li et al. 2015)** (Fig. 3). In the same manner, upon treatment with GLP-1 receptor agonists, PGC-1α promotes mitochondrial biogenesis and anti-oxidation to provide neuroprotection **(An, Chen et al. 2015)**. Lastly, PGC-1α expression was shown to be increased dose-dependently upon free fatty acids exposure and inhibited by glucose treatment **(Zhang, Liu et al. 2005)** (Fig. 3) indicating that PGC-1α is also sensing fatty acids and glucose levels. Taken together, those data show that PGC-1α is sensitive to all the elements that act on the arcuate nucleus to regulate whole body energy homeostasis (Fig. 5). This therefore strongly indicates that PGC-1α could plays a key role in AgRP and POMC neurons by integrating and sensing signals of whole body energy balance sent by peripheral tissues.

In addition to responding to all the regulators of appetite, PGC-1α also steps in many signaling pathways controlling energy balance in the arcuate nucleus (Fig. 5). Thus, SITR1 that activates PGC-1α through de-acetylation is required in AgRP neurons for food intake regulation **(Dietrich, Antunes et al. 2010)**. SIRT1 inhibition disrupts POMC inhibitory tones and AgRP neuronal activity, which leads to impaired food intake induction during the dark period and upon ghrelin treatment, ultimately leading to reductions in both fat and lean mass **(Dietrich, Antunes et al. 2010)**. Intriguingly, SIRT1 loss in POMC neurons leads to an opposite phenotype that is hypersensitive to diet-induced obesity through reduction of energy expenditure

(Ramadori, Fujikawa et al. 2010) which suggest that SIRT1 is regulated by different signals in AgRP and POMC neurons or that it is engaged in different signaling pathways in those two kinds of cells. A second major regulator of PGC-1α through phosphorylation, the AMPK, plays a key role in the control of energy homeostasis (Fig. 5). Indeed, AMPK is activated upon fasting in the arcuate nucleus and promotes the activation of NPY neurons and an induction of food intake that is dependent of NPY **(Kohno, Sone et al. 2011)**. AMPK is responsible for glucose sensing in both AgRP and POMC neurons **(Claret, Smith et al. 2007)**. In addition, similar to SIRT1 deletion, loss of AMPK in AgRP and POMC neurons respectively, leads to a lean phenotype and increased obesity due to reduced energy expenditure and impaired food intake **(Claret, Smith et al. 2007)**. Besides being regulated by elements involved in the arcuate nucleus control of energy balance, PGC-1α interacts with various proteins that are also necessary for AgRP and POMC neuronal activity. For example, the O-GlcNAcylation of PGC-1α by the O-GlcNAc transferase (OGT) in liver prevents PGC-1α from degradation by promoting the binding of the de-ubiquitinase BRCA1 associated protein-1 (BAP1) **(Ruan, Han et al. 2012)**. Glucose availability modulates gluconeogenesis through the stabilization of PGC-1α via the OGT. In turn, PGC-1α binds to the OGT and targets it to the FOXO1 protein for its GlcNAcylation, which increases its transcriptional activity and eventually contributes to the induction of gluconeogenesis **(Housley, Udeshi et al. 2009)**. Interestingly, both OGT and FOXO1 are essential for central control of energy homeostasis (Fig. 5). Upon fasting, OGT expression in the AgRP neurons suppresses WAT browning through the activation of AgRP neuronal activity in order to conserve energy **(Ruan, Dietrich et al. 2014)**. Activation of FOXO1 in AgRP neurons induces food intake while its inactivation inhibits it and promotes leanness through the regulation of G Protein-Coupled Receptor 17 (Gpr17) **(Kitamura, Feng et al. 2006, Ren, Orozco et al. 2012)**. In POMC neurons, FOXO1 inhibits leptin-induction of POMC expression by interacting with STAT3 which prevents its binding to the specificity protein –POMC promoter complex (Fig. 5) **(Yang, Lim et al. 2009, Ma, Fuentes et al. 2015)**. Since PGC-1α is a master regulator of mitochondrial FAO **(Vega, Huss et al. 2000)** and controls the expression of uncoupling protein 2 (UCP2) **(Oberkofler, Klein et al. 2006)** and carnitine O-palmitoyltransferase 1 (CPT1) **(Baldan, Relat et al. 2004)**, it is interesting to note that SIRT1 and AMPK signaling in the AgRP neurons engages a change in the redox status, leading to the generation of ATP and sustained neuronal activity through β-oxidation of

fatty acids via UCP2 and CPT1 (Fig. 5) **(Andrews, Liu et al. 2008, Lopez, Lage et al. 2008, Dietrich, Antunes et al. 2010)**. Consistently, the expression UCP2 is necessary in AgRP neurons to mediate ghrelin-induced food intake **(Andrews, Liu et al. 2008)** and CPT1 has been shown to be a major regulator of feeding (Fig. 5) **(Obici, Feng et al. 2003)**. PPARγ, a well-known binding partner of PGC-1α involved in FAO metabolism **(Puigserver, Wu et al. 1998)**, is important for energy balance maintenance in the arcuate nucleus. Indeed, its activation within AgRP neurons is enough to elevate AgRP/NPY mRNA level and food intake **(Garretson, Teubner et al. 2015)** while during high fat diet, it sensitizes POMC neurons to leptin and mediates their cellular, biological, and functional adaptations **(Long, Toda et al. 2014)**. Aside from mitochondrial FAO, mitochondrial dynamics also represent a crucial element in the signaling pathways that activates AgRP and POMC neurons and that is controlled by PGC-1α (Fig. 5) **(Martin, Lai et al. 2014, Cannavino, Brocca et al. 2015, Greene, Lee et al. 2015)**. In AgRP neurons, food deprivation induces mitochondrial fission whereas over-feeding leads to mitochondrial fusion **(Dietrich, Liu et al. 2013, Nasrallah and Horvath 2014)** mainly through Mfn1 and 2. Indeed, single deletion of both mitofusins proteins in AgRP neurons protects against high fat diet induced mitochondrial fusion and obesity **(Dietrich, Liu et al. 2013)**. Conversely, POMC neuron-specific Mfn2 deletion results in hyperphagia, decreased energy expenditure and morbid obesity in chow-diet fed mice **(Schneeberger, Dietrich et al. 2013, Contreras, Nogueiras et al. 2016)**. This phenotype is driven by the disruption of mitochondrial endoplasmic reticulum association that triggers an increase of ER stress mediated leptin resistance in the POMC neurons (Fig. 5). Moreover, genetic models, inducing ER stress in POMC neurons triggers hepatic gluconeogenesis that is abolished when ER stress is relieved **(Schneeberger, Gomez-Valades et al. 2015)**. In line with previous results, mice overexpressing the stress reliever X-box-binding protein 1 (Xbp1) in POMC neurons display increased energy expenditure and thermogenesis markers BAT and WAT **(Williams, Liu et al. 2014)**. Remarkably, PGC-1α has been shown to mediate the ER stress response to exercise in muscle **(Wu, Ruas et al. 2011)** and could potentially also impact POMC neuronal signaling in the same manner.

Taken together, the facts that PGC-1α senses and responds to all signaling elements altering the arcuate nucleus regulation of energy balance and that it interacts, controls and

regulates many players of the AgRP and POMC signaling pathways (Fig. 3 and 5), strongly indicate that PGC-1α is a keystone in the function of AgRP and POMC neurons. The effects of global and neuronal PGC-1α ablation on energy balance further reinforce this hypothesis. In this view, specific deletion of PGC-1α in AgRP and POMC neurons could provide new essential information about the regulation of energy homeostasis in the arcuate nucleus. In addition, this would also prevent the long array of dysfunctions in multiple tissues and brain area from the other knockout mouse models that might overshadow the selective effects of PGC-1-α deletion in those neurons.

C. PGC-1α and skeletal muscle aging

Similar to the brain, skeletal muscle requires fast metabolic adaptations to switch to different energy metabolism and substrate utilization according to the environmental conditions, the source of energy available and the type of effort it has to produce. For example, in high intensity exercise of short duration, the degradation of glycogen to lactate is used as the primary source of fuel, whereas during prolonged submaximal exercise almost all ATP molecules arises from oxidative metabolism of carbohydrates and lipids **(Withers, Sherman et al. 1991)**. In this aim, living organisms need various mechanisms to sense energy availability inside the muscle cell and at the whole body level and to integrate and execute central commands of contraction. As neurons, skeletal muscle is very demanding in energy, especially for actin–myosin cross-bridge cycling during contraction and in addition to other energy dependent cellular processes such as excitation–contraction coupling. Since the ATP intramuscular concentration is low (5–6 mmol/kg wet weight), a substantial production is required to sustain the energetic demand during exercise. Muscle is therefore another organ where PGC-1-α endorses an important role as a coactivator of mitochondrial biogenesis and as a metabolic sensor. Consequently, it is also very likely involved in mitochondrial dysfunction related myo-pathophysiology including muscle aging.

1. Skeletal muscle aging

By 2050, the world population over 60 years old will have risen from 11% to 22%. 2 billion people will be over 60 years old and 400 million over 80 years old (World Health Organization, http://www.who.int/en/). Increased life expectancy is undoubtedly positive, although it implies higher incidence of chronic health conditions and a rise in prevalence of impairment and disability. Cognitive decline, musculoskeletal disorders, frailty and sarcopenia heavily contribute to elder's disability and loss of independence. The lack of mobility, as well as the daily-required activities, frequently difficult and painful, often leads to further reduced activity, which exacerbates those aging related disorders and creates a vicious cycle, ultimately resulting in a severe decay in health span. Muscle loss of function is a strong predictor for mobility limitation, increased rates of falls and ultimately mortality. For example, an elderly with low muscle strength has a 2.6-fold greater chance of mobility limitation and a 2.1-fold higher risk of mortality than an older adult with high muscle force **(Manini, Visser et al. 2007)**. Understanding the mechanisms of muscle aging and unraveling treatments to improve muscle disorders would definitely enhance the independency and the life quality of our exponentially growing elderly population.

Skeletal muscle loss and dysfunction during aging

The term sarcopenia was used for the first time by Rosenberg to refer to the loss of lean mass with aging **(Rosenberg 1997)**. In the last few years, the definition of sarcopenia was extended and criteria for diagnosis were added, including low muscle mass measured by bioelectrical impedance or dual energy X-ray absorptiometry [DEXA] but also muscle weakness determined by grip strength and altered muscle functions, such as low walking speed, altered sit to stand time or impaired balance **(Cruz-Jentoft, Baeyens et al. 2010)** (Fig. 6). Muscle cross sectional area starts to decline after the fourth decades of age in humans **(Lexell, Taylor et al. 1988, Short, Vittone et al. 2004)**. Muscle mass decreases at 1-2% per year after the age of 50 years **(Hughes, Frontera et al. 2002)** and declines at approximately 25% between 50 and 75 years of age **(Balagopal, Rooyackers et al. 1997)**. The loss of muscle mass further accelerates in advanced elderly, in such a way that the prevalence of sarcopenia is 50% in adults over 80 years

old whereas only 15% of adults over 65 years old are sarcopenic **(Iannuzzi-Sucich, Prestwood et al. 2002)** (Fig. 6). Several studies using computed tomography, MRI and ultrasonography demonstrated that muscle tissue reduction is accompanied by infiltration of fat and connective tissue **(Sipila and Suominen 1994, Jubrias, Odderson et al. 1997, Kent-Braun, Ng et al. 2000)**, a condition known as myosteatosis **(Taaffe, Henwood et al. 2009)**. This further reduces the net contractile muscle mass estimated though cross sectional area measurement. In parallel of muscle mass loss, a decrease of muscle strength is observed in elderly adults (Fig. 6). Isokinetic strength presents a drop of 14% and 16% per decade for knee extensors and knee flexors respectively in both men and women **(Hughes, Frontera et al. 2001)**. Other studies indicated a higher loss of muscle strength and mass in men than women, especially in the lower limbs **(Frontera, Hughes et al. 2000, Hughes, Frontera et al. 2001)**. Importantly, the authors found that muscle strength normalized to free-fat mass also decreases with age, demonstrating that not only muscle mass but also muscle efficiency, or in other words, muscle quality, declines with age. This decline of muscle mass and function with age translates in lower aerobic performance (Fig. 6), as determined by lower maximal oxygen consumption **(Short, Vittone et al. 2004)** and reduced resting body metabolic rate **(Piers, Soares et al. 1998, van Pelt, Dinneno et al. 2001)**. Moreover sarcopenia negatively impacts balance performance (Fig. 6). Elderly adults displaying sarcopenia have a chance to fall increased by 3 fold compared to an elderly person without sarcopenia **(Landi, Liperoti et al. 2012)**. A fall accident that triggers hospitalization, immobilization, reduced muscle and locomotor activity, increases sarcopenia and the risk of falling, reproducing the vicious cycle mentioned above.

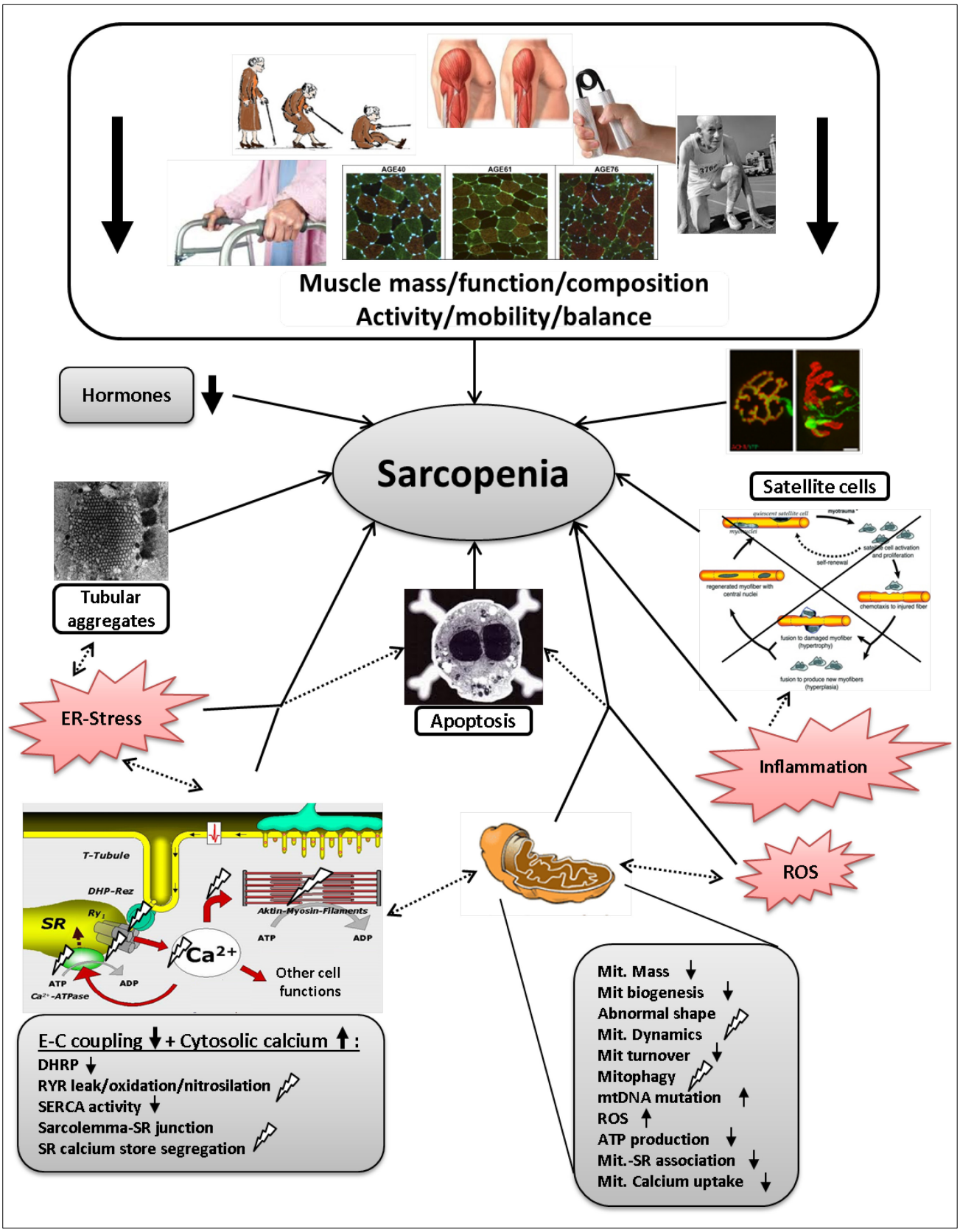

Figure 6: Muscle functional and molecular changes during aging

Age-related changes in the muscle fiber

The decline in muscle mass and whole muscle cross sectional area implies both a decrease in myofiber numbers (hypoplasia) **(Lexell, Taylor et al. 1988)** and size (atrophy) **(Aniansson, Hedberg et al. 1986)**, conversely to disuse atrophy that only triggers loss in muscle fiber cross sectional area but not in numbers (Fig. 6). Muscle fiber atrophy is strongly driven by size reduction of type II fibers, which have been largely demonstrated to be more sensitive to age-related atrophy than type 1. One of the first works in this domain showed that area of type I and type II fibers display a 23% and 42% reduction between 20-29 and 55-60 year old individuals **(Larsson 1978)**. A second study found a 26% decrease in cross sectional areas of type II fibers but no difference in areas of type I fibers between 80 year old and 20 year old subjects **(Lexell, Taylor et al. 1988)**. Higher decrease in type IIb than type IIa fibers has been reported while another work depicted no difference between the 2 subtypes **(Klitgaard, Mantoni et al. 1990, Coggan, Spina et al. 1992)**. However, age-related muscle cell atrophy seems to be muscle dependent as experiments on biceps brachii did not show changes in fiber size between the different fiber types of young and old adults **(Klitgaard, Mantoni et al. 1990)**. In addition, the clear assessment of different fiber types is harder at old age as many fibers co-express MHC-I and –II **(Andersen 2003)**. Alteration of expression of the different isoforms and thusly fiber type shifts are dependent on different processes occurring during aging. Neuropathic denervations touching preferentially fast motor units drive a shift toward slow fiber type **(Campbell, McComas et al. 1973)**. However, muscle disuse and lack of neuromuscular activity promotes expression of fast MHC isoforms driving the opposite shift **(Fluck and Hoppeler 2003, Liu, Schlumberger et al. 2003)** while finally, exercise leads to a shift towards slower fiber type (Fig. 6). Therefore an “aging fiber type signature” does not really exist, as fiber type difference can be the consequence of various events, leading to discrepancy between studies performed in different conditions. For example, while early cross sectional studies found a selective decline of type II fibers promoting an increase of type I/II ratio with age **(Larsson 1978)**, other work found a rather close decline between type I and type II fibers with age **(Lexell, Downham et al. 1986, Lexell, Taylor et al. 1988)**. Finally, individual muscle fibers also produce lower maximal force with age, independent of fiber type and even after normalization for fiber size. This indicates

that reduction of muscle strength cannot be caused only by hypoplasia and atrophy but also implies other age-related muscle changes.

Molecular mechanisms driving muscle aging

Age-related deterioration of the neuromuscular junction (NMJ), the interaction site between the nerve and the muscle, has been widely reported **(Wokke, Jennekens et al. 1990, Balice-Gordon 1997, Deschenes, Roby et al. 2010)** (Fig. 6). However, it is not established whether it is cause or consequence of sarcopenia, or whether these changes are endorsed by pre-synaptic or post-synaptic deteriorations. Muscle atrophy might originate from pre-synaptic dysfunction. Likewise the changes of fiber types described above and fiber type grouping observed in aged muscle **(Lexell and Downham 1991)** constitutes evidences for a denervation/reinnervation process in the aging muscle **(Beermann, Cassens et al. 1977, Kostrominova 2010)**. On the other hand, the rise of central nuclei in old muscle indicates post-synaptic involvements. In addition, NMJ age-associated changes differ among muscle types suggesting that they could be linked to muscle activity levels **(Rosenheimer 1990)**. NMJ alterations encompass various pre- and post- synaptic changes during muscle aging (Fig. 6). In mice tibialis anterior muscle, between 6 and 24 months of age, the number of fragmented NMJ goes from less than 5% to over 80% while faint acetyl-choline receptor from 0% to 8% and fully denervated endplates from 0% to 15% **(Valdez, Tapia et al. 2010)**. In the same muscle, pre-synaptic change includes a rise from 0% to 15% sprouting axons, approximately 1% to over 20% swelling axons and 2% to 40% thin axons between 6 and 24 month old mice. Additionally, age-related increases in area, perimeter, branched synaptic fold and subsarcolemmal vesicles have been reported **(Fahim and Robbins 1982, Fahim 1993)**, as well as lower synaptic vesicles, correlated to increased amplitude of evoked endplate potentials associated with an enhanced neurotransmitter turnover **(Kelly and Robbins 1983)**.

While NMJ deterioration might be a cause or a consequence of muscle loss during aging, satellite cells alterations represent a likely contributor of age-associated muscle atrophy, as they constitute the regenerative power of the muscle (Fig. 6). Especially, decline of satellite cells

associated with fiber type II **(Verdijk, Koopman et al. 2007)** indicates that their deterioration contributes to muscle loss, as it is mainly driven by the atrophy of those fiber types. Both decline in satellite cell numbers and function jeopardizing the regenerative capacity of the whole muscle **(Sousa-Victor, Gutarra et al. 2014)** can be attributed to loss of satellite cells regenerative capacity, exhaustion to forced differentiation and apoptosis. This leads to increased fibrosis due to aberrant repair after injury **(Fry, Lee et al. 2015)**. Besides intrinsic satellite cell alterations during aging, rejuvenation of satellite cell upon exposure to young environment and circulatory system indicates that extrinsic factors impact satellite cell aging **(Villeda, Luo et al. 2011)**. For instance, increasing inflammation in old muscle has been shown to negatively impact stem cell function **(Franceschi, Bonafe et al. 2000, Smythe, Shavlakadze et al. 2008)**. In line with this observation, inhibition of the pro-inflammatory marker, nuclear factor-kappa B and the resulting decrease of IL-6 improve muscle regenerative capacities in aged mice (https://dash.harvard.edu/handle/1/17464788). In addition, increased levels of interleukins 1 and 6, as well as tumor necrosis factor α during aging, enhances catabolic activity in the muscle **(Doherty 2003)**.

Contrary to catabolic factors, extrinsic factors stimulating muscle growth are reduced with age, which further reduces muscle mass (Fig. 6). For example, growth hormone, insulin-like growth factor and testosterone levels are all reduced in older adults **(Corpas, Harman et al. 1993, Morley, Kaiser et al. 1997)**. Likewise, inhibition of proteolysis and activation of protein synthesis by insulin in response to feeding is reduced in aged subjects **(Wilkes, Selby et al. 2009)**. Muscle protein synthesis upon amino acid feeding is also reduced in elderly adults **(Cuthbertson, Smith et al. 2005)**. In parallel, aging inhibits insulin stimulated muscle glucose uptake and disposal, and lipid utilization by the mitochondria **(Cleasby, Jamieson et al. 2016)**, therefore impairing muscle growth and function. In turn, as muscle represent 40-50% of the body mass, it is the primary source of glucose disposal **(Baron, Brechtel et al. 1988)** and its age-associated insulin insensitivity largely contributes to whole body insulin resistance. For the same reason, muscle mass age-related reduction, further contributes to alterations of whole body glucose homeostasis. In line with those observations, sarcopenia has been recently associated with the metabolic syndrome **(Dominguez and Barbagallo 2016, Lee, Hong et al. 2016)** which

includes obesity, hypertension, hyperglycemia and insulin resistance **(Srikanthan, Feyh et al. 2016)**.

In addition of external factors impacting skeletal muscle aging, intra-cellular pathways and processes are impaired in the old myofiber, leading to muscle dysfunction and muscle mass loss. The main function of skeletal muscle, with preservation of body homeostasis, is to allow posture maintenance and body motor functions, both relying on its contractile ability. A key physiological process for muscle contraction affected by aging is the excitation-contraction coupling mechanism that transforms sarcolemmal action potential into muscle force generation **(Delbono 2002)** (Fig. 6). The dihydropyridine receptor (DHRP) is an essential protein for this process. It activates calcium release from the sarcoplasmic reticulum (SR), which in turn will bind troponin C, resulting in actomyosin interaction and muscle contraction. When this excitation-contraction coupling mechanism is altered in any way, muscle displays lower fiber activation, force generation and ultimately reduced whole muscle strength **(Manini and Clark 2012)**. In old muscles, DHRP concentration is reduced (Fig. 6), as well as its coupling with the ryanodine receptor (RYR) and consequently the induced calcium release **(Renganathan, Messi et al. 1997, Delbono 2000)**. Interestingly, authors also demonstrated that muscle overexpression of the insulin growth factor-1 prevents age-related reduction of the DHRP level and function, resulting in improved muscle strength. This shows that age-associated reduction of this factor is not only important for muscle mass as described above but also for muscle function. Alternatively, NMJ alterations could also cause excitation-contraction uncoupling as denervation reduces DHRP and RYR expression **(Delbono 1992, Ray, Kyselovic et al. 1995)**.

Besides lower expression of the excitation-contraction proteins, another study reported changes in the organization and the structure of the junction between SR and transverse tubules during muscle aging of humans **(Boncompagni, d'Amelio et al. 2006)** (Fig. 6). The junctional alterations included morphological and cellular disposition changes, as well as reduced occurrence of the junctional units, which in turn reduces the amount of calcium released from the SR to the myoplasma **(Jimenez-Moreno, Wang et al. 2008)** and its availability for the contractile apparatus, ultimately associated with a lower muscle strength **(Payne, Jimenez-Moreno et al. 2009)**. Additionally, a portion of the SR calcium store is segregated from

the calcium releasing machinery with age (Fig. 6) **(Weisleder, Brotto et al. 2006)** (likely due to the disruption of the sarcolemma-SR junction), further reducing the amount of calcium available for muscle contraction and reinforcing muscle weakness. On the other hand, oxidation and nitrosilation of the RYRs disturbs calbastatin function that limits ryanodine receptor calcium release **(Andersson, Betzenhauser et al. 2011)**. As a consequence, calcium leak from the SR is increased in old muscle and impairs myoplasmic calcium homeostasis **(Boschek, Jones et al. 2008)** (Fig. 6). Impaired calcium re-uptake by the SR, associated to lower sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) activity in elderly adults reinforces intracellular calcium level increase in old muscle **(Hunter, Thompson et al. 1999)**.

Increased myoplasmic calcium levels during muscle aging represents a harmful stress for the muscle, not only because it disrupts excitation-contraction coupling but also because dysregulated calcium cytosolic levels are toxic for the cell **(Kass and Orrenius 1999)** and that proper calcium signaling regulating key cellular functions, such as cell survival/death **(Zhivotovsky and Orrenius 2011) (Miyawaki, Zhou et al. 1996)** and ATP production **(Jouaville, Pinton et al. 1999)**, rely on highly precise calcium homeostasis. Therefore, a large range of mechanisms are used by the cell to buffer possible excess of calcium, including high capacity binding protein, pump and exchanger for SR re-uptake, and the mitochondria which sequester calcium as soon as it overrides the dangerous concentration of 500nM in the cytosol, thus representing a major buffering and detoxifying compartment **(Kass and Orrenius 1999)**. However, prolonged or extreme calcium overload due to failure of those mechanisms, such as the age-related calcium leak or reduced calcium SR-reuptake described above, will lead to cell damages and ultimately cell death **(de Leiris and Boucher 1990, Richter 1993)**. Decreased mitochondrial calcium handling is also observed in old muscle and will be discussed in more detail in in this section and in section V.B.2 of this thesis.

Cytosolic calcium perturbation can in turn promote endoplasmic reticulum stress (ER stress), originally induced to restore homeostasis and overcome stresses and protein misfolding **(Kania, Pajak et al. 2015)** but that might lead to cellular damage and eventually cell death if stresses are too excessive or prolonged **(Sano and Reed 2013)**. Interestingly, although not much

work has been done on ER stress in the old muscle, several lines of evidence indicate SR burden in old muscle (Fig. 6). First, disruption of SR-sarcolemmal junctions as well as SR protein levels alteration discussed above already indicates SR dysregulation in the aged muscle. Second, markers of ER stress induced apoptosis and chaperone protein, also up-regulated to respond to ER stress, are increased with aging in muscles **(Ogata, Machida et al. 2009)**. In other studies, authors showed correlating increase of apoptosis and caspase-12 further indicating ER stress cell death induction in the aging muscle **(Dirks and Leeuwenburgh 2004, Chung and Ng 2006)**. Lastly, old mice display sarcoplasmic reticulum aggregates, named tubular aggregates, which are also a clear sign of the SR burden in the old muscle (Fig. 6). Interestingly, human mutations of the ORAI Calcium Release-Activated Calcium Modulator 1 (ORA1) and of Stromal Interaction Molecule 1 (STIM1), two elements that modulate influx of cellular calcium after intracellular calcium store depletion, both cause tubular aggregate myopathies **(Endo, Noguchi et al. 2015, Okuma, Saito et al. 2016)**. This strongly suggests that tubular aggregates form upon SR-calcium homeostasis dysregulation. Interestingly caslequestrin 1 (CSQ1) presence and SERCA1 crystallization are important for tubular aggregate formation **(Boncompagni, Protasi et al. 2012)**, further suggesting that SR protein alterations might be the trigger of tubular aggregate formation. Alternatively, as tubular aggregates display a calcium buffering capacity **(Salviati, Pierobon-Bormioli et al. 1985)**, they might represent a compensatory mechanism to lower age-related increase of cytosolic calcium.

Oxidative stress is another major type of stress induced during aging that could lead to cellular damage and dysregulation observed in old muscles (Fig. 6). Basal production of reactive oxygen species (ROS) such as O_2O- and H_2O_2, responsible of oxidative stress, are increased with age in both rodents and humans **(Bejma and Ji 1999, Capel, Rimbert et al. 2005)**. Different sites of ROS production exist in cells. Mitochondria are the primary source of ROS in muscle and its role in oxidative stress will be further discussed in the subsection “Mitochondrial dysfunction and muscle aging” of the section I.C.1. Xanthine oxidase catabolism generates O_2O- during purine catabolism **(Hellsten, Ahlborg et al. 1988)** and its enzymatic activity is increased in old muscle possibly resulting in age-associated O_2O- overproduction **(Lambertucci, Levada-Pires et al. 2007, Ryan, Jackson et al. 2011)**. Nicotinamide adenine dinucleotide phosphate oxidases

located in the sarcolemma can also generate ROS **(Ji 2001)** and thereby very likely trigger calcium leak by damaging RYRs. Despite anti-oxidative enzymes adaptation as defense mechanism **(Gianni, Jan et al. 2004)**, humans and other mammals display increased oxidative damage such as DNA or protein oxidation and lipid peroxidation in old muscles. **(Radak, Naito et al. 2002, Lambertucci, Levada-Pires et al. 2007, Feng, Xie et al. 2008)**. In addition, oxidative stress has been shown to affect muscle contractile proteins **(Reid 2008)**, thereby reducing muscle strength and fatigue resistance **(Reid, Khawli et al. 1993)**, to reduce protein synthesis and muscle regeneration, and to increase proteolysis **(Derbre, Gratas-Delamarche et al. 2014)**.

The different cellular stresses accumulate during muscle aging and above a threshold cannot be counteracted by defense mechanism anymore. This leads to increased cellular apoptosis during muscle aging (Fig. 6) **(Leeuwenburgh 2003, Marzetti, Lawler et al. 2008, Marzetti, Privitera et al. 2010)**. Data indicate that oxidative stress resulting in DNA/protein damage, imbalanced protein synthesis and degradation, but also mitochondrial dysfunction, most likely leads to increased apoptosis in old muscle **(Braga, Sinha Hikim et al. 2008, Meng and Yu 2010)** (Fig. 6). Age-associated increased cytosolic calcium levels might also lead to cell death in old muscles through stimulation of the sarcoplasmic reticulum-mediated apoptotic pathway **(Nitahara, Cheng et al. 1998, Meng and Yu 2010)** (Fig. 6). Given both the role and the ability of mitochondria to induce programmed cell death and its age-associated dysfunction, mitochondria-driven cellular apoptosis is considered as a central mechanism leading to muscle cell death during aging (Fig. 6). For example, old animals observe a pro-apoptotic shift in BCL2 Associated X (Bax) / B-Cell CLL/Lymphoma 2 (BCL2) protein ratio **(Pistilli, Jackson et al. 2006, Marzetti, Wohlgemuth et al. 2008)**. In addition, increased susceptibility of mitochondrial permeability transition pore opening (mPTP) leading to mitochondria swelling and cell death ratios have been noticed in aged muscles **(Chabi, Ljubicic et al. 2008)**, correlating with a higher cyclophilin D (CYPD)/ Voltage-dependent anion channel (VDAC) ratio **(Marzetti, Wohlgemuth et al. 2008)** that is known to induced mPTP opening and mitochondrial swelling **(Baines, Kaiser et al. 2005)**.

Mitochondrial dysfunction and muscle aging

In addition to being the main energy producer of the muscle, mitochondria are key organelles that regulate various cellular functions such as cell cycle, signal transduction or apoptosis **(McBride, Neuspiel et al. 2006, Wang and Youle 2009)**. Increasing lines of evidence exhibit age-related mitochondria dysfunction and indicate its key role in muscle aging via multiple mechanisms (Fig. 6). As mentioned above, mitochondria are the primary source of free radicals **(Nakamura, Takamura et al. 2009)** which makes its closely located mitochondrial DNA (mtDNA) and protein, particularly susceptible to age-associated ROS overproduction and oxidative damages **(Mansouri, Muller et al. 2006, Chabi, Ljubicic et al. 2008)**. Supporting the role of ROS in mitochondrial dysfunction during aging, old transgenic mice expressing the human catalase, targeted to the mitochondria, show protection against age-associated oxidative damage and reduced mitochondrial mass. MtDNA is dramatically affected by ROS since 70% of muscle mtDNA present deletions in subjects older than 80 years of age **(Chabi, Mousson de Camaret et al. 2005)**. In addition to mtDNA oxidative damage, mtDNA quantity, reflecting mitochondrial mass is also impaired by aging in muscle. This has been demonstrated by different methods measuring mitochondrial quantities such as electronic microscopy, DNA copy numbers or proteomics **(Barazzoni, Short et al. 2000, Conley, Jubrias et al. 2000, Short, Bigelow et al. 2005)**. Correlating with lower mitochondria numbers, muscle mitochondrial enzymatic activities are reduced with age **(Trounce, Byrne et al. 1989)** as well as basal and maximal ATP production **(Short, Vittone et al. 2004, Short, Bigelow et al. 2005)**. Another study reports a drop of 50% in oxidative capacity per muscle volume and 30% per mitochondrial volume in old compared to young individuals **(Conley, Jubrias et al. 2000)**. In addition to their lower ATP production, higher number of depolarized mitochondria further indicates mitochondrial damages **(Terman, Kurz et al. 2010)**. Morphologically, mitochondria of old muscles are abnormally enlarged with a rounder shape and exhibit shortened cristae and matrix vacuolization **(Beregi, Regius et al. 1988, Shigenaga, Hagen et al. 1994)**. However, it is not clear whether mitochondrial alterations are causing sarcopenia or reflects secondary effects of age-associated muscle insulin resistance and reduced physical activity **(Petersen, Befroy et al. 2003, Lanza, Short et al. 2008)**.

Mitochondria are highly dynamic organelles being constantly adjusted by mitochondrial biogenesis, fusion and fission events and degradative mechanism. Mitochondrial biogenesis greatly influences mitochondrial mass. Alteration in AMPK-induced mitochondrial biogenesis in old muscle **(Reznick, Zong et al. 2007)** suggests that impaired mitochondrial production contributes to reduced mitochondrial densities observed in aged muscle **(Chabi, Ljubicic et al. 2008)**. Mitochondrial fusion is important for energy exchange, equilibration of the mitochondrial proteins and allows mixing of dysfunctional mitochondria with healthy ones, which can alleviate respiratory alterations due to their mtDNA mutations **(Sato, Nakada et al. 2006)**. However, this process also enables accumulation of mutated mtDNA during aging. Therefore, dysregulation of this process during muscle aging could contribute to accumulations of damaged mitochondria. For example, mutation of the key fusion protein optic atrophies 1 (Opa1) in human muscle triggers a reduction in oxidative phosphorylation and ATP production **(Lodi, Tonon et al. 2004)**. Interestingly, another key protein of mitochondrial fusion, mitofusin 2 (Mfn2) is downregulated in old muscle **(Crane, Devries et al. 2010)**. The counterpart of fusion is mitochondrial fission which together with autophagy, regulates mitophagy and the removal of impaired mitochondria **(Benard and Karbowski 2009)**. Age-associated increase of fission 1 (Fis1) protein **(O'Connell and Ohlendieck 2009)** might be the consequence of higher numbers of dysfunctional mitochondria. Reduced mitochondrial fusion and fission in muscle aging, as suggested by reduced amount of fusion and fission protein levels **(Ibebunjo, Chick et al. 2013)**, might lead to reduced mitochondrial turnover which would be exacerbated by decreased mitochondrial protein renewal, as suggested by lower mitochondrial protein synthesis and proteolysis in aged muscle **(Rooyackers, Adey et al. 1996, Combaret, Dardevet et al. 2009)**.

Lastly, some data indicate that mitochondria of old muscle have an impaired calcium handling. First, in aged muscle, the capacity of mitochondria to uptake calcium is altered **(Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**. This could very likely contribute to the increased cytosolic calcium levels depicted previously. Second, mitochondrial association to the sarcoplasmic reticulum, allowed by mitochondria-associated-membranes (MAM), is reduced with age. Old muscles exhibit both lower numbers of mitochondria association with the SR and less tethers associating the two organelles thereby

weakening each association **(Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**. Moreover, Pietrangelo and colleagues demonstrated in the same publication that the distance between SR and mitochondria is enlarged in muscles of old animals. The communication between the two organelles is important for calcium signaling and cellular homeostasis, cell survival, ER stress and mitochondrial function. Therefore, although it has not been extensively studied yet, these data strongly suggest that age-related impaired mitochondrial calcium handling could contribute to muscle stresses and dysfunction observed during aging.

2. The function of PGC-1α in skeletal muscle health and diseases

PGC-1α: a master regulator of mitochondrial and muscle metabolism

PGC-1α is expressed in highly oxidative tissue including skeletal muscle and promotes oxidative metabolism (Fig. 7) when overexpressed in the latter **(Lin, Wu et al. 2002)** or in culture of muscle cells **(Wu, Puigserver et al. 1999)**. Especially, overexpression of PGC-1α in mice white glycolytic muscle turns them into red oxidative muscles, likely through the induction of oxygen-bound cytochrome-c and myoglobin. Muscle and muscle cells with elevated PGC-1α levels also exhibit increased mitochondrial mass and biogenesis (Fig. 7) **(Choi, Befroy et al. 2008)**, as well as enhanced mRNA and protein levels of various mitochondrial metabolic genes and respiratory subunits **(Lin, Wu et al. 2002, St-Pierre, Lin et al. 2003, Wende, Schaeffer et al. 2007)**. In addition, they show ameliorated enzymatic activities for FAO, Krebs cycle and oxidative phosphorylation, which result in improved mitochondrial respiration. In parallel, transcriptional co-activation of PGC-1α also stimulates the production of heme biosynthesis, ion transport such as calcium, mitochondrial translation and protein import, which further improves mitochondrial respiratory capacity **(Wu, Puigserver et al. 1999, St-Pierre, Lin et al. 2003)**. Accordingly, mice overexpressing PGC-1α display higher endurance and fatigue resistance **(Lin, Wu et al. 2002, Calvo, Daniels et al. 2008)**. In line with these data, PGC-1α muscle specific knock-out animals have diminished expression of mitochondrial respiratory chain protein and mRNA,

mitochondrial respiration capacities and endurance performances **(Handschin, Chin et al. 2007, Handschin, Choi et al. 2007)**. Mechanistically, PGC-1α co-activates and even regulates expression of multiple transcription factors that controls the expression of gene essential for mitochondrial biogenesis and function, including ERRα, PPARs and NRF-1 and 2 **(Wu, Puigserver et al. 1999, Kelly and Scarpulla 2004)**. For example, NRF-1 and 2 have been shown to regulate transcription factor A (Tfam) expression, essential for the transcription and replication of the mitochondrial DNA **(Kelly and Scarpulla 2004)**. Other data also suggest that PGC-1α could directly control the transcription of mitochondrially encoded genes. Indeed, PGC-1α and SIRT1 are present in the mitochondrial apparatus and are immunoprecipitated together with Tfam and mitochondrial PGC-1α level increases upon exercise **(Aquilano, Vigilanza et al. 2010, Safdar, Little et al. 2011)**. In addition to control mitochondrial biogenesis, studies also showed that PGC-1α controls mitochondrial dynamics including fusion, fission and mitophagy **(Garnier, Fortin et al. 2005, Soriano, Liesa et al. 2006, Vainshtein, Desjardins et al. 2015, Vainshtein, Tryon et al. 2015, Kang and Ji 2016)**.

Muscle PGC-1α not only regulates mitochondrial genes but also different processes of muscle metabolism. PGC-1α induces the expression of key fatty acids metabolism enzymes such as CPTI, medium-chain acyl-coenzyme A dehydrogenase (MCAD) and fatty acid translocase/CD36 (CD36) in both cell culture and muscle **(Vega, Huss et al. 2000, Wende, Schaeffer et al. 2007, Calvo, Daniels et al. 2008)**. Overexpression of pyruvate dehydrogenase kinase 4 (PDK4) in muscle with elevated PGC-1α levels possibly further contributes to increase FAO in transgenic animals. Finally, PGC-1α increases lactate uptake in the muscle and drives a switch in lactate dehydrogenase (LDH) Isoenzyme-type, promoting transformation of lactate in pyruvate that can be used in the Krebs cycle. Furthermore, muscle PGC-1α enhances the expression of glucose transporter 4 (GLUT4) indicating higher glucose refueling and glycogen synthesis in muscle (Fig. 7) but lowers glycolysis and glucose oxidation, increasing muscle glycogen levels **(Wende, Schaeffer et al. 2007, Calvo, Daniels et al. 2008)**. Similarly, PGC-1α promotes de novo lipid synthesis in muscle, which together with increased glycogenesis are important to replenish energy levels after exercise when PGC-1α levels are concomitantly increased **(Summermatter, Baum et al. 2010)**. Paradoxically, it worsens high fat diet (HFD)

induced insulin resistance in sedentary animals but improves glucose homeostasis in exercised mice fed with the same diet **(Summermatter, Shui et al. 2013)**. Enhanced fatty acids oxidation and mitochondrial metabolism of PGC-1α transgenic mice increases their endurance capacities (Fig. 7) and lowers their respiratory exchange ratio (RER) during exercise **(Calvo, Daniels et al. 2008)**. However, they showed reduced running performance in high intensity exhaustive exercise bouts, very likely because of their inability to use muscle glycogen **(Wende, Schaeffer et al. 2007)**. Together, these data indicate a switch from glycolytic to oxidative metabolism induced by PGC-1α in muscle. Accordingly, mice muscles overexpressing PGC-1α display an increase in the proportion of fiber type I and IIa **(Lin, Wu et al. 2002)**. Interestingly, PGC-1α muscle specific knock out animals have a reduction in the proportion of these types of fibers **(Handschin, Chin et al. 2007)** but not PGC-1α whole body knockout mice indicating compensatory mechanisms **(Arany, He et al. 2005)**. Finally, in line with a switch towards slow fiber types, PGC-1α remodels calcium transient and calcium handling and promotes a shift of the CSQ1 (fast type) towards caslequestrin 2 (CSQ2) (slow type) expression **(Summermatter, Thurnheer et al. 2012)**.

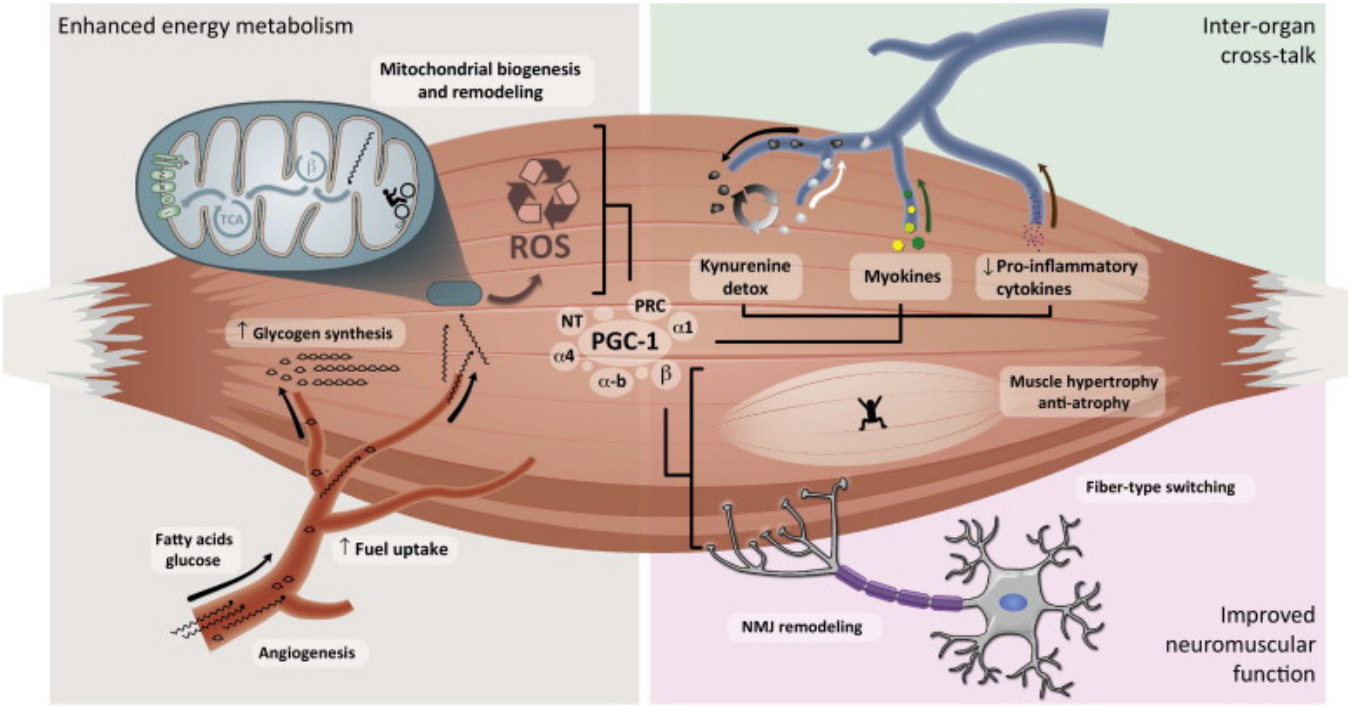

Figure 7: The coordinated actions of peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) in skeletal muscle. (Correia, Ferreira et al. 2015)

PGC-1α and anti-oxidants

Increased mitochondrial metabolism and respiratory capacities potentially increases mitochondrial ROS production and oxidative damages. Consequently, to prevent increased oxidative stress in transgenic animals, PGC-1α enhances the expression of detoxifying enzymes (Fig. 7) such as superoxide dismutase 2 (SOD2) **(Wenz, Rossi et al. 2009)**. Conversely, PGC-1α muscle specific knockout animals exhibit reduced levels of SOD1 and 2 and glutathione peroxidase (GPx)1 mRNA and protein levels in muscle **(Handschin, Choi et al. 2007, Leick, Wojtaszewski et al. 2008, Leick, Lyngby et al. 2010)**. Similar observations were reported upon induction of those enzymes by H_2O_2 in fibroblast, where PGC-1α suppresses ROS **(St-Pierre, Drori et al. 2006)**. Additionally, PGC-1α up-regulates UCP2 and 3 in cells **(St-Pierre, Lin et al. 2003)**, possibly increasing uncoupling and thereby reducing ROS production, as well as sirtuin 3 (SIRT3) **(Kong, Wang et al. 2010)** that is known to deacetylate and activate SOD2 **(Shi, Wang et al. 2005)**.

PGC-1α and muscle secreted factors

While ROS production can induce an inflammatory response through activation of the redox sensitive nuclear factor kappa B (NF-kB) **(Morgan and Liu 2011)**, PGC-1α muscle deletion promotes expression of various inflammatory genes and increases blood interleukin 6 (IL-6) levels **(Handschin, Choi et al. 2007)** (Fig. 7). Those animals also exhibit an exacerbated inflammatory response upon exercise concomitant with increased muscle damage and blood tumor necrosis factor (TNF)α concentrations **(Handschin, Chin et al. 2007)**. However, other studies using PGC-1α muscle deficient mice generated with a different genetic method did not show differences in plasma IL-6 or TNFα concentrations **(Olesen, Larsson et al. 2012, Sczelecki, Besse-Patin et al. 2014)**. In line with the data of the first study, mice overexpressing muscle PGC-1α present lower muscle and plasma IL-6 and TNFα levels **(Wenz, Rossi et al. 2009, Olesen, Larsson et al. 2012)**. In turn, PGC-1α expression is decreased in multiple conditions with increased systemic inflammation such as type 2 diabetes or aging **(Patti, Butte et al. 2003, Sczelecki, Besse-Patin et al. 2014)**. Mechanistically, PGC-1α prevents pro-inflammatory

cytokines expression upon inflammation stimulus and reduces p65 phosphorylation and p65 activation of NF-kB **(Wenz, Rossi et al. 2009, Eisele, Salatino et al. 2013)**.

In skeletal muscle, PGC-1α promotes oxygen and nutrients consumption by increasing mitochondrial respiration and energy metabolism. Importantly, PGC-1α concomitantly enhances angiogenesis that supplies muscle with more oxygen and fuel (Fig. 7) **(Arany, Foo et al. 2008, Tadaishi, Miura et al. 2011)**. Angiogenesis increase upon PGC-1α overexpression in myocytes is mediated by induction and secretion of the vascular endothelial growth factor (VEGF), as well as other angiogenic factors and is independent of the hypoxia –inducible system **(Arany, Foo et al. 2008)**. Increased angiogenesis allows proper recovery after ischemia, which is therefore impaired in mice with PGC-1α deletion and enhanced in animals overexpressing muscle PGC-1α. In line with those data, a recent study showed that PGC-1α induces the formation of functional and non-leaky blood vessels through upregulation of secreted phosphoprotein 1 which recruits macrophages and activates the migration of adjacent cells **(Rowe, Raghuram et al. 2014)**. Importantly, deleting secreted phosphoprotein 1 prevents the formation of proper vessels by PGC-1α. PGC-1α upregulation in skeletal muscle also ameliorates blood flow recovery after hindlimb ischemia of diabetic mice **(Rowe, Raghuram et al. 2014)**. Finally, while PGC-1α is dispensable for basal capillarization, previous work showed that it is required for exercise induced angiogenesis and VEGF upregulation **(Chinsomboon, Ruas et al. 2009, Leick, Hellsten et al. 2009)**.

Besides regulating VEGF expression, as well as inflammatory related factors released by the muscle, PGC-1α controls muscle secretion of several other myokines (Fig. 7) that can trigger different effects in various tissues. First, PGC-1α induces the expression of Fibronectin type III domain-containing protein 5 (FNDC5) mRNA, further transformed in irisin and released in blood vessels **(Bostrom, Wu et al. 2012)**. Irisin will trigger WAT browning and its systemic delivery promotes energy expenditure and improves glucose homeostasis in obese mice **(Bostrom, Wu et al. 2012)**. It is released in the blood after exercise and cold exposure, two major triggers of PGC-1α muscle induction **(Lee, Linderman et al. 2014)**. Interestingly, peripheral FNDC5 gene delivery to mouse liver stimulates expression of brain derived neurotrophic factor (BDNF) in the

hippocampus, which improves synaptic plasticity and memory **(Kuipers and Bramham 2006, Wrann, White et al. 2013)**. Recently, PGC-1α has been shown to mediate the transformation of kynurenine to kynurenic acid (Fig. 7) **(Agudelo, Femenia et al. 2014)**. Since kynurenic acid cannot cross the blood–brain barrier, the conversion alleviates stressed induced depression by preventing kynurenine to reach the brain and to induce neuro-inflammation and synaptic dysfunction. As exercise also induces this PGC-1α-mediated conversion, this process might be how exercise improves depression and various cognitive disorders **(Abe 2012, Adamson, Ensari et al. 2015)**. β-aminoisobutyric acid (BAIBA), product from valine catabolism, is released by muscle and increased in plasma of both exercised and PGC-1α muscle overexpressing mice **(Roberts, Bostrom et al. 2014)**. Following its secretion, BAIBA will activate FAO in the liver and WAT browning **(Roberts, Bostrom et al. 2014)**. This increases energy expenditure, reduces adiposity and improves glucose tolerance. In addition to the secretion of those factors, the isoform PGC-1α4 inhibits muscle secretion of myostatin, a condition that is known to reduce adiposity and increase adipose lipolysis and FAO, thereby promoting WAT browning and increased energy expenditure **(Zhang, McFarlane et al. 2012, Shan, Liang et al. 2013)**. Finally, this isoform and exercise both drive the expression and secretion of meteorin-like (Metrnl), which again increases WAT browning and energy expenditure as well as glucose utilization **(Rao, Long et al. 2014)**.

PGC-1α and NMJ regulation

Several lines of evidence indicate that muscle PGC-1α is important for NMJs structure and function (Fig. 7). PGC-1α muscle deletion reduces the expression of multiple NMJ genes such as acetyl choline receptors or utrophin, and impairs acetylcholine receptor clustering **(Handschin, Kobayashi et al. 2007)**. In light of these elements, we studied pre- and post-synaptic rearrangements of the NMJ in PGC-1α muscle specific knock animals. No substantial changes were observed in PGC-1α muscle deficient mice in comparison to wild type (WT) animals (Fig. 8). We therefore did not further investigate in this direction. Conversely, muscle elevation of PGC-1α levels improves acetylcholine receptor clustering and the transcription of NMJ genes **(Handschin, Kobayashi et al. 2007, Arnold, Gill et al. 2014)**. Its overexpression in

cultured muscle is sufficient to promote the same effects **(Handschin, Kobayashi et al. 2007)**. In muscle, overexpression of PGC-1α improves the NMJ post-synaptic structure, increases NMJ synaptic folding but also ameliorates NMJ function and by the same way muscle fatigue resistance. In addition, PGC-1α increases pre-synaptic branching and complexity **(Chakkalakal, Nishimune et al. 2010, Arnold, Gill et al. 2014)**. PGC-1α delivery in the muscle after P10, post synapse elimination, promotes a shift towards slow motor neurons indicating that pre-synaptic remodeling are mediated by a retrograde signaling from muscle to motor neurons **(Chakkalakal, Nishimune et al. 2010)**.

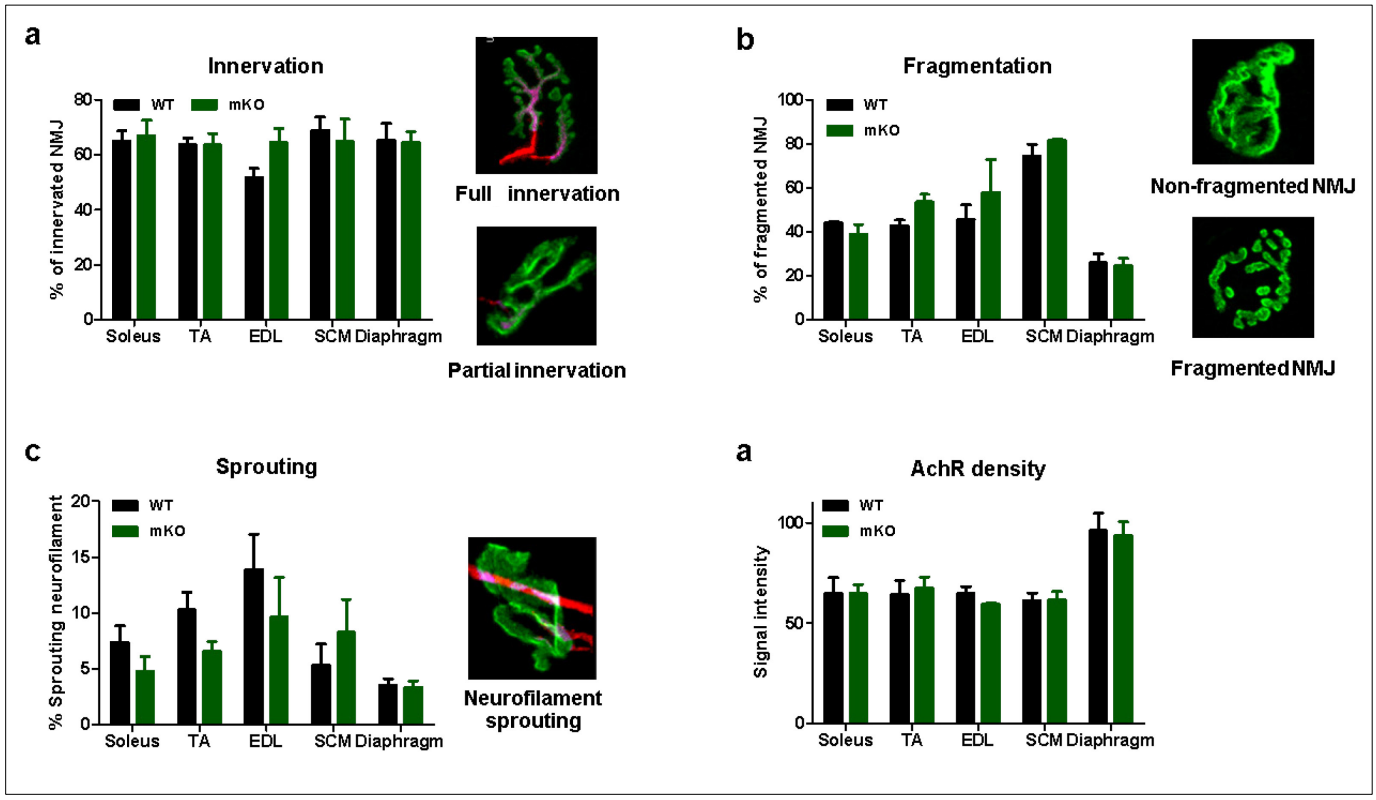

Figure 8: Muscle PGC-1α deletion does not alter the NMJ. (a-d) NMJ pre- and post- synaptic analysis indicating NMJ innervation, fragmentation, neurofilament sprouting and acetyl-choline receptor densities (n=2-3). Values are mean ± SEM.

PGC-1α in muscle related diseases

The maintenance of NMJ integrity by PGC-1α is an asset for the protection against various neuromuscular disorders. For example, PGC-1α improves exercise capacity, lowers creatine kinase levels in blood, and prevents exercise-associated muscle damage and necrosis to ameliorate muscle dystrophy in mdx mice, a model of Duchenne muscular dystrophy **(Handschin, Kobayashi et al. 2007)**. Similarly, PGC-1α improves the muscle phenotype of utrophin-mdx mice, which present higher muscle dysfunction than the previous model **(Chan, Rowe et al. 2014)**. Of note, PGC-1α delivery, after the disease has developed, partially rescues the phenotype by improving strength and fatigue resistance and reducing inflammation **(Selsby, Morine et al. 2012, Hollinger, Gardan-Salmon et al. 2013)**. Likewise during aging, PGC-1α has been shown to improve NMJ integrity **(Wenz, Rossi et al. 2009)**. Lastly, PGC-1α improves NMJ structures and preserves muscle mass in an amyotrophic lateral sclerosis mouse model in which SOD1G37R mutation induces motor neuron degeneration **(Da Cruz, Parone et al. 2012)**. This also suggests that PGC-1α could play an important role to sustain muscle mass in other conditions triggering muscle atrophy.

Reduction of PGC-1α levels in multiple disorders reducing muscle mass further support this hypothesis. For example, muscle PGC-1α mRNA expression decreases in human disuse muscle atrophy **(Suetta, Frandsen et al. 2012, Wall, Dirks et al. 2015)**. Likewise PGC-1α expression is reduced in human **(Safdar, Hamadeh et al. 2010)** and murine **(Sczelecki, Besse-Patin et al. 2014)** muscle aging. Similar observations have been seen in rodent models of muscle-wasting-associated diseases like diabetes or cancer **(Sandri, Lin et al. 2006)**. In addition, PGC-1α reduces muscle fiber atrophy induced by denervation and fasting, and blunts the associated increase of atrophy-related genes in mice **(Sandri, Lin et al. 2006)**. The suggested mechanism is the prevention of Forkhead-Box-Protein O3 (Foxo3) mediated atrophy and atrogin1 transcription activation. Similarly, PGC-1α protects against TNF-related weak inducer of apoptosis (TWEAK–Fn14)-associated muscle wasting. PGC-1α repression and FoxO3- mediated atrophy induced by TWEAK–Fn14 signaling further suggests that it reduces muscle mass by diminishing PGC-1α levels in muscle, which in turn will cause atrophy through the FoxO3

pathway. Finally, PGC-1α4 isoform, induced by resistance exercise training, promotes insulin-like growth factor-1, mammalian target of rapamycin and reduces myostatin expression **(Ruas, White et al. 2012, White, Wrann et al. 2014)** resulting in muscle hypertrophy and improved muscle strength. Accordingly, PGC-1α4 protects from cancer-associated cachexia **(Ruas, White et al. 2012)**.

Similar to muscle wasting conditions, PGC-1α expression is decreased in diabetic disorders. Since mitochondrial dysfunctions are also observed in muscles of diabetic patients, it has been postulated that PGC-1α could play a role in diabetes and muscle insulin resistance. However, mice with PGC-1α deficient muscle present little alteration in glucose handling and insulin sensitivity. Interestingly, high PGC-1α elevation in muscle increases diet-induced insulin resistance, likely due to increased intramuscular lipid accumulation, while moderate increases of PGC-1α, similar to exercise-induced PGC-1α elevation, improves insulin sensitivity in cells and muscles **(Bonen 2009)**. In addition, high PGC-1α overexpression potentiated exercise beneficial effect on insulin sensitivity, further supporting that muscle PGC-1α mediates exercise-associated amelioration of insulin resistance **(Summermatter, Shui et al. 2013)**.

3. PGC-1α and exercise as potential treatments against muscle aging

PGC-1α and aging

Aging is a multifactorial process, but as in muscle, age-related pathologies in other organs are considered to be initiated or at least correlated to mitochondrial dysfunction. Mitochondrial disorders are often associated with neurological disorders, diabetes, endocrinopathies and other systemic diseases **(DiMauro and Schon 2008)**. Interestingly, similar conditions occurring during aging are also correlated with mitochondrial dysfunctions **(Baker, Betik et al. 2006, Civitarese, Carling et al. 2007)**. In addition to regulating mitochondrial function, PGC-1α is linked to different factors involved in the general process of aging, including SIRT1, insulin/IGF signaling and mammalian target of rapamycin **(Fadini, Ceolotto et al. 2011)**.

The versatile role of PGC-1α likely impacts the aging process in different organs affected by mitochondrial dysfunction and by alteration in the mentioned pathways during aging (Fig. 9). For example, in the heart, SIRT1 and PPARα, that control PGC-1α expression and activity, protect against age-associated hypertrophy, metabolic disorders and inflammation **(Planavila, Iglesias et al. 2011)**. Interestingly, similar effects were observed after resveratrol treatment, which also indirectly activates PGC-1α. Moreover, while age-related dysfunctional vasculature contributes to cardiac disorders, PGC-1α is important for the vasculature wall and also promote angiogenesis (Fig. 9) (as described above) **(Rowe, Jiang et al. 2010)**. On top of that, PGC-1α reduction might cause the switch from fatty acids to glucose utilization observed in the aging failing heart **(Huss and Kelly 2005)** as it normally induces mitochondrial function and FAO in this tissue (Fig. 9) **(Huss and Kelly 2004)**. Recently, PGC-1α deletion has also been shown to accelerate vascular aging (Fig. 9) and atherosclerosis, correlating with increased telomere alterations and DNA damages **(Xiong, Patrushev et al. 2015)**.

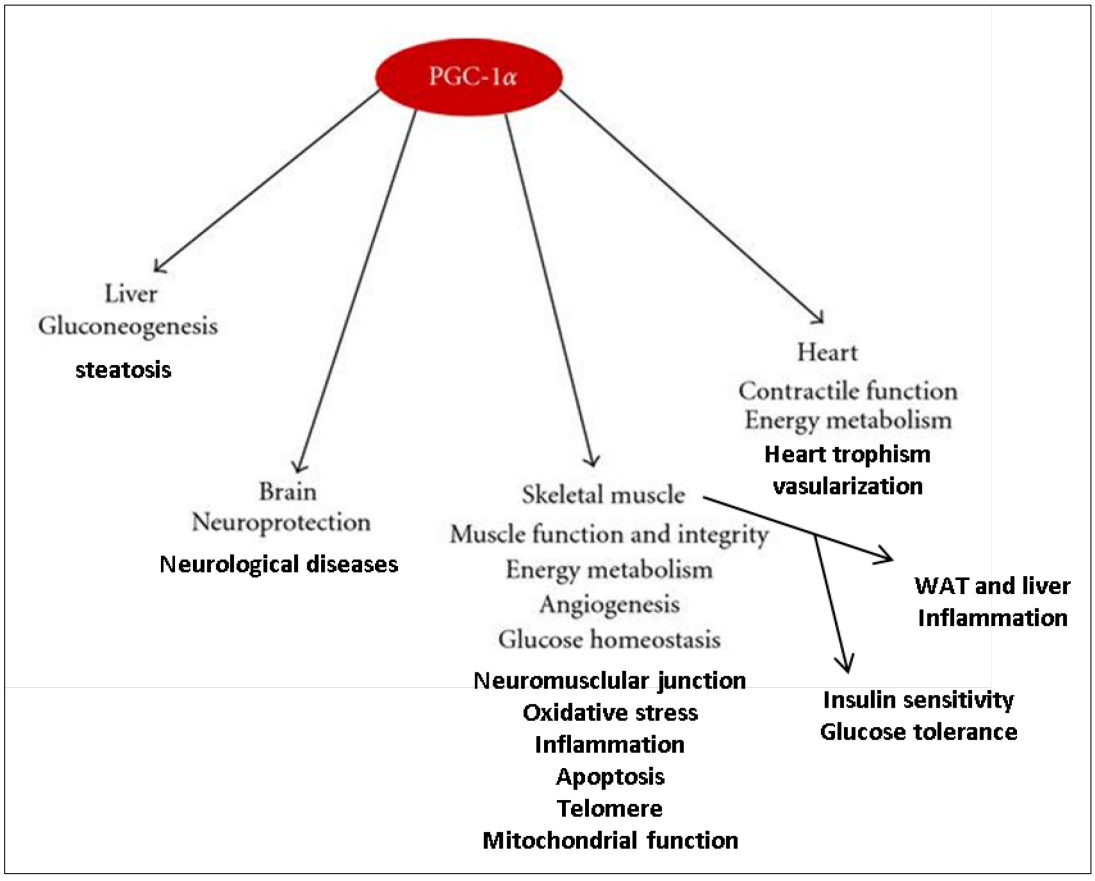

Figure 9: PGC-1α and age-related disorders. Adapted from **(Wenz 2011)**

In the brain, mitochondrial dysfunction is also detrimental during aging **(Bender, Krishnan et al. 2006)**. As described in section I.B.3, PGC-1α plays a major role in this organ to maintain mitochondrial function and strongly affects neuronal diseases (Fig. 9) that are developed with age such as Huntington, Alzheimer or Parkinson diseases. Finally, general mitochondrial dysfunction is considered to be a potential underlying effect of the age developed insulin resistance, whereas increased mitochondrial function improves it **(Petersen, Befroy et al. 2003, Dumas, Simard et al. 2009, Wenz, Rossi et al. 2009)**. Of note, PGC-1α promoter methylation is reduced in muscle of diabetic subjects **(Barres, Osler et al. 2009)** and PGC-1α expression is negatively correlated to steatosis stages (Fig. 9) **(Wang, Kamat et al. 2011)**. In accordance with these results, rosiglitazone, that indirectly activates PGC-1α, improves age-related liver pathology **(Gupte, Liu et al. 2010)**.

In muscle, age-associate PGC-1α expression reduction at the mRNA and protein levels has been largely described in humans and rodents **(Chabi, Ljubicic et al. 2008, Wenz, Rossi et al. 2009, Ghosh, Lertwattanarak et al. 2011, Kang, Chung et al. 2013)**. Correlating with this, aging triggers changes in multiple skeletal muscle processes and structures that are actually affected by PGC-1α levels (as described in I.C.1 and I.C.2), including NMJ, ROS, inflammation and mitochondrial function and numbers (Fig. 9). Those data support an important role for PGC-1α in muscle aging. Accordingly, Wenz and collaborators demonstrated that muscle PGC-1α overexpression positively impacts several aspects of muscle aging in mice **(Wenz, Rossi et al. 2009)**. Muscle PGC-1α upregulation ameliorated age-associated diminished oxidative and anti-oxidant capacities, ATP production, and endurance performance (Fig. 9). Accompanying those ameliorations, PGC-1α reduced inflammatory marker amounts and apoptosis. In addition, PGC-1α improved NMJ integrity and at the systemic level, lean body mass and insulin sensitivity (Fig. 9). Furthermore, PGC-1α muscle overexpression has been shown to delay the myopathy onset of a mouse model of mitochondrial myopathy and to increase both health and lifespan of these animals by improving OXPHOS defect and ATP production (Fig. 9). Interestingly, bezafibrate, a PPAR agonist that activates PGC-1α, recapitulates these positive effects in cells of patients with OXPHOS dysfunctions, demonstrating the potential of PGC-1α to improve myopathies with mitochondrial defects. Finally, other works with PGC-1α overexpression used the coactivator to

reverse the aging phenotype of the mtDNA mutator mouse model that develops a pre-mature aging and mitochondrial dysfunctions **(Dillon, Williams et al. 2012)**, and to improve the phenotype of another mouse model encompassing telomere linked mitochondrial defects, **(Sahin, Colla et al. 2011)** which are also present during aging **(Mikhelson and Gamaley 2012)**. Another study oppositely investigated the consequences of muscle PGC-1α deletion on aging **(Sczelecki, Besse-Patin et al. 2014)**. Authors found as expected that both aging and PGC-1α deletion in young mice decreases electron transport chain gene expression and function but also that PGC-1α deletion exacerbated those changes in old muscles. Interestingly, they also revealed that PGC-1α muscle deletion worsen age-related glucose tolerance decline and further increased fat mass, insulin resistance and WAT and liver expression of inflammatory markers during aging (Fig. 9). However, descriptions of the effect of muscle PGC-1α deletion on muscle function or other muscle metabolic processes have not been reported in this work and are still missing. In addition, when many processes altered by aging are influenced by PGC-1α upregulation, only a part of them have been studied and shown to be altered by the coactivator in an aging context.

Exercise improves muscle aging

Currently, exercise is one of the best interventions against skeletal muscle aging. Resistance training has been shown to be particularly efficient to improve muscle mass and strength in elderly subjects (Fig. 10) **(Fiatarone, Marks et al. 1990, Liu and Latham 2009)** and in very old frail patients **(Fiatarone, Marks et al. 1990, Serra-Rexach, Bustamante-Ara et al. 2011)**. It also ameliorates aerobic capacities, mobility, motor functions and balance in the elderly population (Fig. 10) **(Kortebein, Ferrando et al. 2007, de Vries, van Ravensberg et al. 2012, Gault and Willems 2013)**. In line with improved muscle force, exercise training ameliorates myofiber atrophy, strength and power **(Cartee, Hepple et al. 2016)**. However, the hypertrophic response seems to be blunted in older adults compared to young **(Bickel, Cross et al. 2011)**.

Mitochondrial amelioration and cellular anti-oxidant defense induced by exercise are thought to be main players of the beneficial exercise effects on muscle in the elderly population **(Ji 2001, Tarnopolsky 2009)**, but those adaptations are rapidly abolished after exercise cessation. Also, exercise quantity seems to be more important than exercise intensity for mitochondrial biogenesis induction **(Bishop, Granata et al. 2014)**. Mitochondrial mass, OXPHOS protein expression and mitochondrial enzymatic activities and oxidative capacities are remarkably improved by exercise in aged muscle (Fig. 10) **(Skorjanc, Traub et al. 1998, Pette and Skorjanc 2001, Short, Vittone et al. 2003, Menshikova, Ritov et al. 2006)**. Importantly, several studies demonstrated that mitochondrial adaptation to exercise occurs similarly in young and old humans and rodents **(Farrar, Martin et al. 1981, Short, Vittone et al. 2003, Ghosh, Lertwattanarak et al. 2011)**. Of note, exercise can transform damaged mitochondria back to healthy and functional ones **(Jubrias, Esselman et al. 2001, Conley, Jubrias et al. 2013)**. Exercise also improves expression of mitochondrial dynamic genes in old humans likely contributing to improve mitochondrial wellness **(Konopka, Suer et al. 2014)**. Lastly, similar to PGC-1α, exercise rescues the pre-mature aging phenotype and mitochondrial dysfunctions displayed by the mtDNA mutator mice **(Safdar, Bourgeois et al. 2011)**

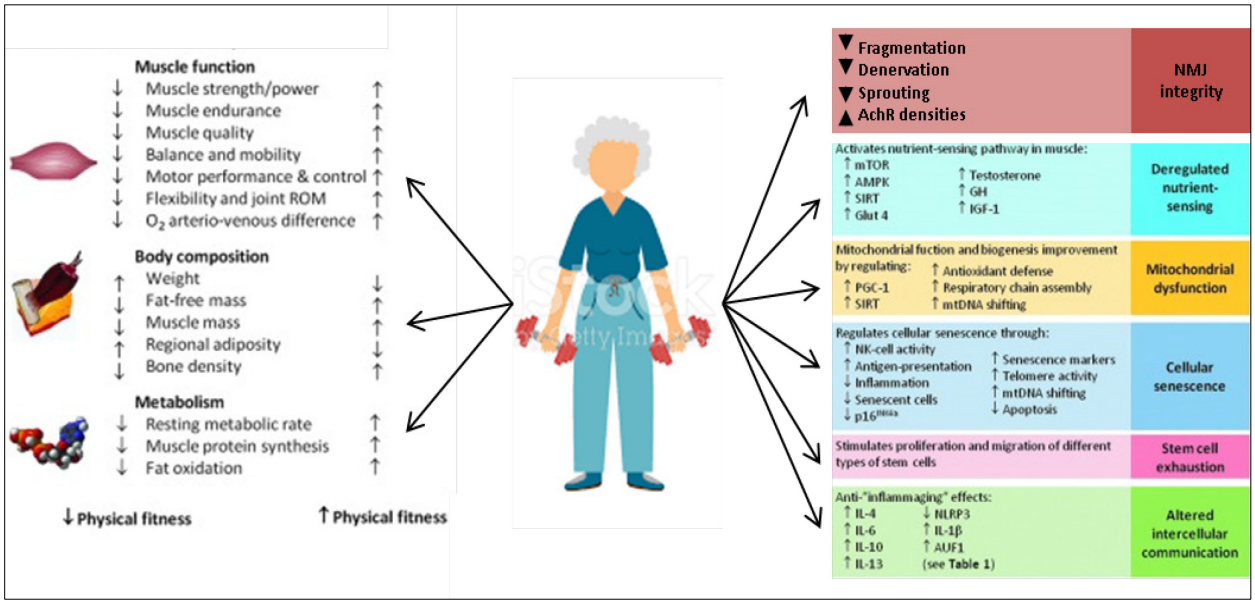

Figure 10: Exercise ameliorations of muscle aging. Adapted from **(Garatachea, Pareja-Galeano et al. 2015)**

In addition to ameliorate mitochondrial function, exercise also improves other processes impaired in old muscles (Fig. 10). Some of those ameliorations are briefly discussed here. For example, amounts of physical activity are negatively correlated to levels of inflammatory markers in both young and elderly adults (Fig. 10) **(Pedersen and Fischer 2007)** and this phenomenon is associated with altered muscle mass in old rats **(Marzetti, Groban et al. 2008)**. Increase in mass of aged muscle after exercise could also be pushed by the restoration of growth factor levels such as IGF-1 (Fig. 10) **(Urso, Fiatarone Singh et al. 2005)**. In addition, satellite cell content and myogenic regulating factors of satellite cells are increased with exercise in older adults **(Kim, Kosek et al. 2005, Dreyer, Blanco et al. 2006)**, which could also contribute to restore muscle mass (Fig. 10). Moreover, exercise reduces apoptosis in old rats, thereby attenuating fiber atrophy (Fig. 10) **(Song, Kwak et al. 2006)**. In this study, exercise showed a particularly strong rescuing effect on apoptotic markers as it decreased by 95-96% caspase-3 cleavage and Bax/Bcl-2 ratio and brought back DNA fragmentation to the level of young animals. Finally, exercise improves age-related NMJ deteriorations, such as NMJ fragmentation, denervation and neurofilament sprouting (Fig. 10) **(Valdez, Tapia et al. 2010)**.
At the systemic level, exercise also rescues impaired whole body energy homeostasis. Especially, insulin sensitivity is restored by exercise in old humans **(Pauli, Ropelle et al. 2010, Wang, Patterson et al. 2013)** and rats the day following exercise. In accordance with these data, muscle insulin-stimulated glucose uptake and Glut4 expression are increased 3 hours post-exercise in old rats **(Sharma, Wang et al. 2015)**. Different types of chronic exercises such as cycling, running or walking can lead to increased insulin sensitivity and GLUT4 levels in older adults and rodents **(Gulve, Rodnick et al. 1993, Evans, Racette et al. 2005, Consitt, Van Meter et al. 2013, Bienso, Olesen et al. 2015)**.

Taken together, those data demonstrate that exercise can slow down the majority of muscle aging disorders. However, its effect is still limited and exercise is not always easy for elderly to practice. Therefore it would be extremely beneficial in the fight against muscle aging to reveal a molecule able to increase exercise effects and to mimic it.

PGC-1α mediates, potentiates and mimics exercise-induced amelioration in young skeletal muscle

When going through the previous sections, what is striking is the number of processes and parameters that are positively impacted by both exercise and PGC-1α expression, many of which are actually impaired with aging. This includes mitochondrial content, function, dynamics, enzymatic activities but also neuromuscular adaptations, oxidative metabolism and fiber type switch towards slow muscle, muscle mass and endurance, myokines and other muscle secreted factors, GLUT4 expression and glycogen storage, insulin signaling, inflammation, angiogenesis and anti-oxidative enzymes induction. Another muscle adaptation to exercise is the unfolded protein response (UPR) that is the consequence of ER stress induced by exercise. On the contrary to UPR increase in old muscle, likely due to chronic ER stress, UPR after exercise constitutes a defense mechanism to restore and conserve cellular homeostasis. Interestingly, a previous study demonstrated that muscle PGC-1α overexpression also mimics exercise in the induction of the UPR **(Wu, Ruas et al. 2011)**. In light of those elements, PGC-1α seems to be a molecule that can strongly mimic exercise in improving muscle function and possibly muscle aging.

The strong exercise-mimetic effect of PGC-1α also suggests that the coactivator is important for muscle adaptation to training. This is supported by the fact that many signaling pathways activated by exercise converge to PGC-1α induction and activation as described in section I.A.1. To further investigate this hypothesis, some studies evaluated the requirement of PGC-1α expression to mediate exercise effect on muscle. Thus, PGC-1α expression has been shown to be required for the UPR induction upon both exercise and ER stress stimulation in muscle **(Wu, Ruas et al. 2011)**. Interestingly, muscles deleted for PGC-1α present higher damage post-exercise than muscles of control animals, supporting the idea that they cannot promote an appropriate defense against exercise-induced muscle stress **(Handschin, Chin et al. 2007)**. In addition, our laboratory recently highlighted that exercise enhances muscle ketolytic capacity only in the presence of PGC-1α in the muscle **(Svensson, Albert et al. 2016)**. Finally, other works showed that muscle PGC-1α is mandatory for exercise induction of angiogenesis and VEGF

(Chinsomboon, Ruas et al. 2009), as well as for exercise stimulation of mitochondrial biogenesis and mitochondrial enzyme expression **(Geng, Li et al. 2010)**. However, a previous study showed that muscle PGC-1α was dispensable for training induced mitochondrial gene expression **(Leick, Wojtaszewski et al. 2008)**. Interestingly, the same authors showed that PGC-1α expression is required by exercise to prevent age-related decline in citrate synthase activity and SOD2 protein content in 13-month-old mice **(Leick, Lyngby et al. 2010)** while another work found that exercise could increase SOD2 expression in young muscle lacking PGC-1α expression **(Geng, Li et al. 2010)**. The discrepancy of those results still leaves the question of the necessity of PGC-1α expression for exercise-stimulated mitochondrial protein expression opened. However the variation of the results could be due to whole-body versus muscle specific deletion of PGC-1α, different exercise protocols and sacrifice timing post-training. In addition, other proteins and mitochondrial measure should be used to characterize in a more comprehensive way the requirement of PGC-1α expression for exercise muscle adaptations.

In addition to mediating some exercise effects, PGC-1α levels affect exercise performance **(Handschin, Chin et al. 2007, Calvo, Daniels et al. 2008, Perez-Schindler, Svensson et al. 2014)** and therefore training capacity. Lower training efficiency might ultimately reduce the beneficial effects expected from exercise. On the other hand, reduced daily activity might be the cause of PGC-1α expression decrease in older adults, resulting in lower mitochondrial content and function **(Chabi, Ljubicic et al. 2008, Wenz, Rossi et al. 2009, Ghosh, Lertwattanarak et al. 2011, Kang, Chung et al. 2013)**. Importantly, a study showed that people over 80 years of age that went through a life-long endurance training, have higher muscle PGC-1α mRNA content than an age matched healthy subject that had normal activity levels **(Lanza, Short et al. 2008)**. Furthermore, exercise training can stimulate PGC-1α expression, and accordingly mitochondrial biogenesis gene expression, enzymatic activity and ATP production in young and old individuals **(Short, Vittone et al. 2003, Lanza, Short et al. 2008, Cobley, Bartlett et al. 2012)**. However, while most of these parameters are induced at similar levels in young and old subjects by exercise, PGC-1α and Tfam protein expression are less exercise-stimulated in old compared to young subject **(Lanza, Short et al. 2008)**.

Finally, studies revealed that PGC-1α overexpression combined with exercise could have synergistic effects. For example, muscle glucose uptake as well as whole-body glucose homeostasis of mice fed with HFD is further improved by exercise in mice with elevated PGC-1α expression than in WT exercised mice **(Summermatter, Shui et al. 2013)**. However, the combination of PGC-1α upregulation and exercise to maximize muscle function improvements is still much understudied and therefore deserves high attention.

D. Aim of the Thesis

Since the identification of the coactivator of transcription factors PGC-1α, many publications have demonstrated its extensive role in regulating energy metabolism processes such as thermogenesis, gluconeogenesis or mitochondrial biogenesis in different organs in response to various stimuli such as cold exposure or exercise. In addition, recent studies in muscle have shown that it regulates crosstalks between different organs through myokines, as well as whole body glucose homeostasis. PGC-1α therefore now represents a muscle metabolic sensor that can impact whole body homeostasis. However the role of PGC-1α in the brain, another major organ regulating whole body homeostasis, is still restricted to local neuronal survival and function. This prompted us to generate mouse models with deletion of PGC-1α specifically in AgRP and POMC neurons, which are in charge of regulating whole body energy balance, in order to investigate whether it could play a similar role those cells. Our findings regarding the role of PGC-1α in AgRP- and POMC- neuronal regulation of energy balance are presented in section II of this thesis and the aims in the first part of this thesis were:

1. **Define the role of PGC-1α in AgRP and POMC neurons for the regulation of food intake and whole body energy balance**
2. **Determine if PGC-1α regulates AgRP and POMC expression upon different energetic stimulus in order to achieve this function**

The second part of the thesis focuses on the role of PGC-1α in muscle aging. Muscle aging involves mitochondrial dysfunctions as a primary cause of muscle disorders but also displays many other alterations such as disruption of calcium homeostasis, ER stress and apoptosis. In the literature, one study examined the consequences of PGC-1α overexpression on mitochondrial dysfunction, inflammation, insulin resistance, oxidative stress and apoptosis during aging while another work focused on the outcomes of PGC-1α muscle deletion on systemic changes during aging. In our study, we used muscle specific PGC-1α overexpression and deletion mouse models to characterize in a broader way the influence of PGC-1α on

mitochondrial and muscle function disorders in the old muscle. We also wanted to determine the repercussions of muscle PGC-1α modulation on other muscle metabolic processes that are potentially regulated by PGC-1α and influenced by aging but not studied in the previous investigations. The results concerning this study are presented in III of this thesis. The general aims of our second project were:

3. **Characterize the impact of PGC-1α modulation on mitochondrial aging and age-associated muscle damage and dysfunction**
4. **Identify new pathways influenced by PGC-1α that leads to age-related muscle alterations**

Finally, we were interested in unraveling the role PGC-1α in the context of exercise treatment against muscle aging. Exercise is one of the most effective treatments to slow down sarcopenia and positively impacts the majority of age-related muscle alterations. As PGC-1α is strongly induced by exercise and share many beneficial repercussions that exercise has on muscle, we hypothesized that it could be the factor that mediates exercise-associated muscle adaptations during aging. In addition, we though that PGC-1α could be a therapeutic target to potentiate exercise effects or to replace exercise training in the fight against muscle aging. The results of those investigations are presented in section IV of this work and the aims can be resumed as follow:

5. **Determine if PGC-1α mediates exercise effects during muscle aging.**
6. **Investigate if PGC-1α modulates exercise-induced muscle adaptation during aging.**

Some results during this thesis led to new ideas and projects that are still ongoing and that will be discussed in section V.

E. References

Abe, K. (2012). "Total daily physical activity and the risk of AD and cognitive decline in older adults." Neurology **79**(10): 1071; author reply 1071.

Adamson, B. C., I. Ensari and R. W. Motl (2015). "Effect of exercise on depressive symptoms in adults with neurologic disorders: a systematic review and meta-analysis." Arch Phys Med Rehabil **96**(7): 1329-1338.

Agapito, M. A., C. Q. Zhang, S. Murugan and D. K. Sarkar (2014). "Fetal Alcohol Exposure Disrupts Metabolic Signaling in Hypothalamic Proopiomelanocortin Neurons via a Circadian Mechanism in Male Mice." Endocrinology **155**(7): 2578-2588.

Agudelo, L. Z., T. Femenia, F. Orhan, M. Porsmyr-Palmertz, M. Goiny, V. Martinez-Redondo, J. C. Correia, M. Izadi, M. Bhat, I. Schuppe-Koistinen, A. T. Pettersson, D. M. Ferreira, A. Krook, R. Barres, J. R. Zierath, S. Erhardt, M. Lindskog and J. L. Ruas (2014). "Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression." Cell **159**(1): 33-45.

Air, E. L., M. Z. Strowski, S. C. Benoit, S. L. Conarello, G. M. Salituro, X. M. Guan, K. Liu, S. C. Woods and B. B. Zhang (2002). "Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity." Nat Med **8**(2): 179-183.

Akimoto, T., S. C. Pohnert, P. Li, M. Zhang, C. Gumbs, P. B. Rosenberg, R. S. Williams and Z. Yan (2005). "Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway." J Biol Chem **280**(20): 19587-19593.

Amat, R., A. Planavila, S. L. Chen, R. Iglesias, M. Giralt and F. Villarroya (2009). "SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma Co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD." J Biol Chem **284**(33): 21872-21880.

Amat, R., A. Planavila, S. L. Chen, R. Iglesias, M. Giralt and F. Villarroya (2009). "SIRT1 controls the transcription of the peroxisome proliferator-activated receptor-gamma Co-activator-1alpha (PGC-1alpha) gene in skeletal muscle through the PGC-1alpha autoregulatory loop and interaction with MyoD." The Journal of biological chemistry **284**(33): 21872-21880.

An, F. M., S. Chen, Z. Xu, L. Yin, Y. Wang, A. R. Liu, W. B. Yao and X. D. Gao (2015). "Glucagon-like peptide-1 regulates mitochondrial biogenesis and tau phosphorylation against advanced glycation end product-induced neuronal insult: Studies in vivo and in vitro." Neuroscience **300**: 75-84.

Anand, B. K. and J. R. Brobeck (1951). "Hypothalamic control of food intake in rats and cats." Yale J Biol Med **24**(2): 123-140.

Anand, B. K. and J. R. Brobeck (1951). "Localization of a "feeding center" in the hypothalamus of the rat." Proc Soc Exp Biol Med **77**(2): 323-324.

Andersen, J. L. (2003). "Muscle fibre type adaptation in the elderly human muscle." Scand J Med Sci Sports **13**(1): 40-47.

Andersson, D. C., M. J. Betzenhauser, S. Reiken, A. C. Meli, A. Umanskaya, W. Xie, T. Shiomi, R. Zalk, A. Lacampagne and A. R. Marks (2011). "Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging." Cell Metab **14**(2): 196-207.

Andrews, Z. B., Z. W. Liu, N. Walllingford, D. M. Erion, E. Borok, J. M. Friedman, M. H. Tschop, M. Shanabrough, G. Cline, G. I. Shulman, A. Coppola, X. B. Gao, T. L. Horvath and S. Diano (2008). "UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals." Nature **454**(7206): 846-851.

Aniansson, A., M. Hedberg, G. B. Henning and G. Grimby (1986). "Muscle morphology, enzymatic activity, and muscle strength in elderly men: a follow-up study." Muscle Nerve **9**(7): 585-591.

Aquilano, K., P. Vigilanza, S. Baldelli, B. Pagliei, G. Rotilio and M. R. Ciriolo (2010). "Peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1alpha) and sirtuin 1 (SIRT1) reside in mitochondria: possible direct function in mitochondrial biogenesis." The Journal of biological chemistry **285**(28): 21590-21599.

Arany, Z., S. Y. Foo, Y. Ma, J. L. Ruas, A. Bommi-Reddy, G. Girnun, M. Cooper, D. Laznik, J. Chinsomboon, S. M. Rangwala, K. H. Baek, A. Rosenzweig and B. M. Spiegelman (2008). "HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha." Nature **451**(7181): 1008-1012.

Arany, Z., H. He, J. Lin, K. Hoyer, C. Handschin, O. Toka, F. Ahmad, T. Matsui, S. Chin, P. H. Wu, Rybkin, II, J. M. Shelton, M. Manieri, S. Cinti, F. J. Schoen, R. Bassel-Duby, A. Rosenzweig, J. S. Ingwall and B. M. Spiegelman (2005). "Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle." Cell metabolism **1**(4): 259-271.

Arany, Z., M. Novikov, S. Chin, Y. Ma, A. Rosenzweig and B. M. Spiegelman (2006). "Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha." Proc Natl Acad Sci U S A **103**(26): 10086-10091.

Arnold, A. S., J. Gill, M. Christe, R. Ruiz, S. McGuirk, J. St-Pierre, L. Tabares and C. Handschin (2014). "Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1alpha." Nature communications **5**: 3569.

Baar, K., A. R. Wende, T. E. Jones, M. Marison, L. A. Nolte, M. Chen, D. P. Kelly and J. O. Holloszy (2002). "Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **16**(14): 1879-1886.

Baines, C. P., R. A. Kaiser, N. H. Purcell, N. S. Blair, H. Osinska, M. A. Hambleton, E. W. Brunskill, M. R. Sayen, R. A. Gottlieb, G. W. Dorn, J. Robbins and J. D. Molkentin (2005). "Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death." Nature **434**(7033): 658-662.

Baker, D. J., A. C. Betik, D. J. Krause and R. T. Hepple (2006). "No decline in skeletal muscle oxidative capacity with aging in long-term calorically restricted rats: effects are independent of mitochondrial DNA integrity." J Gerontol A Biol Sci Med Sci **61**(7): 675-684.

Balagopal, P., O. E. Rooyackers, D. B. Adey, P. A. Ades and K. S. Nair (1997). "Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans." The American journal of physiology **273**(4 Pt 1): E790-800.

Baldan, A., J. Relat, P. F. Marrero and D. Haro (2004). "Functional interaction between peroxisome proliferator-activated receptors-alpha and Mef-2C on human carnitine palmitoyltransferase 1beta (CPT1beta) gene activation." Nucleic Acids Res **32**(16): 4742-4749.

Balice-Gordon, R. J. (1997). "Age-related changes in neuromuscular innervation." Muscle Nerve Suppl **5**: S83-87.

Barazzoni, R., K. R. Short and K. S. Nair (2000). "Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart." The Journal of biological chemistry **275**(5): 3343-3347.

Barazzoni, R., M. Zanetti, M. R. Cattin, L. Visintin, P. Vinci, L. Cattin, M. Stebel and G. Guarnieri (2007). "Ghrelin enhances in vivo skeletal muscle but not liver AKT signaling in rats." Obesity (Silver Spring) **15**(11): 2614-2623.

Barbatelli, G., I. Murano, L. Madsen, Q. Hao, M. Jimenez, K. Kristiansen, J. P. Giacobino, R. De Matteis and S. Cinti (2010). "The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation." Am J Physiol Endocrinol Metab **298**(6): E1244-1253.

Baron, A. D., G. Brechtel, P. Wallace and S. V. Edelman (1988). "Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans." Am J Physiol **255**(6 Pt 1): E769-774.

Barres, R., M. E. Osler, J. Yan, A. Rune, T. Fritz, K. Caidahl, A. Krook and J. R. Zierath (2009). "Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density." Cell metabolism **10**(3): 189-198.

Barsh, G. S. and M. W. Schwartz (2002). "Genetic approaches to studying energy balance: perception and integration." Nat Rev Genet **3**(8): 589-600.

Beermann, D. H., R. G. Cassens, C. C. Couch and F. J. Nagle (1977). "The effects of experimental denervation and reinnervation on skeletal muscle fiber type and intramuscular innervation." J Neurol Sci **31**(2): 207-221.

Bejma, J. and L. L. Ji (1999). "Aging and acute exercise enhance free radical generation in rat skeletal muscle." J Appl Physiol (1985) **87**(1): 465-470.

Benard, G. and M. Karbowski (2009). "Mitochondrial fusion and division: Regulation and role in cell viability." Semin Cell Dev Biol **20**(3): 365-374.

Bender, A., K. J. Krishnan, C. M. Morris, G. A. Taylor, A. K. Reeve, R. H. Perry, E. Jaros, J. S. Hersheson, J. Betts, T. Klopstock, R. W. Taylor and D. M. Turnbull (2006). "High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease." Nat Genet **38**(5): 515-517.

Benoit, S. C., E. L. Air, L. M. Coolen, R. Strauss, A. Jackman, D. J. Clegg, R. J. Seeley and S. C. Woods (2002). "The catabolic action of insulin in the brain is mediated by melanocortins." J Neurosci **22**(20): 9048-9052.

Beregi, E., O. Regius, T. Huttl and Z. Gobl (1988). "Age-related changes in the skeletal muscle cells." Z Gerontol **21**(2): 83-86.

Besseiche, A., J. P. Riveline, J. F. Gautier, B. Breant and B. Blondeau (2015). "Metabolic roles of PGC-1alpha and its implications for type 2 diabetes." Diabetes Metab **41**(5): 347-357.

Bewick, G. A., J. V. Gardiner, W. S. Dhillo, A. S. Kent, N. E. White, Z. Webster, M. A. Ghatei and S. R. Bloom (2005). "Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype." FASEB J **19**(12): 1680-1682.

Bhalla, S., C. Ozalp, S. Fang, L. Xiang and J. K. Kemper (2004). "Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol and glucose metabolism." The Journal of biological chemistry **279**(43): 45139-45147.

Bickel, C. S., J. M. Cross and M. M. Bamman (2011). "Exercise dosing to retain resistance training adaptations in young and older adults." Med Sci Sports Exerc **43**(7): 1177-1187.

Bienso, R. S., J. Olesen, L. Gliemann, J. F. Schmidt, M. S. Matzen, J. F. Wojtaszewski, Y. Hellsten and H. Pilegaard (2015). "Effects of Exercise Training on Regulation of Skeletal Muscle Glucose Metabolism in Elderly Men." J Gerontol A Biol Sci Med Sci **70**(7): 866-872.

Bingham, N. C., K. K. Anderson, A. L. Reuter, N. R. Stallings and K. L. Parker (2008). "Selective loss of leptin receptors in the ventromedial hypothalamic nucleus results in increased adiposity and a metabolic syndrome." Endocrinology **149**(5): 2138-2148.

Bishop, D. J., C. Granata and N. Eynon (2014). "Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content?" Biochim Biophys Acta **1840**(4): 1266-1275.

Boncompagni, S., L. d'Amelio, S. Fulle, G. Fano and F. Protasi (2006). "Progressive disorganization of the excitation-contraction coupling apparatus in aging human skeletal muscle as revealed by electron microscopy: a possible role in the decline of muscle performance." J Gerontol A Biol Sci Med Sci **61**(10): 995-1008.

Boncompagni, S., F. Protasi and C. Franzini-Armstrong (2012). "Sequential stages in the age-dependent gradual formation and accumulation of tubular aggregates in fast twitch muscle fibers: SERCA and calsequestrin involvement." Age (Dordr) **34**(1): 27-41.

Bonen, A. (2009). "PGC-1alpha-induced improvements in skeletal muscle metabolism and insulin sensitivity." Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme **34**(3): 307-314.

Borgius, L. J., K. R. Steffensen, J. A. Gustafsson and E. Treuter (2002). "Glucocorticoid signaling is perturbed by the atypical orphan receptor and corepressor SHP." J Biol Chem **277**(51): 49761-49766.

Boschek, C. B., T. E. Jones, H. S. Smallwood, T. C. Squier and D. J. Bigelow (2008). "Loss of the calmodulin-dependent inhibition of the RyR1 calcium release channel upon oxidation of methionines in calmodulin." Biochemistry **47**(1): 131-142.

Boss, O., E. Bachman, A. Vidal-Puig, C. Y. Zhang, O. Peroni and B. B. Lowell (1999). "Role of the beta(3)-adrenergic receptor and/or a putative beta(4)-adrenergic receptor on the expression of uncoupling

proteins and peroxisome proliferator-activated receptor-gamma coactivator-1." Biochem Biophys Res Commun **261**(3): 870-876.

Bostrom, P., J. Wu, M. P. Jedrychowski, A. Korde, L. Ye, J. C. Lo, K. A. Rasbach, E. A. Bostrom, J. H. Choi, J. Z. Long, S. Kajimura, M. C. Zingaretti, B. F. Vind, H. Tu, S. Cinti, K. Hojlund, S. P. Gygi and B. M. Spiegelman (2012). "A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis." Nature **481**(7382): 463-468.

Bouret, S. G., S. J. Draper and R. B. Simerly (2004). "Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice." J Neurosci **24**(11): 2797-2805.

Braga, M., A. P. Sinha Hikim, S. Datta, M. G. Ferrini, D. Brown, E. L. Kovacheva, N. F. Gonzalez-Cadavid and I. Sinha-Hikim (2008). "Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice." Apoptosis **13**(6): 822-832.

Butler, A. A. and R. D. Cone (2002). "The melanocortin receptors: lessons from knockout models." Neuropeptides **36**(2-3): 77-84.

Calvo, J. A., T. G. Daniels, X. Wang, A. Paul, J. Lin, B. M. Spiegelman, S. C. Stevenson and S. M. Rangwala (2008). "Muscle-specific expression of PPARgamma coactivator-1alpha improves exercise performance and increases peak oxygen uptake." J Appl Physiol (1985) **104**(5): 1304-1312.

Campbell, M. J., A. J. McComas and F. Petito (1973). "Physiological changes in ageing muscles." J Neurol Neurosurg Psychiatry **36**(2): 174-182.

Cannavino, J., L. Brocca, M. Sandri, B. Grassi, R. Bottinelli and M. A. Pellegrino (2015). "The role of alterations in mitochondrial dynamics and PGC-1alpha over-expression in fast muscle atrophy following hindlimb unloading." The Journal of physiology **593**(8): 1981-1995.

Cao, W., Q. F. Collins, T. C. Becker, J. Robidoux, E. G. Lupo, Jr., Y. Xiong, K. W. Daniel, L. Floering and S. Collins (2005). "p38 Mitogen-activated protein kinase plays a stimulatory role in hepatic gluconeogenesis." J Biol Chem **280**(52): 42731-42737.

Cao, W., K. W. Daniel, J. Robidoux, P. Puigserver, A. V. Medvedev, X. Bai, L. M. Floering, B. M. Spiegelman and S. Collins (2004). "p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene." Mol Cell Biol **24**(7): 3057-3067.

Capel, F., V. Rimbert, D. Lioger, A. Diot, P. Rousset, P. P. Mirand, Y. Boirie, B. Morio and L. Mosoni (2005). "Due to reverse electron transfer, mitochondrial H2O2 release increases with age in human vastus lateralis muscle although oxidative capacity is preserved." Mech Ageing Dev **126**(4): 505-511.

Cartee, G. D., R. T. Hepple, M. M. Bamman and J. R. Zierath (2016). "Exercise Promotes Healthy Aging of Skeletal Muscle." Cell metabolism **23**(6): 1034-1047.

Cettour-Rose, P. and F. Rohner-Jeanrenaud (2002). "The leptin-like effects of 3-d peripheral administration of a melanocortin agonist are more marked in genetically obese Zucker (fa/fa) than in lean rats." Endocrinology **143**(6): 2277-2283.

Chabi, B., V. Ljubicic, K. J. Menzies, J. H. Huang, A. Saleem and D. A. Hood (2008). "Mitochondrial function and apoptotic susceptibility in aging skeletal muscle." Aging Cell **7**(1): 2-12.

Chabi, B., B. Mousson de Camaret, A. Chevrollier, S. Boisgard and G. Stepien (2005). "Random mtDNA deletions and functional consequence in aged human skeletal muscle." Biochem Biophys Res Commun **332**(2): 542-549.

Chakkalakal, J. V., H. Nishimune, J. L. Ruas, B. M. Spiegelman and J. R. Sanes (2010). "Retrograde influence of muscle fibers on their innervation revealed by a novel marker for slow motoneurons." Development **137**(20): 3489-3499.

Chan, M. C., G. C. Rowe, S. Raghuram, I. S. Patten, C. Farrell and Z. Arany (2014). "Post-natal induction of PGC-1alpha protects against severe muscle dystrophy independently of utrophin." Skeletal muscle **4**(1): 2.

Chaturvedi, R. K. and M. Flint Beal (2013). "Mitochondrial diseases of the brain." Free Radic Biol Med **63**: 1-29.

Chen, H. Y., M. E. Trumbauer, A. S. Chen, D. T. Weingarth, J. R. Adams, E. G. Frazier, Z. Shen, D. J. Marsh, S. D. Feighner, X. M. Guan, Z. Ye, R. P. Nargund, R. G. Smith, L. H. Van der Ploeg, A. D. Howard, D. J. MacNeil and S. Qian (2004). "Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein." Endocrinology **145**(6): 2607-2612.

Chinsomboon, J., J. Ruas, R. K. Gupta, R. Thom, J. Shoag, G. C. Rowe, N. Sawada, S. Raghuram and Z. Arany (2009). "The transcriptional coactivator PGC-1alpha mediates exercise-induced angiogenesis in skeletal muscle." Proceedings of the National Academy of Sciences of the United States of America **106**(50): 21401-21406.

Choi, C. S., D. E. Befroy, R. Codella, S. Kim, R. M. Reznick, Y. J. Hwang, Z. X. Liu, H. Y. Lee, A. Distefano, V. T. Samuel, D. Zhang, G. W. Cline, C. Handschin, J. Lin, K. F. Petersen, B. M. Spiegelman and G. I. Shulman (2008). "Paradoxical effects of increased expression of PGC-1alpha on muscle mitochondrial function and insulin-stimulated muscle glucose metabolism." Proceedings of the National Academy of Sciences of the United States of America **105**(50): 19926-19931.

Chung, L. and Y. C. Ng (2006). "Age-related alterations in expression of apoptosis regulatory proteins and heat shock proteins in rat skeletal muscle." Biochimica et biophysica acta **1762**(1): 103-109.

Ciron, C., S. Lengacher, J. Dusonchet, P. Aebischer and B. L. Schneider (2012). "Sustained expression of PGC-1alpha in the rat nigrostriatal system selectively impairs dopaminergic function." Human molecular genetics **21**(8): 1861-1876.

Civitarese, A. E., S. Carling, L. K. Heilbronn, M. H. Hulver, B. Ukropcova, W. A. Deutsch, S. R. Smith, E. Ravussin and C. P. Team (2007). "Calorie restriction increases muscle mitochondrial biogenesis in healthy humans." PLoS Med **4**(3): e76.

Claret, M., M. A. Smith, R. L. Batterham, C. Selman, A. I. Choudhury, L. G. Fryer, M. Clements, H. Al-Qassab, H. Heffron, A. W. Xu, J. R. Speakman, G. S. Barsh, B. Viollet, S. Vaulont, M. L. Ashford, D. Carling and D. J. Withers (2007). "AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons." The Journal of clinical investigation **117**(8): 2325-2336.

Cleasby, M. E., P. M. Jamieson and P. J. Atherton (2016). "Insulin resistance and sarcopenia: mechanistic links between common co-morbidities." J Endocrinol **229**(2): R67-81.

Cobley, J. N., J. D. Bartlett, A. Kayani, S. W. Murray, J. Louhelainen, T. Donovan, S. Waldron, W. Gregson, J. G. Burniston, J. P. Morton and G. L. Close (2012). "PGC-1alpha transcriptional response and mitochondrial adaptation to acute exercise is maintained in skeletal muscle of sedentary elderly males." Biogerontology **13**(6): 621-631.

Coggan, A. R., R. J. Spina, D. S. King, M. A. Rogers, M. Brown, P. M. Nemeth and J. O. Holloszy (1992). "Histochemical and enzymatic comparison of the gastrocnemius muscle of young and elderly men and women." J Gerontol **47**(3): B71-76.

Combaret, L., D. Dardevet, D. Bechet, D. Taillandier, L. Mosoni and D. Attaix (2009). "Skeletal muscle proteolysis in aging." Curr Opin Clin Nutr Metab Care **12**(1): 37-41.

Conley, K. E., S. A. Jubrias, M. E. Cress and P. C. Esselman (2013). "Elevated energy coupling and aerobic capacity improves exercise performance in endurance-trained elderly subjects." Experimental physiology **98**(4): 899-907.

Conley, K. E., S. A. Jubrias and P. C. Esselman (2000). "Oxidative capacity and ageing in human muscle." J Physiol **526 Pt 1**: 203-210.

Consitt, L. A., J. Van Meter, C. A. Newton, D. N. Collier, M. S. Dar, J. F. Wojtaszewski, J. T. Treebak, C. J. Tanner and J. A. Houmard (2013). "Impairments in site-specific AS160 phosphorylation and effects of exercise training." Diabetes **62**(10): 3437-3447.

Contreras, C., R. Nogueiras, C. Dieguez, G. Medina-Gomez and M. Lopez (2016). "Hypothalamus and thermogenesis: Heating the BAT, browning the WAT." Mol Cell Endocrinol.

Coppari, R., G. Ramadori and J. K. Elmquist (2009). "The role of transcriptional regulators in central control of appetite and body weight." Nature clinical practice. Endocrinology & metabolism **5**(3): 160-166.

Corpas, E., S. M. Harman and M. R. Blackman (1993). "Human growth hormone and human aging." Endocr Rev **14**(1): 20-39.

Correia, J. C., D. M. Ferreira and J. L. Ruas (2015). "Intercellular: local and systemic actions of skeletal muscle PGC-1s." Trends Endocrinol Metab **26**(6): 305-314.

Cowell, R. M., K. R. Blake and J. W. Russell (2007). "Localization of the transcriptional coactivator PGC-1alpha to GABAergic neurons during maturation of the rat brain." The Journal of comparative neurology **502**(1): 1-18.

Crane, J. D., M. C. Devries, A. Safdar, M. J. Hamadeh and M. A. Tarnopolsky (2010). "The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure." J Gerontol A Biol Sci Med Sci **65**(2): 119-128.

Cruz-Jentoft, A. J., J. P. Baeyens, J. M. Bauer, Y. Boirie, T. Cederholm, F. Landi, F. C. Martin, J. P. Michel, Y. Rolland, S. M. Schneider, E. Topinkova, M. Vandewoude, M. Zamboni and P. European Working Group on

Sarcopenia in Older (2010). "Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People." Age Ageing **39**(4): 412-423.

Cui, L., H. Jeong, F. Borovecki, C. N. Parkhurst, N. Tanese and D. Krainc (2006). "Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration." Cell **127**(1): 59-69.

Cummings, D. E., K. Clement, J. Q. Purnell, C. Vaisse, K. E. Foster, R. S. Frayo, M. W. Schwartz, A. Basdevant and D. S. Weigle (2002). "Elevated plasma ghrelin levels in Prader Willi syndrome." Nat Med **8**(7): 643-644.

Currie, P. J. and D. V. Coscina (1995). "Dissociated feeding and hypothermic effects of neuropeptide Y in the paraventricular and perifornical hypothalamus." Peptides **16**(4): 599-604.

Cuthbertson, D., K. Smith, J. Babraj, G. Leese, T. Waddell, P. Atherton, H. Wackerhage, P. M. Taylor and M. J. Rennie (2005). "Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle." FASEB J **19**(3): 422-424.

Da Cruz, S., P. A. Parone, V. S. Lopes, C. Lillo, M. McAlonis-Downes, S. K. Lee, A. P. Vetto, S. Petrosyan, M. Marsala, A. N. Murphy, D. S. Williams, B. M. Spiegelman and D. W. Cleveland (2012). "Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS." Cell Metab **15**(5): 778-786.

Daitoku, H., K. Yamagata, H. Matsuzaki, M. Hatta and A. Fukamizu (2003). "Regulation of PGC-1 promoter activity by protein kinase B and the forkhead transcription factor FKHR." Diabetes **52**(3): 642-649.

Davis, H. R., Jr., D. E. Mullins, J. M. Pines, L. M. Hoos, C. F. France, D. S. Compton, M. P. Graziano, E. J. Sybertz, C. D. Strader and M. Van Heek (1998). "Effect of chronic central administration of glucagon-like peptide-1 (7-36) amide on food consumption and body weight in normal and obese rats." Obes Res **6**(2): 147-156.

Dayalu, P. and R. L. Albin (2015). "Huntington disease: pathogenesis and treatment." Neurol Clin **33**(1): 101-114.

de Leiris, J. and F. Boucher (1990). "Ischemic myocardial cell necrosis: calcium overload or oxygen free-radicals?" Rev Port Cardiol **9**(2): 153-158.

de Vries, N. M., C. D. van Ravensberg, J. S. Hobbelen, M. G. Olde Rikkert, J. B. Staal and M. W. Nijhuis-van der Sanden (2012). "Effects of physical exercise therapy on mobility, physical functioning, physical activity and quality of life in community-dwelling older adults with impaired mobility, physical disability and/or multi-morbidity: a meta-analysis." Ageing Res Rev **11**(1): 136-149.

Delbono, O. (1992). "Calcium current activation and charge movement in denervated mammalian skeletal muscle fibres." J Physiol **451**: 187-203.

Delbono, O. (2000). "Regulation of excitation contraction coupling by insulin-like growth factor-1 in aging skeletal muscle." J Nutr Health Aging **4**(3): 162-164.

Delbono, O. (2002). "Molecular mechanisms and therapeutics of the deficit in specific force in ageing skeletal muscle." Biogerontology **3**(5): 265-270.

Delerive, P., Y. Wu, T. P. Burris, W. W. Chin and C. S. Suen (2002). "PGC-1 functions as a transcriptional coactivator for the retinoid X receptors." J Biol Chem **277**(6): 3913-3917.

Derbre, F., A. Gratas-Delamarche, M. C. Gomez-Cabrera and J. Vina (2014). "Inactivity-induced oxidative stress: a central role in age-related sarcopenia?" Eur J Sport Sci **14 Suppl 1**: S98-108.

Deschenes, M. R., M. A. Roby, M. K. Eason and M. B. Harris (2010). "Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers." Exp Gerontol **45**(5): 389-393.

Dietrich, M. O., C. Antunes, G. Geliang, Z. W. Liu, E. Borok, Y. Nie, A. W. Xu, D. O. Souza, Q. Gao, S. Diano, X. B. Gao and T. L. Horvath (2010). "Agrp neurons mediate Sirt1's action on the melanocortin system and energy balance: roles for Sirt1 in neuronal firing and synaptic plasticity." The Journal of neuroscience : the official journal of the Society for Neuroscience **30**(35): 11815-11825.

Dietrich, M. O., Z. W. Liu and T. L. Horvath (2013). "Mitochondrial dynamics controlled by mitofusins regulate Agrp neuronal activity and diet-induced obesity." Cell **155**(1): 188-199.

Dillon, L. M., S. L. Williams, A. Hida, J. D. Peacock, T. A. Prolla, J. Lincoln and C. T. Moraes (2012). "Increased mitochondrial biogenesis in muscle improves aging phenotypes in the mtDNA mutator mouse." Hum Mol Genet **21**(10): 2288-2297.

DiMauro, S. and E. A. Schon (2008). "Mitochondrial disorders in the nervous system." Annu Rev Neurosci **31**: 91-123.

Dirks, A. J. and C. Leeuwenburgh (2004). "Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12." Free Radical Biology and Medicine **36**(1): 27-39.

Dodd, G. T., S. Decherf, K. Loh, S. E. Simonds, F. Wiede, E. Balland, T. L. Merry, H. Munzberg, Z. Y. Zhang, B. B. Kahn, B. G. Neel, K. K. Bence, Z. B. Andrews, M. A. Cowley and T. Tiganis (2015). "Leptin and insulin act on POMC neurons to promote the browning of white fat." Cell **160**(1-2): 88-104.

Doherty, T. J. (2003). "Invited review: Aging and sarcopenia." Journal of applied physiology **95**(4): 1717-1727.

Dominguez, L. J. and M. Barbagallo (2016). "The biology of the metabolic syndrome and aging." Curr Opin Clin Nutr Metab Care **19**(1): 5-11.

Dominy, J. E., Jr., Y. Lee, Z. Gerhart-Hines and P. Puigserver (2010). "Nutrient-dependent regulation of PGC-1alpha's acetylation state and metabolic function through the enzymatic activities of Sirt1/GCN5." Biochim Biophys Acta **1804**(8): 1676-1683.

Dougherty, S. E., A. F. Bartley, E. K. Lucas, J. J. Hablitz, L. E. Dobrunz and R. M. Cowell (2014). "Mice lacking the transcriptional coactivator PGC-1alpha exhibit alterations in inhibitory synaptic transmission in the motor cortex." Neuroscience **271**: 137-148.

Draper, S., M. Kirigiti, M. Glavas, B. Grayson, C. N. Chong, B. Jiang, M. S. Smith, L. M. Zeltser and K. L. Grove (2010). "Differential gene expression between neuropeptide Y expressing neurons of the dorsomedial nucleus of the hypothalamus and the arcuate nucleus: microarray analysis study." Brain research **1350**: 139-150.

Dreyer, H. C., C. E. Blanco, F. R. Sattler, E. T. Schroeder and R. A. Wiswell (2006). "Satellite cell numbers in young and older men 24 hours after eccentric exercise." Muscle Nerve **33**(2): 242-253.

Dumas, J. F., G. Simard, M. Flamment, P. H. Ducluzeau and P. Ritz (2009). "Is skeletal muscle mitochondrial dysfunction a cause or an indirect consequence of insulin resistance in humans?" Diabetes Metab **35**(3): 159-167.

Egawa, M., H. Yoshimatsu and G. A. Bray (1991). "Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats." Am J Physiol **260**(2 Pt 2): R328-334.

Eisele, P. S., S. Salatino, J. Sobek, M. O. Hottiger and C. Handschin (2013). "The peroxisome proliferator-activated receptor gamma coactivator 1alpha/beta (PGC-1) coactivators repress the transcriptional activity of NF-kappaB in skeletal muscle cells." J Biol Chem **288**(4): 2246-2260.

Elias, C. F., C. Aschkenasi, C. Lee, J. Kelly, R. S. Ahima, C. Bjorbaek, J. S. Flier, C. B. Saper and J. K. Elmquist (1999). "Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area." Neuron **23**(4): 775-786.

Endo, Y., S. Noguchi, Y. Hara, Y. K. Hayashi, K. Motomura, S. Miyatake, N. Murakami, S. Tanaka, S. Yamashita, R. Kizu, M. Bamba, Y. Goto, N. Matsumoto, I. Nonaka and I. Nishino (2015). "Dominant mutations in ORAI1 cause tubular aggregate myopathy with hypocalcemia via constitutive activation of store-operated Ca(2)(+) channels." Hum Mol Genet **24**(3): 637-648.

Esterbauer, H., H. Oberkofler, F. Krempler and W. Patsch (1999). "Human peroxisome proliferator activated receptor gamma coactivator 1 (PPARGC1) gene: cDNA sequence, genomic organization, chromosomal localization, and tissue expression." Genomics **62**(1): 98-102.

Evans, E. M., S. B. Racette, L. R. Peterson, D. T. Villareal, J. S. Greiwe and J. O. Holloszy (2005). "Aerobic power and insulin action improve in response to endurance exercise training in healthy 77-87 yr olds." J Appl Physiol (1985) **98**(1): 40-45.

Fadini, G. P., G. Ceolotto, E. Pagnin, S. de Kreutzenberg and A. Avogaro (2011). "At the crossroads of longevity and metabolism: the metabolic syndrome and lifespan determinant pathways." Aging Cell **10**(1): 10-17.

Fahim, M. A. (1993). "Morphological correlates of physiological responses in partially denervated mouse muscle during aging." Int J Dev Neurosci **11**(3): 303-310.

Fahim, M. A. and N. Robbins (1982). "Ultrastructural studies of young and old mouse neuromuscular junctions." J Neurocytol **11**(4): 641-656.

Fan, M., J. Rhee, J. St-Pierre, C. Handschin, P. Puigserver, J. Lin, S. Jaeger, H. Erdjument-Bromage, P. Tempst and B. M. Spiegelman (2004). "Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1alpha: modulation by p38 MAPK." Genes & development **18**(3): 278-289.

Farrar, R. P., T. P. Martin and C. M. Ardies (1981). "The interaction of aging and endurance exercise upon the mitochondrial function of skeletal muscle." J Gerontol **36**(6): 642-647.

Feng, J., H. Xie, D. L. Meany, L. V. Thompson, E. A. Arriaga and T. J. Griffin (2008). "Quantitative proteomic profiling of muscle type-dependent and age-dependent protein carbonylation in rat skeletal muscle mitochondria." J Gerontol A Biol Sci Med Sci **63**(11): 1137-1152.

Fernandez-Marcos, P. J. and J. Auwerx (2011). "Regulation of PGC-1alpha, a nodal regulator of mitochondrial biogenesis." The American journal of clinical nutrition **93**(4): 884S-890.

Fernandez-Sanz, C., M. Ruiz-Meana, E. Miro-Casas, E. Nunez, J. Castellano, M. Loureiro, I. Barba, M. Poncelas, A. Rodriguez-Sinovas, J. Vazquez and D. Garcia-Dorado (2014). "Defective sarcoplasmic reticulum-mitochondria calcium exchange in aged mouse myocardium." Cell Death Dis **5**: e1573.

Fiatarone, M. A., E. C. Marks, N. D. Ryan, C. N. Meredith, L. A. Lipsitz and W. J. Evans (1990). "High-intensity strength training in nonagenarians. Effects on skeletal muscle." JAMA **263**(22): 3029-3034.

Fisher, F. M., S. Kleiner, N. Douris, E. C. Fox, R. J. Mepani, F. Verdeguer, J. Wu, A. Kharitonenkov, J. S. Flier, E. Maratos-Flier and B. M. Spiegelman (2012). "FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis." Genes & development **26**(3): 271-281.

Fluck, M. and H. Hoppeler (2003). "Molecular basis of skeletal muscle plasticity--from gene to form and function." Rev Physiol Biochem Pharmacol **146**: 159-216.

Franceschi, C., M. Bonafe, S. Valensin, F. Olivieri, M. De Luca, E. Ottaviani and G. De Benedictis (2000). "Inflamm-aging. An evolutionary perspective on immunosenescence." Ann N Y Acad Sci **908**: 244-254.

Francis, G. A., E. Fayard, F. Picard and J. Auwerx (2003). "Nuclear receptors and the control of metabolism." Annu Rev Physiol **65**: 261-311.

Frederich, R. C., B. Lollmann, A. Hamann, A. Napolitano-Rosen, B. B. Kahn, B. B. Lowell and J. S. Flier (1995). "Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity." J Clin Invest **96**(3): 1658-1663.

Frontera, W. R., V. A. Hughes, R. A. Fielding, M. A. Fiatarone, W. J. Evans and R. Roubenoff (2000). "Aging of skeletal muscle: a 12-yr longitudinal study." J Appl Physiol (1985) **88**(4): 1321-1326.

Fry, C. S., J. D. Lee, J. Mula, T. J. Kirby, J. R. Jackson, F. Liu, L. Yang, C. L. Mendias, E. E. Dupont-Versteegden, J. J. McCarthy and C. A. Peterson (2015). "Inducible depletion of satellite cells in adult, sedentary mice impairs muscle regenerative capacity without affecting sarcopenia." Nat Med **21**(1): 76-80.

Garatachea, N., H. Pareja-Galeano, F. Sanchis-Gomar, A. Santos-Lozano, C. Fiuza-Luces, M. Moran, E. Emanuele, M. J. Joyner and A. Lucia (2015). "Exercise attenuates the major hallmarks of aging." Rejuvenation research **18**(1): 57-89.

Garnier, A., D. Fortin, J. Zoll, B. N'Guessan, B. Mettauer, E. Lampert, V. Veksler and R. Ventura-Clapier (2005). "Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle." FASEB J **19**(1): 43-52.

Garretson, J. T., B. J. Teubner, K. L. Grove, A. Vazdarjanova, V. Ryu and T. J. Bartness (2015). "Peroxisome proliferator-activated receptor gamma controls ingestive behavior, agouti-related protein, and neuropeptide Y mRNA in the arcuate hypothalamus." J Neurosci **35**(11): 4571-4581.

Gault, M. L. and M. E. Willems (2013). "Aging, functional capacity and eccentric exercise training." Aging Dis **4**(6): 351-363.

Geng, T., P. Li, M. Okutsu, X. Yin, J. Kwek, M. Zhang and Z. Yan (2010). "PGC-1alpha plays a functional role in exercise-induced mitochondrial biogenesis and angiogenesis but not fiber-type transformation in mouse skeletal muscle." American journal of physiology. Cell physiology **298**(3): C572-579.

Gerhart-Hines, Z., J. T. Rodgers, O. Bare, C. Lerin, S. H. Kim, R. Mostoslavsky, F. W. Alt, Z. Wu and P. Puigserver (2007). "Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha." The EMBO journal **26**(7): 1913-1923.

German, J., F. Kim, G. J. Schwartz, P. J. Havel, C. J. Rhodes, M. W. Schwartz and G. J. Morton (2009). "Hypothalamic leptin signaling regulates hepatic insulin sensitivity via a neurocircuit involving the vagus nerve." Endocrinology **150**(10): 4502-4511.

Ghosh, S., R. Lertwattanarak, N. Lefort, M. Molina-Carrion, J. Joya-Galeana, B. P. Bowen, J. Garduno-Garcia Jde, M. Abdul-Ghani, A. Richardson, R. A. DeFronzo, L. Mandarino, H. Van Remmen and N. Musi (2011). "Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance." Diabetes **60**(8): 2051-2060.

Gianni, P., K. J. Jan, M. J. Douglas, P. M. Stuart and M. A. Tarnopolsky (2004). "Oxidative stress and the mitochondrial theory of aging in human skeletal muscle." Exp Gerontol **39**(9): 1391-1400.

Goldenthal, M. J., H. R. Weiss and J. Marin-Garcia (2004). "Bioenergetic remodeling of heart mitochondria by thyroid hormone." Mol Cell Biochem **265**(1-2): 97-106.

Greene, N. P., D. E. Lee, J. L. Brown, M. E. Rosa, L. A. Brown, R. A. Perry, J. N. Henry and T. A. Washington (2015). "Mitochondrial quality control, promoted by PGC-1alpha, is dysregulated by Western diet-induced obesity and partially restored by moderate physical activity in mice." Physiol Rep **3**(7).

Gropp, E., M. Shanabrough, E. Borok, A. W. Xu, R. Janoschek, T. Buch, L. Plum, N. Balthasar, B. Hampel, A. Waisman, G. S. Barsh, T. L. Horvath and J. C. Bruning (2005). "Agouti-related peptide-expressing neurons are mandatory for feeding." Nat Neurosci **8**(10): 1289-1291.

Gulve, E. A., K. J. Rodnick, E. J. Henriksen and J. O. Holloszy (1993). "Effects of wheel running on glucose transporter (GLUT4) concentration in skeletal muscle of young adult and old rats." Mech Ageing Dev **67**(1-2): 187-200.

Gupte, A. A., J. Z. Liu, Y. Ren, L. J. Minze, J. R. Wiles, A. R. Collins, C. J. Lyon, D. Pratico, M. J. Finegold, S. T. Wong, P. Webb, J. D. Baxter, D. D. Moore and W. A. Hsueh (2010). "Rosiglitazone attenuates age- and diet-associated nonalcoholic steatohepatitis in male low-density lipoprotein receptor knockout mice." Hepatology **52**(6): 2001-2011.

Hahn, T. M., J. F. Breininger, D. G. Baskin and M. W. Schwartz (1998). "Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons." Nat Neurosci **1**(4): 271-272.

Hamilton, B. S. and H. N. Doods (2002). "Chronic application of MTII in a rat model of obesity results in sustained weight loss." Obes Res **10**(3): 182-187.

Handschin, C., S. Chin, P. Li, F. Liu, E. Maratos-Flier, N. K. Lebrasseur, Z. Yan and B. M. Spiegelman (2007). "Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals." The Journal of biological chemistry **282**(41): 30014-30021.

Handschin, C., C. S. Choi, S. Chin, S. Kim, D. Kawamori, A. J. Kurpad, N. Neubauer, J. Hu, V. K. Mootha, Y. B. Kim, R. N. Kulkarni, G. I. Shulman and B. M. Spiegelman (2007). "Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk." The Journal of clinical investigation **117**(11): 3463-3474.

Handschin, C., Y. M. Kobayashi, S. Chin, P. Seale, K. P. Campbell and B. M. Spiegelman (2007). "PGC-1alpha regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy." Genes & development **21**(7): 770-783.

Handschin, C., J. Lin, J. Rhee, A. K. Peyer, S. Chin, P. H. Wu, U. A. Meyer and B. M. Spiegelman (2005). "Nutritional regulation of hepatic heme biosynthesis and porphyria through PGC-1alpha." Cell **122**(4): 505-515.

Handschin, C., J. Rhee, J. Lin, P. T. Tarr and B. M. Spiegelman (2003). "An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle." Proc Natl Acad Sci U S A **100**(12): 7111-7116.

Handschin, C. and B. M. Spiegelman (2006). "Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism." Endocr Rev **27**(7): 728-735.

Harlan, S. M., D. A. Morgan, K. Agassandian, D. F. Guo, M. D. Cassell, C. D. Sigmund, A. L. Mark and K. Rahmouni (2011). "Ablation of the leptin receptor in the hypothalamic arcuate nucleus abrogates leptin-induced sympathetic activation." Circ Res **108**(7): 808-812.

Heilig, M., L. Vecsei and E. Widerlov (1989). "Opposite effects of centrally administered neuropeptide Y (NPY) on locomotor activity of spontaneously hypertensive (SH) and normal rats." Acta Physiol Scand **137**(2): 243-248.

Hellsten, Y., G. Ahlborg, M. Jensen-Urstad and B. Sjodin (1988). "Indication of in vivo xanthine oxidase activity in human skeletal muscle during exercise." Acta Physiol Scand **134**(1): 159-160.

Herrmann, C., R. Goke, G. Richter, H. C. Fehmann, R. Arnold and B. Goke (1995). "Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients." Digestion **56**(2): 117-126.

Herzig, S., F. Long, U. S. Jhala, S. Hedrick, R. Quinn, A. Bauer, D. Rudolph, G. Schutz, C. Yoon, P. Puigserver, B. Spiegelman and M. Montminy (2001). "CREB regulates hepatic gluconeogenesis through the coactivator PGC-1." Nature **413**(6852): 179-183.

Hollinger, K., D. Gardan-Salmon, C. Santana, D. Rice, E. Snella and J. T. Selsby (2013). "Rescue of dystrophic skeletal muscle by PGC-1alpha involves restored expression of dystrophin-associated protein complex components and satellite cell signaling." American journal of physiology. Regulatory, integrative and comparative physiology **305**(1): R13-23.

Holst, J. J., T. W. Schwartz, N. A. Lovgreen, O. Pedersen and H. Beck-Nielsen (1983). "Diurnal profile of pancreatic polypeptide, pancreatic glucagon, gut glucagon and insulin in human morbid obesity." Int J Obes **7**(6): 529-538.

Hondares, E., O. Mora, P. Yubero, M. Rodriguez de la Concepcion, R. Iglesias, M. Giralt and F. Villarroya (2006). "Thiazolidinediones and rexinoids induce peroxisome proliferator-activated receptor-coactivator (PGC)-1alpha gene transcription: an autoregulatory loop controls PGC-1alpha expression in adipocytes via peroxisome proliferator-activated receptor-gamma coactivation." Endocrinology **147**(6): 2829-2838.

Housley, M. P., N. D. Udeshi, J. T. Rodgers, J. Shabanowitz, P. Puigserver, D. F. Hunt and G. W. Hart (2009). "A PGC-1alpha-O-GlcNAc transferase complex regulates FoxO transcription factor activity in response to glucose." The Journal of biological chemistry **284**(8): 5148-5157.

Houten, S. M. and J. Auwerx (2004). "PGC-1alpha: turbocharging mitochondria." Cell **119**(1): 5-7.

Houtkooper, R. H., C. Canto, R. J. Wanders and J. Auwerx (2010). "The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways." Endocr Rev **31**(2): 194-223.

Hsu, W. H., B. H. Lee and T. M. Pan (2015). "Leptin-induced mitochondrial fusion mediates hepatic lipid accumulation." Int J Obes (Lond) **39**(12): 1750-1756.

Hu, J., Y. Lang, T. Zhang, S. Ni and H. Lu (2016). "Lentivirus-mediated PGC-1alpha overexpression protects against traumatic spinal cord injury in rats." Neuroscience **328**: 40-49.

Huang, H., S. H. Lee, C. Ye, I. S. Lima, B. C. Oh, B. B. Lowell, J. M. Zabolotny and Y. B. Kim (2013). "ROCK1 in AgRP neurons regulates energy expenditure and locomotor activity in male mice." Endocrinology **154**(10): 3660-3670.

Hughes, V. A., W. R. Frontera, R. Roubenoff, W. J. Evans and M. A. Singh (2002). "Longitudinal changes in body composition in older men and women: role of body weight change and physical activity." Am J Clin Nutr **76**(2): 473-481.

Hughes, V. A., W. R. Frontera, M. Wood, W. J. Evans, G. E. Dallal, R. Roubenoff and M. A. Fiatarone Singh (2001). "Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health." J Gerontol A Biol Sci Med Sci **56**(5): B209-217.

Hunter, S. K., M. W. Thompson, P. A. Ruell, A. R. Harmer, J. M. Thom, T. H. Gwinn and R. D. Adams (1999). "Human skeletal sarcoplasmic reticulum Ca2+ uptake and muscle function with aging and strength training." J Appl Physiol (1985) **86**(6): 1858-1865.

Huo, L., K. Gamber, S. Greeley, J. Silva, N. Huntoon, X. H. Leng and C. Bjorbaek (2009). "Leptin-dependent control of glucose balance and locomotor activity by POMC neurons." Cell Metab **9**(6): 537-547.

Huss, J. M. and D. P. Kelly (2004). "Nuclear receptor signaling and cardiac energetics." Circ Res **95**(6): 568-578.

Huss, J. M. and D. P. Kelly (2005). "Mitochondrial energy metabolism in heart failure: a question of balance." J Clin Invest **115**(3): 547-555.

Huss, J. M., R. P. Kopp and D. P. Kelly (2002). "Peroxisome proliferator-activated receptor coactivator-1alpha (PGC-1alpha) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor-alpha and -gamma. Identification of novel leucine-rich interaction motif within PGC-1alpha." The Journal of biological chemistry **277**(43): 40265-40274.

Huszar, D., C. A. Lynch, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, L. R. Berkemeier, W. Gu, R. A. Kesterson, B. A. Boston, R. D. Cone, F. J. Smith, L. A. Campfield, P. Burn and F. Lee (1997). "Targeted disruption of the melanocortin-4 receptor results in obesity in mice." Cell **88**(1): 131-141.

Hwa, J. J., M. B. Witten, P. Williams, L. Ghibaudi, J. Gao, B. G. Salisbury, D. Mullins, F. Hamud, C. D. Strader and E. M. Parker (1999). "Activation of the NPY Y5 receptor regulates both feeding and energy expenditure." Am J Physiol **277**(5 Pt 2): R1428-1434.

Iannuzzi-Sucich, M., K. M. Prestwood and A. M. Kenny (2002). "Prevalence of sarcopenia and predictors of skeletal muscle mass in healthy, older men and women." J Gerontol A Biol Sci Med Sci **57**(12): M772-777.

Ibebunjo, C., J. M. Chick, T. Kendall, J. K. Eash, C. Li, Y. Zhang, C. Vickers, Z. Wu, B. A. Clarke, J. Shi, J. Cruz, B. Fournier, S. Brachat, S. Gutzwiller, Q. Ma, J. Markovits, M. Broome, M. Steinkrauss, E. Skuba, J. R. Galarneau, S. P. Gygi and D. J. Glass (2013). "Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia." Mol Cell Biol **33**(2): 194-212.

Jager, S., C. Handschin, J. St-Pierre and B. M. Spiegelman (2007). "AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha." Proceedings of the National Academy of Sciences of the United States of America **104**(29): 12017-12022.

Ji, L. L. (2001). "Exercise at old age: does it increase or alleviate oxidative stress?" Ann N Y Acad Sci **928**: 236-247.

Jiang, H., S. U. Kang, S. Zhang, S. Karuppagounder, J. Xu, Y. K. Lee, B. G. Kang, Y. Lee, J. Zhang, O. Pletnikova, J. C. Troncoso, S. Pirooznia, S. A. Andrabi, V. L. Dawson and T. M. Dawson (2016). "Adult Conditional Knockout of PGC-1alpha Leads to Loss of Dopamine Neurons." eNeuro **3**(4).

Jimenez-Moreno, R., Z. M. Wang, R. C. Gerring and O. Delbono (2008). "Sarcoplasmic reticulum Ca2+ release declines in muscle fibers from aging mice." Biophys J **94**(8): 3178-3188.

Jorgensen, S. B., J. F. Wojtaszewski, B. Viollet, F. Andreelli, J. B. Birk, Y. Hellsten, P. Schjerling, S. Vaulont, P. D. Neufer, E. A. Richter and H. Pilegaard (2005). "Effects of alpha-AMPK knockout on exercise-induced gene activation in mouse skeletal muscle." FASEB J **19**(9): 1146-1148.

Jouaville, L. S., P. Pinton, C. Bastianutto, G. A. Rutter and R. Rizzuto (1999). "Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming." Proc Natl Acad Sci U S A **96**(24): 13807-13812.

Jubrias, S. A., P. C. Esselman, L. B. Price, M. E. Cress and K. E. Conley (2001). "Large energetic adaptations of elderly muscle to resistance and endurance training." J Appl Physiol (1985) **90**(5): 1663-1670.

Jubrias, S. A., I. R. Odderson, P. C. Esselman and K. E. Conley (1997). "Decline in isokinetic force with age: muscle cross-sectional area and specific force." Pflugers Arch **434**(3): 246-253.

Jun, H. J., T. W. Gettys and J. S. Chang (2012). "Transcriptional Activity of PGC-1alpha and NT-PGC-1alpha Is Differentially Regulated by Twist-1 in Brown Fat Metabolism." PPAR Res **2012**: 320454.

Kakuma, T., Z. W. Wang, W. Pan, R. H. Unger and Y. T. Zhou (2000). "Role of leptin in peroxisome proliferator-activated receptor gamma coactivator-1 expression." Endocrinology **141**(12): 4576-4582.

Kang, C., E. Chung, G. Diffee and L. L. Ji (2013). "Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1alpha." Experimental gerontology **48**(11): 1343-1350.

Kang, C. and L. L. Ji (2016). "PGC-1alpha overexpression via local transfection attenuates mitophagy pathway in muscle disuse atrophy." Free Radic Biol Med **93**: 32-40.

Kania, E., B. Pajak and A. Orzechowski (2015). "Calcium homeostasis and ER stress in control of autophagy in cancer cells." Biomed Res Int **2015**: 352794.

Kass, G. E. and S. Orrenius (1999). "Calcium signaling and cytotoxicity." Environ Health Perspect **107 Suppl 1**: 25-35.

Katsouri, L., C. Parr, N. Bogdanovic, M. Willem and M. Sastre (2011). "PPARgamma co-activator-1alpha (PGC-1alpha) reduces amyloid-beta generation through a PPARgamma-dependent mechanism." Journal of Alzheimer's disease : JAD **25**(1): 151-162.

Kelly, D. P. and R. C. Scarpulla (2004). "Transcriptional regulatory circuits controlling mitochondrial biogenesis and function." Genes Dev **18**(4): 357-368.

Kelly, S. S. and N. Robbins (1983). "Progression of age changes in synaptic transmission at mouse neuromuscular junctions." J Physiol **343**: 375-383.

Kent-Braun, J. A., A. V. Ng and K. Young (2000). "Skeletal muscle contractile and noncontractile components in young and older women and men." J Appl Physiol (1985) **88**(2): 662-668.

Kim, D., M. D. Nguyen, M. M. Dobbin, A. Fischer, F. Sananbenesi, J. T. Rodgers, I. Delalle, J. A. Baur, G. Sui, S. M. Armour, P. Puigserver, D. A. Sinclair and L. H. Tsai (2007). "SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis." EMBO J **26**(13): 3169-3179.

Kim, J. S., D. J. Kosek, J. K. Petrella, J. M. Cross and M. M. Bamman (2005). "Resting and load-induced levels of myogenic gene transcripts differ between older adults with demonstrable sarcopenia and young men and women." J Appl Physiol (1985) **99**(6): 2149-2158.

Kimmel, H. L., W. Gong, S. D. Vechia, R. G. Hunter and M. J. Kuhar (2000). "Intra-ventral tegmental area injection of rat cocaine and amphetamine-regulated transcript peptide 55-102 induces locomotor activity and promotes conditioned place preference." J Pharmacol Exp Ther **294**(2): 784-792.

Kitamura, T., Y. Feng, Y. I. Kitamura, S. C. Chua, Jr., A. W. Xu, G. S. Barsh, L. Rossetti and D. Accili (2006). "Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake." Nature medicine **12**(5): 534-540.

Klitgaard, H., M. Mantoni, S. Schiaffino, S. Ausoni, L. Gorza, C. Laurent-Winter, P. Schnohr and B. Saltin (1990). "Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds." Acta Physiol Scand **140**(1): 41-54.

Knutti, D., A. Kaul and A. Kralli (2000). "A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen." Mol Cell Biol **20**(7): 2411-2422.

Knutti, D., D. Kressler and A. Kralli (2001). "Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor." Proc Natl Acad Sci U S A **98**(17): 9713-9718.

Kohno, D., H. Sone, S. Tanaka, H. Kurita, D. Gantulga and T. Yada (2011). "AMP-activated protein kinase activates neuropeptide Y neurons in the hypothalamic arcuate nucleus to increase food intake in rats." Neurosci Lett **499**(3): 194-198.

Kong, X., R. Wang, Y. Xue, X. Liu, H. Zhang, Y. Chen, F. Fang and Y. Chang (2010). "Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis." PloS one **5**(7): e11707.

Konner, A. C., R. Janoschek, L. Plum, S. D. Jordan, E. Rother, X. Ma, C. Xu, P. Enriori, B. Hampel, G. S. Barsh, C. R. Kahn, M. A. Cowley, F. M. Ashcroft and J. C. Bruning (2007). "Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production." Cell Metab **5**(6): 438-449.

Konopka, A. R., M. K. Suer, C. A. Wolff and M. P. Harber (2014). "Markers of human skeletal muscle mitochondrial biogenesis and quality control: effects of age and aerobic exercise training." The journals of gerontology. Series A, Biological sciences and medical sciences **69**(4): 371-378.

Koo, S. H., H. Satoh, S. Herzig, C. H. Lee, S. Hedrick, R. Kulkarni, R. M. Evans, J. Olefsky and M. Montminy (2004). "PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3." Nat Med **10**(5): 530-534.

Kortebein, P., A. Ferrando, J. Lombeida, R. Wolfe and W. J. Evans (2007). "Effect of 10 days of bed rest on skeletal muscle in healthy older adults." JAMA **297**(16): 1772-1774.

Kostrominova, T. Y. (2010). "Advanced age-related denervation and fiber-type grouping in skeletal muscle of SOD1 knockout mice." Free radical biology & medicine **49**(10): 1582-1593.

Kuipers, S. D. and C. R. Bramham (2006). "Brain-derived neurotrophic factor mechanisms and function in adult synaptic plasticity: new insights and implications for therapy." Curr Opin Drug Discov Devel **9**(5): 580-586.

Kupr, B. and C. Handschin (2015). "Complex Coordination of Cell Plasticity by a PGC-1alpha-controlled Transcriptional Network in Skeletal Muscle." Front Physiol **6**: 325.

Lai, L., T. C. Leone, C. Zechner, P. J. Schaeffer, S. M. Kelly, D. P. Flanagan, D. M. Medeiros, A. Kovacs and D. P. Kelly (2008). "Transcriptional coactivators PGC-1alpha and PGC-lbeta control overlapping programs required for perinatal maturation of the heart." Genes & development **22**(14): 1948-1961.

Lambertucci, R. H., A. C. Levada-Pires, L. V. Rossoni, R. Curi and T. C. Pithon-Curi (2007). "Effects of aerobic exercise training on antioxidant enzyme activities and mRNA levels in soleus muscle from young and aged rats." Mech Ageing Dev **128**(3): 267-275.

Landi, F., R. Liperoti, A. Russo, S. Giovannini, M. Tosato, E. Capoluongo, R. Bernabei and G. Onder (2012). "Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study." Clin Nutr **31**(5): 652-658.

Lanza, I. R., D. K. Short, K. R. Short, S. Raghavakaimal, R. Basu, M. J. Joyner, J. P. McConnell and K. S. Nair (2008). "Endurance exercise as a countermeasure for aging." Diabetes **57**(11): 2933-2942.

Larsson, L. (1978). "Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study." Acta Physiol Scand Suppl **457**: 1-36.

Lee, J., Y. P. Hong, H. J. Shin and W. Lee (2016). "Associations of Sarcopenia and Sarcopenic Obesity With Metabolic Syndrome Considering Both Muscle Mass and Muscle Strength." J Prev Med Public Health **49**(1): 35-44.

Lee, P., J. D. Linderman, S. Smith, R. J. Brychta, J. Wang, C. Idelson, R. M. Perron, C. D. Werner, G. Q. Phan, U. S. Kammula, E. Kebebew, K. Pacak, K. Y. Chen and F. S. Celi (2014). "Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans." Cell Metab **19**(2): 302-309.

Leeuwenburgh, C. (2003). "Role of apoptosis in sarcopenia." J Gerontol A Biol Sci Med Sci **58**(11): 999-1001.

Lehman, J. J., P. M. Barger, A. Kovacs, J. E. Saffitz, D. M. Medeiros and D. P. Kelly (2000). "Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis." J Clin Invest **106**(7): 847-856.

Lehman, J. J., S. Boudina, N. H. Banke, N. Sambandam, X. Han, D. M. Young, T. C. Leone, R. W. Gross, E. D. Lewandowski, E. D. Abel and D. P. Kelly (2008). "The transcriptional coactivator PGC-1alpha is essential for maximal and efficient cardiac mitochondrial fatty acid oxidation and lipid homeostasis." American journal of physiology. Heart and circulatory physiology **295**(1): H185-196.

Lehman, J. J. and D. P. Kelly (2002). "Transcriptional activation of energy metabolic switches in the developing and hypertrophied heart." Clin Exp Pharmacol Physiol **29**(4): 339-345.

Leick, L., Y. Hellsten, J. Fentz, S. S. Lyngby, J. F. Wojtaszewski, J. Hidalgo and H. Pilegaard (2009). "PGC-1alpha mediates exercise-induced skeletal muscle VEGF expression in mice." American journal of physiology. Endocrinology and metabolism **297**(1): E92-103.

Leick, L., S. S. Lyngby, J. F. Wojtaszewski and H. Pilegaard (2010). "PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle." Experimental gerontology **45**(5): 336-342.

Leick, L., J. F. Wojtaszewski, S. T. Johansen, K. Kiilerich, G. Comes, Y. Hellsten, J. Hidalgo and H. Pilegaard (2008). "PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle." American journal of physiology. Endocrinology and metabolism **294**(2): E463-474.

LeMoine, C. M., S. C. Lougheed and C. D. Moyes (2010). "Modular evolution of PGC-1alpha in vertebrates." Journal of molecular evolution **70**(5): 492-505.

Leone, T. C., J. J. Lehman, B. N. Finck, P. J. Schaeffer, A. R. Wende, S. Boudina, M. Courtois, D. F. Wozniak, N. Sambandam, C. Bernal-Mizrachi, Z. Chen, J. O. Holloszy, D. M. Medeiros, R. E. Schmidt, J. E. Saffitz, E. D. Abel, C. F. Semenkovich and D. P. Kelly (2005). "PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis." PLoS biology **3**(4): e101.

Lerin, C., J. T. Rodgers, D. E. Kalume, S. H. Kim, A. Pandey and P. Puigserver (2006). "GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1alpha." Cell metabolism **3**(6): 429-438.

Lexell, J., D. Downham and M. Sjostrom (1986). "Distribution of different fibre types in human skeletal muscles. Fibre type arrangement in m. vastus lateralis from three groups of healthy men between 15 and 83 years." J Neurol Sci **72**(2-3): 211-222.

Lexell, J. and D. Y. Downham (1991). "The occurrence of fibre-type grouping in healthy human muscle: a quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years." Acta Neuropathol **81**(4): 377-381.

Lexell, J., C. C. Taylor and M. Sjostrom (1988). "What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men." J Neurol Sci **84**(2-3): 275-294.

Li, S., C. Liu, N. Li, T. Hao, T. Han, D. E. Hill, M. Vidal and J. D. Lin (2008). "Genome-wide coactivation analysis of PGC-1alpha identifies BAF60a as a regulator of hepatic lipid metabolism." Cell metabolism **8**(2): 105-117.

Li, X., B. Monks, Q. Ge and M. J. Birnbaum (2007). "Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1alpha transcription coactivator." Nature **447**(7147): 1012-1016.

Liang, H. and W. F. Ward (2006). "PGC-1alpha: a key regulator of energy metabolism." Advances in physiology education **30**(4): 145-151.

Lin, J., C. Handschin and B. M. Spiegelman (2005). "Metabolic control through the PGC-1 family of transcription coactivators." Cell Metab **1**(6): 361-370.

Lin, J., H. Wu, P. T. Tarr, C. Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby and B. M. Spiegelman (2002). "Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres." Nature **418**(6899): 797-801.

Lin, J., P. H. Wu, P. T. Tarr, K. S. Lindenberg, J. St-Pierre, C. Y. Zhang, V. K. Mootha, S. Jager, C. R. Vianna, R. M. Reznick, L. Cui, M. Manieri, M. X. Donovan, Z. Wu, M. P. Cooper, M. C. Fan, L. M. Rohas, A. M. Zavacki, S. Cinti, G. I. Shulman, B. B. Lowell, D. Krainc and B. M. Spiegelman (2004). "Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice." Cell **119**(1): 121-135.

Lin, J., R. Yang, P. T. Tarr, P. H. Wu, C. Handschin, S. Li, W. Yang, L. Pei, M. Uldry, P. Tontonoz, C. B. Newgard and B. M. Spiegelman (2005). "Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP." Cell **120**(2): 261-273.

Liu, C., S. Li, T. Liu, J. Borjigin and J. D. Lin (2007). "Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism." Nature **447**(7143): 477-481.

Liu, C. J. and N. K. Latham (2009). "Progressive resistance strength training for improving physical function in older adults." Cochrane Database Syst Rev(3): CD002759.

Liu, Y., A. Schlumberger, K. Wirth, D. Schmidtbleicher and J. M. Steinacker (2003). "Different effects on human skeletal myosin heavy chain isoform expression: strength vs. combination training." J Appl Physiol (1985) **94**(6): 2282-2288.

Lodi, R., C. Tonon, M. L. Valentino, S. Iotti, V. Clementi, E. Malucelli, P. Barboni, L. Longanesi, S. Schimpf, B. Wissinger, A. Baruzzi, B. Barbiroli and V. Carelli (2004). "Deficit of in vivo mitochondrial ATP production in OPA1-related dominant optic atrophy." Ann Neurol **56**(5): 719-723.

Long, L., C. Toda, J. K. Jeong, T. L. Horvath and S. Diano (2014). "PPARgamma ablation sensitizes proopiomelanocortin neurons to leptin during high-fat feeding." J Clin Invest **124**(9): 4017-4027.

Lopez, M., R. Lage, A. K. Saha, D. Perez-Tilve, M. J. Vazquez, L. Varela, S. Sangiao-Alvarellos, S. Tovar, K. Raghay, S. Rodriguez-Cuenca, R. M. Deoliveira, T. Castaneda, R. Datta, J. Z. Dong, M. Culler, M. W. Sleeman, C. V. Alvarez, R. Gallego, C. J. Lelliott, D. Carling, M. H. Tschop, C. Dieguez and A. Vidal-Puig (2008). "Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin." Cell Metab **7**(5): 389-399.

Lucas, E. K., S. E. Dougherty, L. J. McMeekin, A. T. Trinh, C. S. Reid and R. M. Cowell (2012). "Developmental alterations in motor coordination and medium spiny neuron markers in mice lacking pgc-1alpha." PloS one **7**(8): e42878.

Luquet, S., F. A. Perez, T. S. Hnasko and R. D. Palmiter (2005). "NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates." Science **310**(5748): 683-685.

Lustig, Y., J. L. Ruas, J. L. Estall, J. C. Lo, S. Devarakonda, D. Laznik, J. H. Choi, H. Ono, J. V. Olsen and B. M. Spiegelman (2011). "Separation of the gluconeogenic and mitochondrial functions of PGC-1{alpha} through S6 kinase." Genes Dev **25**(12): 1232-1244.

Ma, D., S. Li, E. K. Lucas, R. M. Cowell and J. D. Lin (2010). "Neuronal inactivation of peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) protects mice from diet-induced obesity and leads to degenerative lesions." The Journal of biological chemistry **285**(50): 39087-39095.

Ma, W., G. Fuentes, X. Shi, C. Verma, G. K. Radda and W. Han (2015). "FoxO1 negatively regulates leptin-induced POMC transcription through its direct interaction with STAT3." Biochem J **466**(2): 291-298.

Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan and et al. (1995). "Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects." Nat Med **1**(11): 1155-1161.

Manini, T. M. and B. C. Clark (2012). "Dynapenia and aging: an update." J Gerontol A Biol Sci Med Sci **67**(1): 28-40.

Manini, T. M., M. Visser, S. Won-Park, K. V. Patel, E. S. Strotmeyer, H. Chen, B. Goodpaster, N. De Rekeneire, A. B. Newman, E. M. Simonsick, S. B. Kritchevsky, K. Ryder, A. V. Schwartz and T. B. Harris (2007). "Knee extension strength cutpoints for maintaining mobility." J Am Geriatr Soc **55**(3): 451-457.

Mansouri, A., F. L. Muller, Y. Liu, R. Ng, J. Faulkner, M. Hamilton, A. Richardson, T. T. Huang, C. J. Epstein and H. Van Remmen (2006). "Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging." Mech Ageing Dev **127**(3): 298-306.

Martin, O. J., L. Lai, M. M. Soundarapandian, T. C. Leone, A. Zorzano, M. P. Keller, A. D. Attie, D. M. Muoio and D. P. Kelly (2014). "A role for peroxisome proliferator-activated receptor gamma coactivator-1 in the control of mitochondrial dynamics during postnatal cardiac growth." Circ Res **114**(4): 626-636.

Martinez-Redondo, V., A. T. Pettersson and J. L. Ruas (2015). "The hitchhiker's guide to PGC-1alpha isoform structure and biological functions." Diabetologia **58**(9): 1969-1977.

Marzetti, E., L. Groban, S. E. Wohlgemuth, H. A. Lees, M. Lin, H. Jobe, S. Giovannini, C. Leeuwenburgh and C. S. Carter (2008). "Effects of short-term GH supplementation and treadmill exercise training on physical performance and skeletal muscle apoptosis in old rats." Am J Physiol Regul Integr Comp Physiol **294**(2): R558-567.

Marzetti, E., J. M. Lawler, A. Hiona, T. Manini, A. Y. Seo and C. Leeuwenburgh (2008). "Modulation of age-induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle." Free Radic Biol Med **44**(2): 160-168.

Marzetti, E., G. Privitera, V. Simili, S. E. Wohlgemuth, L. Aulisa, M. Pahor and C. Leeuwenburgh (2010). "Multiple pathways to the same end: mechanisms of myonuclear apoptosis in sarcopenia of aging." TheScientificWorldJournal **10**: 340-349.

Marzetti, E., S. E. Wohlgemuth, H. A. Lees, H. Y. Chung, S. Giovannini and C. Leeuwenburgh (2008). "Age-related activation of mitochondrial caspase-independent apoptotic signaling in rat gastrocnemius muscle." Mech Ageing Dev **129**(9): 542-549.

McBride, H. M., M. Neuspiel and S. Wasiak (2006). "Mitochondria: more than just a powerhouse." Curr Biol **16**(14): R551-560.

McGill, J. K. and M. F. Beal (2006). "PGC-1alpha, a new therapeutic target in Huntington's disease?" Cell **127**(3): 465-468.

Meng, S. J. and L. J. Yu (2010). "Oxidative stress, molecular inflammation and sarcopenia." Int J Mol Sci **11**(4): 1509-1526.

Menshikova, E. V., V. B. Ritov, L. Fairfull, R. E. Ferrell, D. E. Kelley and B. H. Goodpaster (2006). "Effects of exercise on mitochondrial content and function in aging human skeletal muscle." J Gerontol A Biol Sci Med Sci **61**(6): 534-540.

Mesaros, A., S. B. Koralov, E. Rother, F. T. Wunderlich, M. B. Ernst, G. S. Barsh, K. Rajewsky and J. C. Bruning (2008). "Activation of Stat3 signaling in AgRP neurons promotes locomotor activity." Cell Metab **7**(3): 236-248.

Michael, L. F., Z. Wu, R. B. Cheatham, P. Puigserver, G. Adelmant, J. J. Lehman, D. P. Kelly and B. M. Spiegelman (2001). "Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1." Proc Natl Acad Sci U S A **98**(7): 3820-3825.

Mikhelson, V. M. and I. A. Gamaley (2012). "Telomere shortening is a sole mechanism of aging in mammals." Curr Aging Sci **5**(3): 203-208.

Millington, G. W. (2007). "The role of proopiomelanocortin (POMC) neurones in feeding behaviour." Nutr Metab (Lond) **4**: 18.

Minokoshi, Y., M. S. Haque and T. Shimazu (1999). "Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats." Diabetes **48**(2): 287-291.

Miselis, R. R. and A. N. Epstein (1975). "Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat." Am J Physiol **229**(5): 1438-1447.

Miura, S., Y. Kai, Y. Kamei and O. Ezaki (2008). "Isoform-specific increases in murine skeletal muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) mRNA in response to beta2-adrenergic receptor activation and exercise." Endocrinology **149**(9): 4527-4533.

Miyawaki, H., X. Zhou and M. Ashraf (1996). "Calcium preconditioning elicits strong protection against ischemic injury via protein kinase C signaling pathway." Circ Res **79**(1): 137-146.

Monsalve, M., Z. Wu, G. Adelmant, P. Puigserver, M. Fan and B. M. Spiegelman (2000). "Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1." Mol Cell **6**(2): 307-316.

Morgan, M. J. and Z. G. Liu (2011). "Crosstalk of reactive oxygen species and NF-kappaB signaling." Cell Res **21**(1): 103-115.

Morley, J. E., F. E. Kaiser, H. M. Perry, 3rd, P. Patrick, P. M. Morley, P. M. Stauber, B. Vellas, R. N. Baumgartner and P. J. Garry (1997). "Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men." Metabolism **46**(4): 410-413.

Morselli, E., E. Fuente-Martin, B. Finan, M. Kim, A. Frank, C. Garcia-Caceres, C. R. Navas, R. Gordillo, M. Neinast, S. P. Kalainayakan, D. L. Li, Y. Gao, C. X. Yi, L. Hahner, B. F. Palmer, M. H. Tschop and D. J. Clegg (2014). "Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha." Cell Rep **9**(2): 633-645.

Morton, G. J., T. H. Meek and M. W. Schwartz (2014). "Neurobiology of food intake in health and disease." Nat Rev Neurosci **15**(6): 367-378.

Mudo, G., J. Makela, V. Di Liberto, T. V. Tselykh, M. Olivieri, P. Piepponen, O. Eriksson, A. Malkia, A. Bonomo, M. Kairisalo, J. A. Aguirre, L. Korhonen, N. Belluardo and D. Lindholm (2012). "Transgenic expression and activation of PGC-1alpha protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease." Cellular and molecular life sciences : CMLS **69**(7): 1153-1165.

Murphy, B., C. N. Nunes, J. J. Ronan, M. Hanaway, A. M. Fairhurst and T. N. Mellin (2000). "Centrally administered MTII affects feeding, drinking, temperature, and activity in the Sprague-Dawley rat." J Appl Physiol (1985) **89**(1): 273-282.

Myers, M. G., Jr. and D. P. Olson (2012). "Central nervous system control of metabolism." Nature **491**(7424): 357-363.

Nakamura, S., T. Takamura, N. Matsuzawa-Nagata, H. Takayama, H. Misu, H. Noda, S. Nabemoto, S. Kurita, T. Ota, H. Ando, K. Miyamoto and S. Kaneko (2009). "Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria." J Biol Chem **284**(22): 14809-14818.

Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa and S. Matsukura (2001). "A role for ghrelin in the central regulation of feeding." Nature **409**(6817): 194-198.

Naslund, E., B. Barkeling, N. King, M. Gutniak, J. E. Blundell, J. J. Holst, S. Rossner and P. M. Hellstrom (1999). "Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men." Int J Obes Relat Metab Disord **23**(3): 304-311.

Nasrallah, C. M. and T. L. Horvath (2014). "Mitochondrial dynamics in the central regulation of metabolism." Nature reviews. Endocrinology **10**(11): 650-658.

Nijland, P. G., M. E. Witte, B. van het Hof, S. van der Pol, J. Bauer, H. Lassmann, P. van der Valk, H. E. de Vries and J. van Horssen (2014). "Astroglial PGC-1alpha increases mitochondrial antioxidant capacity and suppresses inflammation: implications for multiple sclerosis." Acta Neuropathol Commun **2**: 170.

Nisoli, E., E. Clementi, C. Paolucci, V. Cozzi, C. Tonello, C. Sciorati, R. Bracale, A. Valerio, M. Francolini, S. Moncada and M. O. Carruba (2003). "Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide." Science **299**(5608): 896-899.

Nitahara, J. A., W. Cheng, Y. Liu, B. Li, A. Leri, P. Li, D. Mogul, S. R. Gambert, J. Kajstura and P. Anversa (1998). "Intracellular calcium, DNase activity and myocyte apoptosis in aging Fischer 344 rats." J Mol Cell Cardiol **30**(3): 519-535.

Nogueiras, R., P. Wiedmer, D. Perez-Tilve, C. Veyrat-Durebex, J. M. Keogh, G. M. Sutton, P. T. Pfluger, T. R. Castaneda, S. Neschen, S. M. Hofmann, P. N. Howles, D. A. Morgan, S. C. Benoit, I. Szanto, B. Schrott, A. Schurmann, H. G. Joost, C. Hammond, D. Y. Hui, S. C. Woods, K. Rahmouni, A. A. Butler, I. S. Farooqi, S. O'Rahilly, F. Rohner-Jeanrenaud and M. H. Tschop (2007). "The central melanocortin system directly controls peripheral lipid metabolism." J Clin Invest **117**(11): 3475-3488.

O'Connell, K. and K. Ohlendieck (2009). "Proteomic DIGE analysis of the mitochondria-enriched fraction from aged rat skeletal muscle." Proteomics **9**(24): 5509-5524.

Oberkofler, H., K. Klein, T. K. Felder, F. Krempler and W. Patsch (2006). "Role of peroxisome proliferator-activated receptor-gamma coactivator-1alpha in the transcriptional regulation of the human uncoupling protein 2 gene in INS-1E cells." Endocrinology **147**(2): 966-976.

Obici, S., Z. Feng, A. Arduini, R. Conti and L. Rossetti (2003). "Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production." Nat Med **9**(6): 756-761.

Obici, S., Z. Feng, K. Morgan, D. Stein, G. Karkanias and L. Rossetti (2002). "Central administration of oleic acid inhibits glucose production and food intake." Diabetes **51**(2): 271-275.

Ogata, T., S. Machida, Y. Oishi, M. Higuchi and I. Muraoka (2009). "Differential cell death regulation between adult-unloaded and aged rat soleus muscle." Mech Ageing Dev **130**(5): 328-336.

Okuma, H., F. Saito, J. Mitsui, Y. Hara, Y. Hatanaka, M. Ikeda, T. Shimizu, K. Matsumura, J. Shimizu, S. Tsuji and M. Sonoo (2016). "Tubular aggregate myopathy caused by a novel mutation in the cytoplasmic domain of STIM1." Neurol Genet **2**(1): e50.

Olesen, J., K. Kiilerich and H. Pilegaard (2010). "PGC-1alpha-mediated adaptations in skeletal muscle." Pflugers Archiv : European journal of physiology **460**(1): 153-162.

Olesen, J., S. Larsson, N. Iversen, S. Yousafzai, Y. Hellsten and H. Pilegaard (2012). "Skeletal muscle PGC-1alpha is required for maintaining an acute LPS-induced TNFalpha response." PloS one **7**(2): e32222.

Ollmann, M. M., B. D. Wilson, Y. K. Yang, J. A. Kerns, Y. Chen, I. Gantz and G. S. Barsh (1997). "Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein." Science **278**(5335): 135-138.

Otto, B., U. Cuntz, E. Fruehauf, R. Wawarta, C. Folwaczny, R. L. Riepl, M. L. Heiman, P. Lehnert, M. Fichter and M. Tschop (2001). "Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa." Eur J Endocrinol **145**(5): 669-673.

Pagel-Langenickel, I., J. Bao, J. J. Joseph, D. R. Schwartz, B. S. Mantell, X. Xu, N. Raghavachari and M. N. Sack (2008). "PGC-1alpha integrates insulin signaling, mitochondrial regulation, and bioenergetic function in skeletal muscle." The Journal of biological chemistry **283**(33): 22464-22472.

Pagel-Langenickel, I., J. Bao, J. J. Joseph, D. R. Schwartz, B. S. Mantell, X. Xu, N. Raghavachari and M. N. Sack (2008). "PGC-1alpha integrates insulin signaling, mitochondrial regulation, and bioenergetic function in skeletal muscle." J Biol Chem **283**(33): 22464-22472.

Patti, M. E., A. J. Butte, S. Crunkhorn, K. Cusi, R. Berria, S. Kashyap, Y. Miyazaki, I. Kohane, M. Costello, R. Saccone, E. J. Landaker, A. B. Goldfine, E. Mun, R. DeFronzo, J. Finlayson, C. R. Kahn and L. J. Mandarino (2003). "Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1." Proc Natl Acad Sci U S A **100**(14): 8466-8471.

Pauli, J. R., E. R. Ropelle, D. E. Cintra, C. T. De Souza, A. S. da Silva, J. C. Moraes, P. O. Prada, J. A. de Almeida Leme, E. Luciano, L. A. Velloso, J. B. Carvalheira and M. J. Saad (2010). "Acute exercise reverses aged-induced impairments in insulin signaling in rodent skeletal muscle." Mech Ageing Dev **131**(5): 323-329.

Payne, A. M., R. Jimenez-Moreno, Z. M. Wang, M. L. Messi and O. Delbono (2009). "Role of Ca2+, membrane excitability, and Ca2+ stores in failing muscle contraction with aging." Exp Gerontol **44**(4): 261-273.

Pedersen, B. K. and C. P. Fischer (2007). "Physiological roles of muscle-derived interleukin-6 in response to exercise." Curr Opin Clin Nutr Metab Care **10**(3): 265-271.

Perez-Schindler, J., S. Summermatter, S. Salatino, F. Zorzato, M. Beer, P. J. Balwierz, E. van Nimwegen, J. N. Feige, J. Auwerx and C. Handschin (2012). "The corepressor NCoR1 antagonizes PGC-1alpha and estrogen-related receptor alpha in the regulation of skeletal muscle function and oxidative metabolism." Mol Cell Biol **32**(24): 4913-4924.

Perez-Schindler, J., K. Svensson, E. Vargas-Fernandez, G. Santos, W. Wahli and C. Handschin (2014). "The coactivator PGC-1alpha regulates skeletal muscle oxidative metabolism independently of the nuclear receptor PPARbeta/delta in sedentary mice fed a regular chow diet." Diabetologia **57**(11): 2405-2412.

Petersen, K. F., D. Befroy, S. Dufour, J. Dziura, C. Ariyan, D. L. Rothman, L. DiPietro, G. W. Cline and G. I. Shulman (2003). "Mitochondrial dysfunction in the elderly: possible role in insulin resistance." Science **300**(5622): 1140-1142.

Pette, D. and D. Skorjanc (2001). "Adaptive potentials of skeletal muscle in young and aging rats." Int J Sport Nutr Exerc Metab **11 Suppl**: S3-8.

Piers, L. S., M. J. Soares, L. M. McCormack and K. O'Dea (1998). "Is there evidence for an age-related reduction in metabolic rate?" J Appl Physiol (1985) **85**(6): 2196-2204.

Pietrangelo, L., A. D'Incecco, A. Ainbinder, A. Michelucci, H. Kern, R. T. Dirksen, S. Boncompagni and F. Protasi (2015). "Age-dependent uncoupling of mitochondria from Ca2(+) release units in skeletal muscle." Oncotarget **6**(34): 35358-35371.

Pilegaard, H., B. Saltin and P. D. Neufer (2003). "Exercise induces transient transcriptional activation of the PGC-1alpha gene in human skeletal muscle." The Journal of physiology **546**(Pt 3): 851-858.

Pistilli, E. E., J. R. Jackson and S. E. Alway (2006). "Death receptor-associated pro-apoptotic signaling in aged skeletal muscle." Apoptosis **11**(12): 2115-2126.

Planavila, A., R. Iglesias, M. Giralt and F. Villarroya (2011). "Sirt1 acts in association with PPARalpha to protect the heart from hypertrophy, metabolic dysregulation, and inflammation." Cardiovasc Res **90**(2): 276-284.

Polonsky, K. S., B. D. Given and E. Van Cauter (1988). "Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects." J Clin Invest **81**(2): 442-448.

Porte, D., Jr., D. G. Baskin and M. W. Schwartz (2002). "Leptin and insulin action in the central nervous system." Nutr Rev **60**(10 Pt 2): S20-29; discussion S68-84, 85-27.

Puigserver, P., G. Adelmant, Z. Wu, M. Fan, J. Xu, B. O'Malley and B. M. Spiegelman (1999). "Activation of PPARgamma coactivator-1 through transcription factor docking." Science **286**(5443): 1368-1371.

Puigserver, P., J. Rhee, J. Donovan, C. J. Walkey, J. C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili and B. M. Spiegelman (2003). "Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction." Nature **423**(6939): 550-555.

Puigserver, P., J. Rhee, J. Lin, Z. Wu, J. C. Yoon, C. Y. Zhang, S. Krauss, V. K. Mootha, B. B. Lowell and B. M. Spiegelman (2001). "Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1." Mol Cell **8**(5): 971-982.

Puigserver, P., Z. Wu, C. W. Park, R. Graves, M. Wright and B. M. Spiegelman (1998). "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis." Cell **92**(6): 829-839.

Qin, W., V. Haroutunian, P. Katsel, C. P. Cardozo, L. Ho, J. D. Buxbaum and G. M. Pasinetti (2009). "PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia." Archives of neurology **66**(3): 352-361.

Radak, Z., H. Naito, T. Kaneko, S. Tahara, H. Nakamoto, R. Takahashi, F. Cardozo-Pelaez and S. Goto (2002). "Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle." Pflugers Arch **445**(2): 273-278.

Rahmouni, K. and D. A. Morgan (2007). "Hypothalamic arcuate nucleus mediates the sympathetic and arterial pressure responses to leptin." Hypertension **49**(3): 647-652.

Ramadori, G., T. Fujikawa, M. Fukuda, J. Anderson, D. A. Morgan, R. Mostoslavsky, R. C. Stuart, M. Perello, C. R. Vianna, E. A. Nillni, K. Rahmouni and R. Coppari (2010). "SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity." Cell Metab **12**(1): 78-87.

Ranganath, L. R., J. M. Beety, L. M. Morgan, J. W. Wright, R. Howland and V. Marks (1996). "Attenuated GLP-1 secretion in obesity: cause or consequence?" Gut **38**(6): 916-919.

Rao, R. R., J. Z. Long, J. P. White, K. J. Svensson, J. Lou, I. Lokurkar, M. P. Jedrychowski, J. L. Ruas, C. D. Wrann, J. C. Lo, D. M. Camera, J. Lachey, S. Gygi, J. Seehra, J. A. Hawley and B. M. Spiegelman (2014). "Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis." Cell **157**(6): 1279-1291.

Ray, A., J. Kyselovic, J. J. Leddy, J. T. Wigle, B. J. Jasmin and B. S. Tuana (1995). "Regulation of dihydropyridine and ryanodine receptor gene expression in skeletal muscle. Role of nerve, protein kinase C, and cAMP pathways." J Biol Chem **270**(43): 25837-25844.

Reid, M. B. (2008). "Free radicals and muscle fatigue: Of ROS, canaries, and the IOC." Free Radic Biol Med **44**(2): 169-179.

Reid, M. B., F. A. Khawli and M. R. Moody (1993). "Reactive oxygen in skeletal muscle. III. Contractility of unfatigued muscle." J Appl Physiol (1985) **75**(3): 1081-1087.

Ren, H., I. J. Orozco, Y. Su, S. Suyama, R. Gutierrez-Juarez, T. L. Horvath, S. L. Wardlaw, L. Plum, O. Arancio and D. Accili (2012). "FoxO1 target Gpr17 activates AgRP neurons to regulate food intake." Cell **149**(6): 1314-1326.

Renganathan, M., M. L. Messi and O. Delbono (1997). "Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle." J Membr Biol **157**(3): 247-253.

Reznick, R. M., H. Zong, J. Li, K. Morino, I. K. Moore, H. J. Yu, Z. X. Liu, J. Dong, K. J. Mustard, S. A. Hawley, D. Befroy, M. Pypaert, D. G. Hardie, L. H. Young and G. I. Shulman (2007). "Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis." Cell Metab **5**(2): 151-156.

Rhee, J., Y. Inoue, J. C. Yoon, P. Puigserver, M. Fan, F. J. Gonzalez and B. M. Spiegelman (2003). "Regulation of hepatic fasting response by PPARgamma coactivator-1alpha (PGC-1): requirement for hepatocyte nuclear factor 4alpha in gluconeogenesis." Proc Natl Acad Sci U S A **100**(7): 4012-4017.

Richter, C. (1993). "Pro-oxidants and mitochondrial Ca2+: their relationship to apoptosis and oncogenesis." FEBS Lett **325**(1-2): 104-107.

Roberts, L. D., P. Bostrom, J. F. O'Sullivan, R. T. Schinzel, G. D. Lewis, A. Dejam, Y. K. Lee, M. J. Palma, S. Calhoun, A. Georgiadi, M. H. Chen, V. S. Ramachandran, M. G. Larson, C. Bouchard, T. Rankinen, A. L. Souza, C. B. Clish, T. J. Wang, J. L. Estall, A. A. Soukas, C. A. Cowan, B. M. Spiegelman and R. E. Gerszten (2014). "beta-Aminoisobutyric acid induces browning of white fat and hepatic beta-oxidation and is inversely correlated with cardiometabolic risk factors." Cell Metab **19**(1): 96-108.

Rodgers, J. T., C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman and P. Puigserver (2005). "Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1." Nature **434**(7029): 113-118.

Rooyackers, O. E., D. B. Adey, P. A. Ades and K. S. Nair (1996). "Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle." Proc Natl Acad Sci U S A **93**(26): 15364-15369.

Rosenberg, I. H. (1997). "Sarcopenia: origins and clinical relevance." J Nutr **127**(5 Suppl): 990S-991S.

Rosenheimer, J. L. (1990). "Ultraterminal sprouting in innervated and partially denervated adult and aged rat muscle." Neuroscience **38**(3): 763-770.

Rowe, G. C., A. Jiang and Z. Arany (2010). "PGC-1 coactivators in cardiac development and disease." Circulation research **107**(7): 825-838.

Rowe, G. C., S. Raghuram, C. Jang, J. A. Nagy, I. S. Patten, A. Goyal, M. C. Chan, L. X. Liu, A. Jiang, K. C. Spokes, D. Beeler, H. Dvorak, W. C. Aird and Z. Arany (2014). "PGC-1alpha induces SPP1 to activate macrophages and orchestrate functional angiogenesis in skeletal muscle." Circulation research **115**(5): 504-517.

Ruan, H. B., M. O. Dietrich, Z. W. Liu, M. R. Zimmer, M. D. Li, J. P. Singh, K. Zhang, R. Yin, J. Wu, T. L. Horvath and X. Yang (2014). "O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat." Cell **159**(2): 306-317.

Ruan, H. B., X. Han, M. D. Li, J. P. Singh, K. Qian, S. Azarhoush, L. Zhao, A. M. Bennett, V. T. Samuel, J. Wu, J. R. Yates, 3rd and X. Yang (2012). "O-GlcNAc transferase/host cell factor C1 complex regulates gluconeogenesis by modulating PGC-1alpha stability." Cell metabolism **16**(2): 226-237.

Ruas, J. L., J. P. White, R. R. Rao, S. Kleiner, K. T. Brannan, B. C. Harrison, N. P. Greene, J. Wu, J. L. Estall, B. A. Irving, I. R. Lanza, K. A. Rasbach, M. Okutsu, K. S. Nair, Z. Yan, L. A. Leinwand and B. M. Spiegelman (2012). "A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy." Cell **151**(6): 1319-1331.

Russell, L. K., C. M. Mansfield, J. J. Lehman, A. Kovacs, M. Courtois, J. E. Saffitz, D. M. Medeiros, M. L. Valencik, J. A. McDonald and D. P. Kelly (2004). "Cardiac-specific induction of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator-1alpha promotes mitochondrial biogenesis and reversible cardiomyopathy in a developmental stage-dependent manner." Circ Res **94**(4): 525-533.

Ryan, M. J., J. R. Jackson, Y. Hao, S. S. Leonard and S. E. Alway (2011). "Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice." Free Radic Biol Med **51**(1): 38-52.

Safdar, A., J. M. Bourgeois, D. I. Ogborn, J. P. Little, B. P. Hettinga, M. Akhtar, J. E. Thompson, S. Melov, N. J. Mocellin, G. C. Kujoth, T. A. Prolla and M. A. Tarnopolsky (2011). "Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice." Proceedings of the National Academy of Sciences of the United States of America **108**(10): 4135-4140.

Safdar, A., M. J. Hamadeh, J. J. Kaczor, S. Raha, J. Debeer and M. A. Tarnopolsky (2010). "Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults." PLoS One **5**(5): e10778.

Safdar, A., J. P. Little, A. J. Stokl, B. P. Hettinga, M. Akhtar and M. A. Tarnopolsky (2011). "Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis." The Journal of biological chemistry **286**(12): 10605-10617.

Sahin, E., S. Colla, M. Liesa, J. Moslehi, F. L. Muller, M. Guo, M. Cooper, D. Kotton, A. J. Fabian, C. Walkey, R. S. Maser, G. Tonon, F. Foerster, R. Xiong, Y. A. Wang, S. A. Shukla, M. Jaskelioff, E. S. Martin, T. P. Heffernan, A. Protopopov, E. Ivanova, J. E. Mahoney, M. Kost-Alimova, S. R. Perry, R. Bronson, R. Liao, R. Mulligan, O. S. Shirihai, L. Chin and R. A. DePinho (2011). "Telomere dysfunction induces metabolic and mitochondrial compromise." Nature **470**(7334): 359-365.

Sainz, N., A. Rodriguez, V. Catalan, S. Becerril, B. Ramirez, J. Gomez-Ambrosi and G. Fruhbeck (2009). "Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1alpha in ob/ob mice." PloS one **4**(9): e6808.

Salviati, G., S. Pierobon-Bormioli, R. Betto, E. Damiani, C. Angelini, S. P. Ringel, S. Salvatori and A. Margreth (1985). "Tubular aggregates: sarcoplasmic reticulum origin, calcium storage ability, and functional implications." Muscle & nerve **8**(4): 299-306.

Sandoval, D., D. Cota and R. J. Seeley (2008). "The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation." Annual review of physiology **70**: 513-535.

Sandri, M., J. Lin, C. Handschin, W. Yang, Z. P. Arany, S. H. Lecker, A. L. Goldberg and B. M. Spiegelman (2006). "PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription." Proceedings of the National Academy of Sciences of the United States of America **103**(44): 16260-16265.

Sano, M., S. Tokudome, N. Shimizu, N. Yoshikawa, C. Ogawa, K. Shirakawa, J. Endo, T. Katayama, S. Yuasa, M. Ieda, S. Makino, F. Hattori, H. Tanaka and K. Fukuda (2007). "Intramolecular control of protein stability, subnuclear compartmentalization, and coactivator function of peroxisome proliferator-activated receptor gamma coactivator 1alpha." J Biol Chem **282**(35): 25970-25980.

Sano, R. and J. C. Reed (2013). "ER stress-induced cell death mechanisms." Biochim Biophys Acta **1833**(12): 3460-3470.

Sato, A., K. Nakada and J. Hayashi (2006). "Mitochondrial dynamics and aging: Mitochondrial interaction preventing individuals from expression of respiratory deficiency caused by mutant mtDNA." Biochim Biophys Acta **1763**(5-6): 473-481.

Savontaus, E., I. M. Conwell and S. L. Wardlaw (2002). "Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats." Brain Res **958**(1): 130-138.

Schneeberger, M., M. O. Dietrich, D. Sebastian, M. Imbernon, C. Castano, A. Garcia, Y. Esteban, A. Gonzalez-Franquesa, I. C. Rodriguez, A. Bortolozzi, P. M. Garcia-Roves, R. Gomis, R. Nogueiras, T. L. Horvath, A. Zorzano and M. Claret (2013). "Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance." Cell **155**(1): 172-187.

Schneeberger, M., A. G. Gomez-Valades, J. Altirriba, D. Sebastian, S. Ramirez, A. Garcia, Y. Esteban, A. Drougard, A. Ferres-Coy, A. Bortolozzi, P. M. Garcia-Roves, J. G. Jones, B. Manadas, A. Zorzano, R. Gomis and M. Claret (2015). "Reduced alpha-MSH Underlies Hypothalamic ER-Stress-Induced Hepatic Gluconeogenesis." Cell Rep **12**(3): 361-370.

Schreiber, S. N., R. Emter, M. B. Hock, D. Knutti, J. Cardenas, M. Podvinec, E. J. Oakeley and A. Kralli (2004). "The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis." Proceedings of the National Academy of Sciences of the United States of America **101**(17): 6472-6477.

Schwartz, M. W., D. G. Baskin, T. R. Bukowski, J. L. Kuijper, D. Foster, G. Lasser, D. E. Prunkard, D. Porte, Jr., S. C. Woods, R. J. Seeley and D. S. Weigle (1996). "Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice." Diabetes **45**(4): 531-535.

Schwartz, M. W., A. J. Sipols, J. L. Marks, G. Sanacora, J. D. White, A. Scheurink, S. E. Kahn, D. G. Baskin, S. C. Woods, D. P. Figlewicz and et al. (1992). "Inhibition of hypothalamic neuropeptide Y gene expression by insulin." Endocrinology **130**(6): 3608-3616.

Sczelecki, S., A. Besse-Patin, A. Abboud, S. Kleiner, D. Laznik-Bogoslavski, C. D. Wrann, J. L. Ruas, B. Haibe-Kains and J. L. Estall (2014). "Loss of Pgc-1alpha expression in aging mouse muscle potentiates glucose intolerance and systemic inflammation." American journal of physiology. Endocrinology and metabolism **306**(2): E157-167.

Selsby, J. T., K. J. Morine, K. Pendrak, E. R. Barton and H. L. Sweeney (2012). "Rescue of dystrophic skeletal muscle by PGC-1alpha involves a fast to slow fiber type shift in the mdx mouse." PloS one **7**(1): e30063.

Serra-Rexach, J. A., N. Bustamante-Ara, M. Hierro Villaran, P. Gonzalez Gil, M. J. Sanz Ibanez, N. Blanco Sanz, V. Ortega Santamaria, N. Gutierrez Sanz, A. B. Marin Prada, C. Gallardo, G. Rodriguez Romo, J. R. Ruiz and A. Lucia (2011). "Short-term, light- to moderate-intensity exercise training improves leg muscle strength in the oldest old: a randomized controlled trial." J Am Geriatr Soc **59**(4): 594-602.

Shan, T., X. Liang, P. Bi and S. Kuang (2013). "Myostatin knockout drives browning of white adipose tissue through activating the AMPK-PGC1alpha-Fndc5 pathway in muscle." FASEB J **27**(5): 1981-1989.

Sharma, N., H. Wang, E. B. Arias, C. M. Castorena and G. D. Cartee (2015). "Mechanisms for independent and combined effects of calorie restriction and acute exercise on insulin-stimulated glucose uptake by skeletal muscle of old rats." Am J Physiol Endocrinol Metab **308**(7): E603-612.

Shi, T., F. Wang, E. Stieren and Q. Tong (2005). "SIRT3, a mitochondrial sirtuin deacetylase, regulates mitochondrial function and thermogenesis in brown adipocytes." J Biol Chem **280**(14): 13560-13567.

Shi, Y. C., J. Lau, Z. Lin, H. Zhang, L. Zhai, G. Sperk, R. Heilbronn, M. Mietzsch, S. Weger, X. F. Huang, R. F. Enriquez, P. A. Baldock, L. Zhang, A. Sainsbury, H. Herzog and S. Lin (2013). "Arcuate NPY controls

sympathetic output and BAT function via a relay of tyrosine hydroxylase neurons in the PVN." Cell Metab **17**(2): 236-248.

Shigenaga, M. K., T. M. Hagen and B. N. Ames (1994). "Oxidative damage and mitochondrial decay in aging." Proc Natl Acad Sci U S A **91**(23): 10771-10778.

Shin, J. H., H. S. Ko, H. Kang, Y. Lee, Y. I. Lee, O. Pletinkova, J. C. Troconso, V. L. Dawson and T. M. Dawson (2011). "PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease." Cell **144**(5): 689-702.

Short, K. R., M. L. Bigelow, J. Kahl, R. Singh, J. Coenen-Schimke, S. Raghavakaimal and K. S. Nair (2005). "Decline in skeletal muscle mitochondrial function with aging in humans." Proceedings of the National Academy of Sciences of the United States of America **102**(15): 5618-5623.

Short, K. R., J. L. Vittone, M. L. Bigelow, D. N. Proctor and K. S. Nair (2004). "Age and aerobic exercise training effects on whole body and muscle protein metabolism." Am J Physiol Endocrinol Metab **286**(1): E92-101.

Short, K. R., J. L. Vittone, M. L. Bigelow, D. N. Proctor, R. A. Rizza, J. M. Coenen-Schimke and K. S. Nair (2003). "Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity." Diabetes **52**(8): 1888-1896.

Sipila, S. and H. Suominen (1994). "Knee extension strength and walking speed in relation to quadriceps muscle composition and training in elderly women." Clin Physiol **14**(4): 433-442.

Skorjanc, D., I. Traub and D. Pette (1998). "Identical responses of fast muscle to sustained activity by low-frequency stimulation in young and aging rats." J Appl Physiol (1985) **85**(2): 437-441.

Smythe, G. M., T. Shavlakadze, P. Roberts, M. J. Davies, J. K. McGeachie and M. D. Grounds (2008). "Age influences the early events of skeletal muscle regeneration: studies of whole muscle grafts transplanted between young (8 weeks) and old (13-21 months) mice." Exp Gerontol **43**(6): 550-562.

Song, W., H. B. Kwak and J. M. Lawler (2006). "Exercise training attenuates age-induced changes in apoptotic signaling in rat skeletal muscle." Antioxid Redox Signal **8**(3-4): 517-528.

Soriano, F. X., M. Liesa, D. Bach, D. C. Chan, M. Palacin and A. Zorzano (2006). "Evidence for a mitochondrial regulatory pathway defined by peroxisome proliferator-activated receptor-gamma coactivator-1 alpha, estrogen-related receptor-alpha, and mitofusin 2." Diabetes **55**(6): 1783-1791.

Sousa-Victor, P., S. Gutarra, L. Garcia-Prat, J. Rodriguez-Ubreva, L. Ortet, V. Ruiz-Bonilla, M. Jardi, E. Ballestar, S. Gonzalez, A. L. Serrano, E. Perdiguero and P. Munoz-Canoves (2014). "Geriatric muscle stem cells switch reversible quiescence into senescence." Nature **506**(7488): 316-321.

Southgate, R. J., C. R. Bruce, A. L. Carey, G. R. Steinberg, K. Walder, R. Monks, M. J. Watt, J. A. Hawley, M. J. Birnbaum and M. A. Febbraio (2005). "PGC-1alpha gene expression is down-regulated by Akt-mediated phosphorylation and nuclear exclusion of FoxO1 in insulin-stimulated skeletal muscle." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **19**(14): 2072-2074.

Spiegelman, B. M. and J. S. Flier (2001). "Obesity and the regulation of energy balance." Cell **104**(4): 531-543.

Srikanthan, K., A. Feyh, H. Visweshwar, J. I. Shapiro and K. Sodhi (2016). "Systematic Review of Metabolic Syndrome Biomarkers: A Panel for Early Detection, Management, and Risk Stratification in the West Virginian Population." Int J Med Sci **13**(1): 25-38.

St-Pierre, J., S. Drori, M. Uldry, J. M. Silvaggi, J. Rhee, S. Jager, C. Handschin, K. Zheng, J. Lin, W. Yang, D. K. Simon, R. Bachoo and B. M. Spiegelman (2006). "Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators." Cell **127**(2): 397-408.

St-Pierre, J., J. Lin, S. Krauss, P. T. Tarr, R. Yang, C. B. Newgard and B. M. Spiegelman (2003). "Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells." The Journal of biological chemistry **278**(29): 26597-26603.

Ste Marie, L., G. I. Miura, D. J. Marsh, K. Yagaloff and R. D. Palmiter (2000). "A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors." Proc Natl Acad Sci U S A **97**(22): 12339-12344.

Stephens, T. W., M. Basinski, P. K. Bristow, J. M. Bue-Valleskey, S. G. Burgett, L. Craft, J. Hale, J. Hoffmann, H. M. Hsiung, A. Kriauciunas and et al. (1995). "The role of neuropeptide Y in the antiobesity action of the obese gene product." Nature **377**(6549): 530-532.

Su, X., Y. Chu, J. H. Kordower, B. Li, H. Cao, L. Huang, M. Nishida, L. Song, D. Wang and H. J. Federoff (2015). "PGC-1alpha Promoter Methylation in Parkinson's Disease." PLoS One **10**(8): e0134087.

Suetta, C., U. Frandsen, L. Jensen, M. M. Jensen, J. G. Jespersen, L. G. Hvid, M. Bayer, S. J. Petersson, H. D. Schroder, J. L. Andersen, K. M. Heinemeier, P. Aagaard, P. Schjerling and M. Kjaer (2012). "Aging affects the transcriptional regulation of human skeletal muscle disuse atrophy." PLoS One **7**(12): e51238.

Summermatter, S., O. Baum, G. Santos, H. Hoppeler and C. Handschin (2010). "Peroxisome proliferator-activated receptor {gamma} coactivator 1{alpha} (PGC-1{alpha}) promotes skeletal muscle lipid refueling in vivo by activating de novo lipogenesis and the pentose phosphate pathway." The Journal of biological chemistry **285**(43): 32793-32800.

Summermatter, S., G. Shui, D. Maag, G. Santos, M. R. Wenk and C. Handschin (2013). "PGC-1alpha improves glucose homeostasis in skeletal muscle in an activity-dependent manner." Diabetes **62**(1): 85-95.

Summermatter, S., R. Thurnheer, G. Santos, B. Mosca, O. Baum, S. Treves, H. Hoppeler, F. Zorzato and C. Handschin (2012). "Remodeling of calcium handling in skeletal muscle through PGC-1alpha: impact on force, fatigability, and fiber type." American journal of physiology. Cell physiology **302**(1): C88-99.

Suwa, M., H. Nakano and S. Kumagai (2003). "Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles." J Appl Physiol (1985) **95**(3): 960-968.

Svensson, K., V. Albert, B. Cardel, S. Salatino and C. Handschin (2016). "Skeletal muscle PGC-1alpha modulates systemic ketone body homeostasis and ameliorates diabetic hyperketonemia in mice." FASEB J **30**(5): 1976-1986.

Taaffe, D. R., T. R. Henwood, M. A. Nalls, D. G. Walker, T. F. Lang and T. B. Harris (2009). "Alterations in muscle attenuation following detraining and retraining in resistance-trained older adults." Gerontology **55**(2): 217-223.

Tadaishi, M., S. Miura, Y. Kai, Y. Kano, Y. Oishi and O. Ezaki (2011). "Skeletal muscle-specific expression of PGC-1alpha-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake." PloS one **6**(12): e28290.

Taherzadeh-Fard, E., C. Saft, D. A. Akkad, S. Wieczorek, A. Haghikia, A. Chan, J. T. Epplen and L. Arning (2011). "PGC-1alpha downstream transcription factors NRF-1 and TFAM are genetic modifiers of Huntington disease." Molecular neurodegeneration **6**(1): 32.

Tamaki, M., A. Hagiwara, K. Miyashita, S. Wakino, H. Inoue, K. Fujii, C. Fujii, M. Sato, M. Mitsuishi, A. Muraki, K. Hayashi, T. Doi and H. Itoh (2015). "Improvement of Physical Decline Through Combined Effects of Muscle Enhancement and Mitochondrial Activation by a Gastric Hormone Ghrelin in Male 5/6Nx CKD Model Mice." Endocrinology **156**(10): 3638-3648.

Tamura, H., J. Kamegai, T. Shimizu, S. Ishii, H. Sugihara and S. Oikawa (2002). "Ghrelin stimulates GH but not food intake in arcuate nucleus ablated rats." Endocrinology **143**(9): 3268-3275.

Tarnopolsky, M. A. (2009). "Mitochondrial DNA shifting in older adults following resistance exercise training." Appl Physiol Nutr Metab **34**(3): 348-354.

Tcherepanova, I., P. Puigserver, J. D. Norris, B. M. Spiegelman and D. P. McDonnell (2000). "Modulation of estrogen receptor-alpha transcriptional activity by the coactivator PGC-1." J Biol Chem **275**(21): 16302-16308.

Terman, A., T. Kurz, M. Navratil, E. A. Arriaga and U. T. Brunk (2010). "Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging." Antioxid Redox Signal **12**(4): 503-535.

Teyssier, C., H. Ma, R. Emter, A. Kralli and M. R. Stallcup (2005). "Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation." Genes & development **19**(12): 1466-1473.

Tiraby, C., G. Tavernier, C. Lefort, D. Larrouy, F. Bouillaud, D. Ricquier and D. Langin (2003). "Acquirement of brown fat cell features by human white adipocytes." J Biol Chem **278**(35): 33370-33376.

Trounce, I., E. Byrne and S. Marzuki (1989). "Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing." Lancet **1**(8639): 637-639.

Tschop, M., D. L. Smiley and M. L. Heiman (2000). "Ghrelin induces adiposity in rodents." Nature **407**(6806): 908-913.

Tsunemi, T. and A. R. La Spada (2011). "PGC-1alpha at the intersection of bioenergetics regulation and neuron function: From Huntington's disease to Parkinson's disease and beyond." Progress in neurobiology.

Urso, M. L., M. A. Fiatarone Singh, W. Ding, W. J. Evans, A. C. Cosmas and T. G. Manfredi (2005). "Exercise training effects on skeletal muscle plasticity and IGF-1 receptors in frail elders." Age (Dordr) **27**(2): 117-125.

Vainshtein, A., E. M. Desjardins, A. Armani, M. Sandri and D. A. Hood (2015). "PGC-1alpha modulates denervation-induced mitophagy in skeletal muscle." Skeletal muscle **5**: 9.

Vainshtein, A., L. D. Tryon, M. Pauly and D. A. Hood (2015). "Role of PGC-1alpha during acute exercise-induced autophagy and mitophagy in skeletal muscle." Am J Physiol Cell Physiol **308**(9): C710-719.

Valdez, G., J. C. Tapia, H. Kang, G. D. Clemenson, Jr., F. H. Gage, J. W. Lichtman and J. R. Sanes (2010). "Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise." Proceedings of the National Academy of Sciences of the United States of America **107**(33): 14863-14868.

Valdez, G., J. C. Tapia, H. Kang, G. D. Clemenson, Jr., F. H. Gage, J. W. Lichtman and J. R. Sanes (2010). "Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise." Proc Natl Acad Sci U S A **107**(33): 14863-14868.

van Pelt, R. E., F. A. Dinneno, D. R. Seals and P. P. Jones (2001). "Age-related decline in RMR in physically active men: relation to exercise volume and energy intake." Am J Physiol Endocrinol Metab **281**(3): E633-639.

Vega, R. B., J. M. Huss and D. P. Kelly (2000). "The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes." Mol Cell Biol **20**(5): 1868-1876.

Verdijk, L. B., R. Koopman, G. Schaart, K. Meijer, H. H. Savelberg and L. J. van Loon (2007). "Satellite cell content is specifically reduced in type II skeletal muscle fibers in the elderly." Am J Physiol Endocrinol Metab **292**(1): E151-157.

Villeda, S. A., J. Luo, K. I. Mosher, B. Zou, M. Britschgi, G. Bieri, T. M. Stan, N. Fainberg, Z. Ding, A. Eggel, K. M. Lucin, E. Czirr, J. S. Park, S. Couillard-Despres, L. Aigner, G. Li, E. R. Peskind, J. A. Kaye, J. F. Quinn, D. R. Galasko, X. S. Xie, T. A. Rando and T. Wyss-Coray (2011). "The ageing systemic milieu negatively regulates neurogenesis and cognitive function." Nature **477**(7362): 90-94.

Villena, J. A. (2015). "New insights into PGC-1 coactivators: redefining their role in the regulation of mitochondrial function and beyond." The FEBS journal **282**(4): 647-672.

Wall, B. T., M. L. Dirks, T. Snijders, F. B. Stephens, J. M. Senden, M. L. Verscheijden and L. J. van Loon (2015). "Short-term muscle disuse atrophy is not associated with increased intramuscular lipid deposition or a decline in the maximal activity of key mitochondrial enzymes in young and older males." Exp Gerontol **61**: 76-83.

Wallberg, A. E., S. Yamamura, S. Malik, B. M. Spiegelman and R. G. Roeder (2003). "Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1alpha." Molecular cell **12**(5): 1137-1149.

Wallenius, V., K. Wallenius, B. Ahren, M. Rudling, H. Carlsten, S. L. Dickson, C. Ohlsson and J. O. Jansson (2002). "Interleukin-6-deficient mice develop mature-onset obesity." Nat Med **8**(1): 75-79.

Wang, C., Q. Li, W. Wang, L. Guo, C. Guo, Y. Sun and J. Zhang (2015). "GLP-1 contributes to increases in PGC-1alpha expression by downregulating miR-23a to reduce apoptosis." Biochem Biophys Res Commun **466**(1): 33-39.

Wang, C. and R. J. Youle (2009). "The role of mitochondria in apoptosis*." Annual review of genetics **43**: 95-118.

Wang, L., J. Liu, P. Saha, J. Huang, L. Chan, B. Spiegelman and D. D. Moore (2005). "The orphan nuclear receptor SHP regulates PGC-1alpha expression and energy production in brown adipocytes." Cell metabolism **2**(4): 227-238.

Wang, S., A. Kamat, P. Pergola, A. Swamy, F. Tio and K. Cusi (2011). "Metabolic factors in the development of hepatic steatosis and altered mitochondrial gene expression in vivo." Metabolism **60**(8): 1090-1099.

Wang, X., B. W. Patterson, G. I. Smith, J. Kampelman, D. N. Reeds, S. A. Sullivan and B. Mittendorfer (2013). "A ~60-min brisk walk increases insulin-stimulated glucose disposal but has no effect on hepatic and adipose tissue insulin sensitivity in older women." J Appl Physiol (1985) **114**(11): 1563-1568.

Wang, Y. X., C. H. Lee, S. Tiep, R. T. Yu, J. Ham, H. Kang and R. M. Evans (2003). "Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity." Cell **113**(2): 159-170.

Warne, J. P., J. M. Varonin, S. S. Nielsen, L. E. Olofsson, C. B. Kaelin, S. Chua, Jr., G. S. Barsh, S. K. Koliwad and A. W. Xu (2013). "Coordinated regulation of hepatic energy stores by leptin and hypothalamic agouti-related protein." J Neurosci **33**(29): 11972-11985.

Waterson, M. J. and T. L. Horvath (2015). "Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding." Cell Metab **22**(6): 962-970.

Wei, P., D. Pan, C. Mao and Y. X. Wang (2012). "RNF34 is a cold-regulated E3 ubiquitin ligase for PGC-1alpha and modulates brown fat cell metabolism." Molecular and cellular biology **32**(2): 266-275.

Weisleder, N., M. Brotto, S. Komazaki, Z. Pan, X. Zhao, T. Nosek, J. Parness, H. Takeshima and J. Ma (2006). "Muscle aging is associated with compromised Ca2+ spark signaling and segregated intracellular Ca2+ release." J Cell Biol **174**(5): 639-645.

Weitzel, J. M., C. Radtke and H. J. Seitz (2001). "Two thyroid hormone-mediated gene expression patterns in vivo identified by cDNA expression arrays in rat." Nucleic Acids Res **29**(24): 5148-5155.

Wende, A. R., P. J. Schaeffer, G. J. Parker, C. Zechner, D. H. Han, M. M. Chen, C. R. Hancock, J. J. Lehman, J. M. Huss, D. A. McClain, J. O. Holloszy and D. P. Kelly (2007). "A role for the transcriptional coactivator PGC-1alpha in muscle refueling." The Journal of biological chemistry **282**(50): 36642-36651.

Wenz, T. (2011). "Mitochondria and PGC-1alpha in Aging and Age-Associated Diseases." Journal of aging research **2011**: 810619.

Wenz, T., S. G. Rossi, R. L. Rotundo, B. M. Spiegelman and C. T. Moraes (2009). "Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging." Proceedings of the National Academy of Sciences of the United States of America **106**(48): 20405-20410.

Weydt, P., V. V. Pineda, A. E. Torrence, R. T. Libby, T. F. Satterfield, E. R. Lazarowski, M. L. Gilbert, G. J. Morton, T. K. Bammler, A. D. Strand, L. Cui, R. P. Beyer, C. N. Easley, A. C. Smith, D. Krainc, S. Luquet, I. R. Sweet, M. W. Schwartz and A. R. La Spada (2006). "Thermoregulatory and metabolic defects in

Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration." Cell metabolism **4**(5): 349-362.

Weydt, P., S. M. Soyal, C. Gellera, S. Didonato, C. Weidinger, H. Oberkofler, G. B. Landwehrmeyer and W. Patsch (2009). "The gene coding for PGC-1alpha modifies age at onset in Huntington's Disease." Molecular neurodegeneration **4**: 3.

White, A. T. and S. Schenk (2012). "NAD(+)/NADH and skeletal muscle mitochondrial adaptations to exercise." Am J Physiol Endocrinol Metab **303**(3): E308-321.

White, J. P., C. D. Wrann, R. R. Rao, S. K. Nair, M. P. Jedrychowski, J. S. You, V. Martinez-Redondo, S. P. Gygi, J. L. Ruas, T. A. Hornberger, Z. Wu, D. J. Glass, X. Piao and B. M. Spiegelman (2014). "G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy." Proc Natl Acad Sci U S A **111**(44): 15756-15761.

Wilkes, E. A., A. L. Selby, P. J. Atherton, R. Patel, D. Rankin, K. Smith and M. J. Rennie (2009). "Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia." Am J Clin Nutr **90**(5): 1343-1350.

Williams, K. W., T. Liu, X. Kong, M. Fukuda, Y. Deng, E. D. Berglund, Z. Deng, Y. Gao, T. Liu, J. W. Sohn, L. Jia, T. Fujikawa, D. Kohno, M. M. Scott, S. Lee, C. E. Lee, K. Sun, Y. Chang, P. E. Scherer and J. K. Elmquist (2014). "Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis." Cell Metab **20**(3): 471-482.

Withers, R. T., W. M. Sherman, D. G. Clark, P. C. Esselbach, S. R. Nolan, M. H. Mackay and M. Brinkman (1991). "Muscle metabolism during 30, 60 and 90 s of maximal cycling on an air-braked ergometer." Eur J Appl Physiol Occup Physiol **63**(5): 354-362.

Wokke, J. H., F. G. Jennekens, C. J. van den Oord, H. Veldman, L. M. Smit and G. J. Leppink (1990). "Morphological changes in the human end plate with age." J Neurol Sci **95**(3): 291-310.

Woods, S. C., E. Decke and J. R. Vasselli (1974). "Metabolic hormones and regulation of body weight." Psychol Rev **81**(1): 26-43.

Wrann, C. D., J. P. White, J. Salogiannnis, D. Laznik-Bogoslavski, J. Wu, D. Ma, J. D. Lin, M. E. Greenberg and B. M. Spiegelman (2013). "Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway." Cell Metab **18**(5): 649-659.

Wren, A. M., C. J. Small, C. R. Abbott, W. S. Dhillo, L. J. Seal, M. A. Cohen, R. L. Batterham, S. Taheri, S. A. Stanley, M. A. Ghatei and S. R. Bloom (2001). "Ghrelin causes hyperphagia and obesity in rats." Diabetes **50**(11): 2540-2547.

Wu, H., S. B. Kanatous, F. A. Thurmond, T. Gallardo, E. Isotani, R. Bassel-Duby and R. S. Williams (2002). "Regulation of mitochondrial biogenesis in skeletal muscle by CaMK." Science **296**(5566): 349-352.

Wu, J., J. L. Ruas, J. L. Estall, K. A. Rasbach, J. H. Choi, L. Ye, P. Bostrom, H. M. Tyra, R. W. Crawford, K. P. Campbell, D. T. Rutkowski, R. J. Kaufman and B. M. Spiegelman (2011). "The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex." Cell metabolism **13**(2): 160-169.

Wu, Z., P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell and R. Scarpulla (1999). "Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1." Cell **98**(1): 115-124.

Xiong, S., N. Patrushev, F. Forouzandeh, L. Hilenski and R. W. Alexander (2015). "PGC-1alpha Modulates Telomere Function and DNA Damage in Protecting against Aging-Related Chronic Diseases." Cell reports **12**(9): 1391-1399.

Xu, A. W., C. B. Kaelin, G. J. Morton, K. Ogimoto, K. Stanhope, J. Graham, D. G. Baskin, P. Havel, M. W. Schwartz and G. S. Barsh (2005). "Effects of hypothalamic neurodegeneration on energy balance." PLoS biology **3**(12): 2168-2176.

Xu, Y., T. P. Nedungadi, L. Zhu, N. Sobhani, B. G. Irani, K. E. Davis, X. Zhang, F. Zou, L. M. Gent, L. D. Hahner, S. A. Khan, C. F. Elias, J. K. Elmquist and D. J. Clegg (2011). "Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction." Cell Metab **14**(4): 453-465.

Yang, G., C. Y. Lim, C. Li, X. Xiao, G. K. Radda, C. Li, X. Cao and W. Han (2009). "FoxO1 inhibits leptin regulation of pro-opiomelanocortin promoter activity by blocking STAT3 interaction with specificity protein 1." J Biol Chem **284**(6): 3719-3727.

Yasuda, T., T. Masaki, T. Kakuma and H. Yoshimatsu (2004). "Hypothalamic melanocortin system regulates sympathetic nerve activity in brown adipose tissue." Exp Biol Med (Maywood) **229**(3): 235-239.

Yasuda, T., T. Masaki, T. Sakata and H. Yoshimatsu (2004). "Hypothalamic neuronal histamine regulates sympathetic nerve activity and expression of uncoupling protein 1 mRNA in brown adipose tissue in rats." Neuroscience **125**(3): 535-540.

Yoon, J. C., P. Puigserver, G. Chen, J. Donovan, Z. Wu, J. Rhee, G. Adelmant, J. Stafford, C. R. Kahn, D. K. Granner, C. B. Newgard and B. M. Spiegelman (2001). "Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1." Nature **413**(6852): 131-138.

You, Y., Y. Hou, X. Zhai, Z. Li, L. Li, Y. Zhao and J. Zhao (2016). "Protective effects of PGC-1alpha via the mitochondrial pathway in rat brains after intracerebral hemorrhage." Brain Res **1646**: 34-43.

Yulyaningsih, E., L. Zhang, H. Herzog and A. Sainsbury (2011). "NPY receptors as potential targets for anti-obesity drug development." Br J Pharmacol **163**(6): 1170-1202.

Zhang, C., C. McFarlane, S. Lokireddy, S. Masuda, X. Ge, P. D. Gluckman, M. Sharma and R. Kambadur (2012). "Inhibition of myostatin protects against diet-induced obesity by enhancing fatty acid oxidation and promoting a brown adipose phenotype in mice." Diabetologia **55**(1): 183-193.

Zhang, P., C. Liu, C. Zhang, Y. Zhang, P. Shen, J. Zhang and C. Y. Zhang (2005). "Free fatty acids increase PGC-1alpha expression in isolated rat islets." FEBS letters **579**(6): 1446-1452.

Zhang, Y., L. W. Castellani, C. J. Sinal, F. J. Gonzalez and P. A. Edwards (2004). "Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) regulates triglyceride metabolism by activation of the nuclear receptor FXR." Genes Dev **18**(2): 157-169.

Zhang, Y., P. Huypens, A. W. Adamson, J. S. Chang, T. M. Henagan, A. Boudreau, N. R. Lenard, D. Burk, J. Klein, N. Perwitz, J. Shin, M. Fasshauer, A. Kralli and T. W. Gettys (2009). "Alternative mRNA splicing

produces a novel biologically active short isoform of PGC-1alpha." The Journal of biological chemistry **284**(47): 32813-32826.

Zhang, Y., G. E. Kilroy, T. M. Henagan, V. Prpic-Uhing, W. G. Richards, A. W. Bannon, R. L. Mynatt and T. W. Gettys (2005). "Targeted deletion of melanocortin receptor subtypes 3 and 4, but not CART, alters nutrient partitioning and compromises behavioral and metabolic responses to leptin." FASEB J **19**(11): 1482-1491.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J. M. Friedman (1994). "Positional cloning of the mouse obese gene and its human homologue." Nature **372**(6505): 425-432.

Zhao, W., M. Varghese, S. Yemul, Y. Pan, A. Cheng, P. Marano, S. Hassan, P. Vempati, F. Chen, X. Qian and G. M. Pasinetti (2011). "Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis." Mol Neurodegener **6**(1): 51.

Zheng, B., Z. Liao, J. J. Locascio, K. A. Lesniak, S. S. Roderick, M. L. Watt, A. C. Eklund, Y. Zhang-James, P. D. Kim, M. A. Hauser, E. Grunblatt, L. B. Moran, S. A. Mandel, P. Riederer, R. M. Miller, H. J. Federoff, U. Wullner, S. Papapetropoulos, M. B. Youdim, I. Cantuti-Castelvetri, A. B. Young, J. M. Vance, R. L. Davis, J. C. Hedreen, C. H. Adler, T. G. Beach, M. B. Graeber, F. A. Middleton, J. C. Rochet and C. R. Scherzer (2010). "PGC-1alpha, a potential therapeutic target for early intervention in Parkinson's disease." Science translational medicine **2**(52): 52ra73.

Zhivotovsky, B. and S. Orrenius (2011). "Calcium and cell death mechanisms: a perspective from the cell death community." Cell Calcium **50**(3): 211-221.

Ziotopoulou, M., D. M. Erani, S. M. Hileman, C. Bjorbaek and C. S. Mantzoros (2000). "Unlike leptin, ciliary neurotrophic factor does not reverse the starvation-induced changes of serum corticosterone and hypothalamic neuropeptide levels but induces expression of hypothalamic inhibitors of leptin signaling." Diabetes **49**(11): 1890-1896.

II. Manuscript 1: PGC-1α expression in murine AgRP neurons regulates food intake and energy balance

Jonathan F. Gill, Julien Delezie, Gesa Santos, and Christoph Handschin

Biozentrum, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

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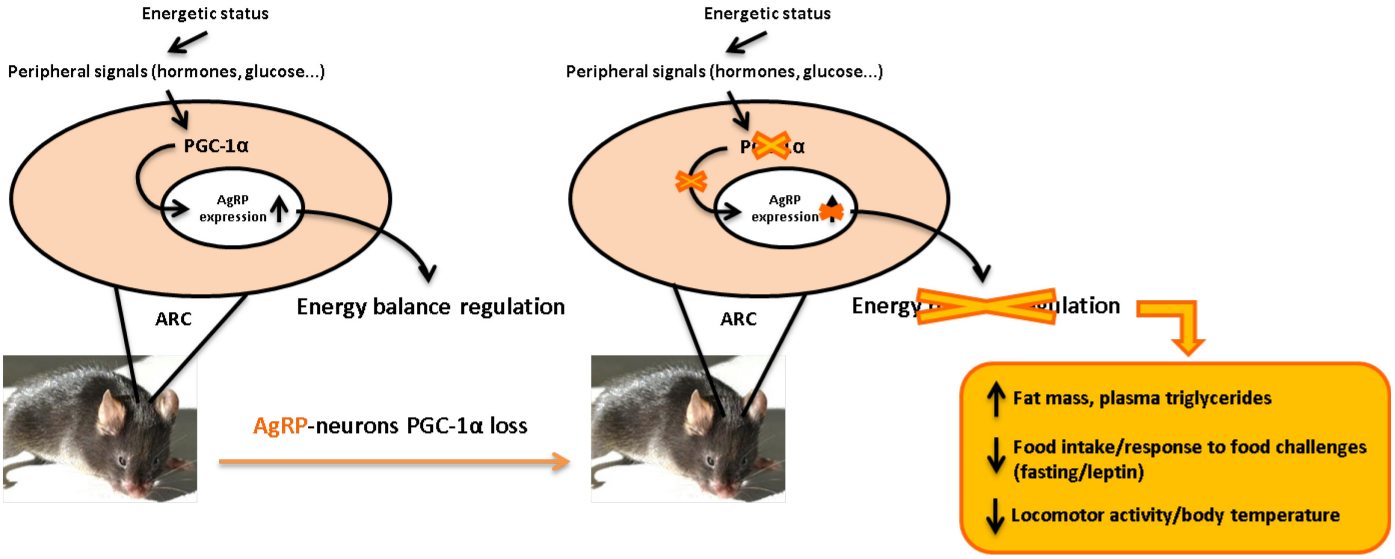


Graphical abstract

A. Abstract

Objective.

Food intake and whole-body energy homeostasis are controlled by agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) neurons located in the arcuate nucleus of the hypothalamus. Key energy sensors, such as the AMP-activated protein kinase (AMPK) or sirtuin 1 (SIRT1), are essential in AgRP and POMC cells to ensure proper energy balance. In peripheral tissues, the transcriptional coactivator PGC-1α closely associates with these sensors to regulate cellular metabolism. The role of PGC-1α in the ARC nucleus however remains unknown.

Methods.

Using AgRP and POMC neurons specific knockout (KO) mouse models we studied the consequences of PGC-1α deletion on metabolic parameters during fed and fasted states and on ghrelin and leptin responses. We also took advantage of an immortalized AgRP cell line to assess the impact of PGC-1α modulation on fasting induced AgRP expression.

Results.

PGC-1α is dispensable for POMC functions in both fed and fasted states. In stark contrast, mice carrying a specific deletion of PGC-1α in AgRP neurons display increased adiposity concomitant with significantly lower body temperature and RER values during nighttime. In addition, the absence of PGC-1α in AgRP neurons reduces food intake in the fed and fasted states, and alters the response to leptin. Finally, both in vivo and in immortalized AgRP cell line, PGC-1α modulates AgRP expression induction upon fasting.

Conclusions.

Collectively, our results highlight a role for PGC-1α in the regulation of AgRP neuronal functions in the control of food intake and peripheral metabolism.

B. Introduction

The arcuate nucleus of the hypothalamus receives and integrates different inputs from peripheral organs and subsequently controls food intake and energy expenditure according to the energy status of the body It **(Cone, Cowley et al. 2001, Sandoval, Cota et al. 2008, Woods 2009, Joly-Amado, Cansell et al. 2014)**. Two major cell populations in the ARC nucleus, namely the orexigenic agouti-related protein (AgRP) and anorexigenic pro-opiomelanocortin (POMC) neurons, secrete diverse neuropeptides including AgRP, neuropeptide Y (NPY), α-melanocyte stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART), respectively. The activity of both AgRP and POMC neurons are regulated by hormonal inputs such as ghrelin **(Ruan, Dietrich et al. 2014, Wang, Liu et al. 2014)**, leptin and insulin **(Varela and Horvath 2012)** and nutrients such as glucose **(Stanley, Wynne et al. 2005)**. As a result, in the fasted state, AgRP neurons stimulate appetite and decrease energy expenditure while POMC neuron activation in the fed state leads to food satiety and enhanced energy production **(Neary, Small et al. 2003, Stanley, Wynne et al. 2005, Coll, Farooqi et al. 2007)**. Interestingly, different energy sensors have been implicated in the cellular mechanisms in the ARC nucleus that ultimately regulate whole-body metabolism. For example, AMP-activated protein kinase (AMPK) is necessary for glucose sensing in both AgRP and POMC neurons and thereby for the control of energy balance **(Claret, Smith et al. 2007, Mountjoy and Rutter 2007)**. Deletion of the NAD+-dependent protein deacetylase sirtuin-1 (SIRT1) in AgRP neurons impairs the response to ghrelin and thus affects energy homeostasis **(Dietrich, Antunes et al. 2010)**. Finally, the forkhead protein FoxO1 mediates leptin inhibition of AgRP expression and food intake **(Kitamura, Feng et al. 2006)**.

The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a major coregulator of transcription factors involved in the control of cellular metabolism **(Martinez-Redondo, Pettersson et al. 2015)**. Intriguingly, key energy sensors that are part of the hypothalamic network controlling energy balance engage PGC-1α in peripheral tissues. For example, SIRT1 interacts with and deacetylates PGC-1α to coordinately induce the expression of gluconeogenic and mitochondrial genes in the liver **(Rodgers, Lerin et al. 2005)**. Similarly, AMPK

activation leads to transcriptional induction and activating phosphorylation events of the PGC-1α gene and protein, respectively **(Jager, Handschin et al. 2007)**. In hepatocytes, Foxo1 engages PGC-1α in the context of insulin-regulated gluconeogenesis **(Puigserver, Rhee et al. 2003)**. Importantly, global as well as deletion of PGC-1α specifically in the brain protects mice from diet-induced obesity **(Lin, Wu et al. 2004, Ma, Li et al. 2010)**. Furthermore, PGC-1α levels in the hypothalamus are increased in response to fasting **(Ma, Li et al. 2010)**, suggesting that PGC-1α may act as a metabolic integrator of different signaling pathways in the ARC nucleus for the regulation of whole body energy homeostasis **(Coppari, Ramadori et al. 2009)**. However, due to the phenotypic complexity of global and brain-specific PGC-1α knockout animals, it is unclear whether this coactivator exerts a direct role in AgRP and POMC neurons.

The present study aimed at investigating the contribution of PGC-1α expression in the ARC nucleus to whole-body energy balance. We therefore generated mouse models with specific deletions of PGC-1α either in AgRP or in POMC neurons and studied their energy homeostasis and their response to different metabolic challenges.

C. Experimental procedures

Animals

Male mice were kept under a 12h/12h light/dark cycle with lights on from 06:00 to 18:00 humidity-controlled rooms at 23°C. All animals had free access to regular chow diet (Provimi Kliba 3432) or High Fat Diet (HFD) (Provimi Kliba 2127) and water. Animals with a specific PGC-1α knock-out in AgRP and POMC neurons, (AgRP- and POMC-PGC1α KO), were generated by crossing PGC-1αloxP/loxP mice with transgenic AgRPCre/+ and POMCCre/+ mice, respectively. The PGC-1αloxP/loxP mice have been described previously **(Lin, Wu et al. 2004)**. AgRPCre/+ (Agrptm1(cre)Lowl, Jax #012899) and POMCCre/+ (STOCK tg(Pomc1-cre)16Lowl/J, Jax #005965) were purchased from Jackson Laboratories. AgRP- and POMC-PGC1α KO or AgRPCre/+ and POMCCre/+ were crossed with Rosa26-EGFP reporter mice carrying an EGFP sequence in the Rosa26 locus to generate AgRP- or POMC-EGFP-Cre and AgRP- or POMC-EGFP-Cre-PGC1α KO mice expressing EGFP in AgRP or POMC neurons. Animals used in all experiments besides weight curves measurement were between 16 and 20 weeks old, except for ghrelin and leptin experiments, for which 8 weeks old mice were used. PGC-1αloxP/loxP littermates mice without AgRPCre/+ and POMCCre/+ sites were used as controls (ctr). The genotype of AgRP-, POMC-PGC1α KO and littermate control animals was assessed by PCR using specific primer pairs (listed in the DNA/RNA extraction and qPCR section) to detect the presence of AgRPCre/+, POMCCre/+ and loxP sites. Aberrant expression of the Cre transgene is sporadically detected in germ cells in both AgRP and POMC lines. Whole body PGC-1α knock-out animals were therefore identified by PCR and excluded from the experiments (approximately 50% of AgRP-PGC1α KO and 2% of POMC-PGC1α KO mice). All experiments were performed in accordance with the federal guidelines for animal experimentation and were approved by the Kantonales Veterinäramt of the Kanton Basel-Stadt.

Body weight curves and composition analysis

Body weight was measured the same day of the week in the morning from 4 to 22 weeks of age and subsequently every month until the age of 50 weeks. Body composition was

evaluated with an EchoMRI-100 analyzer (EchoMRI Medical Systems). Fat and lean mass were normalized to body weight. A HFD experiment was started with 6 weeks old mice and body composition was evaluated after 8 weeks of HFD treatment.

COBAS blood analysis

Blood was harvested after the mice were killed. Blood samples were centrifuged for 10 min at 2000g in tri-potassium-EDTA tubes and plasma was collected. Plasma glucose and triglycerides levels were measured with a COBAS c111 analyzer (Roche Diagnostics).

Glucose tolerance test

A bolus of 2g (glucose)/kg (body weight) was injected intraperitoneally into mice fasted for 16 hours. Blood glucose was measured in the tail vein 0, 15, 30, 45, 60, 90 and 120 minutes after glucose injection with a glucose meter (Accu-Chek, Roche Diagnostics). All mice were acclimatized to handling before the experiment.

Comprehensive laboratory animal monitoring system (CLAMS)

Whole body metabolism was assessed with an indirect calorimetric system (CLAMS, Columbus Instruments). Food intake, locomotor activity, VO2 and VCO2 were recorded in 15 min intervals. Data were analyzed after one day of acclimatization. The plotted values represent 3 days of measurements in fed, 24-h fasted and 24-h refed animals.

Voluntary wheel-running activity and body temperature measurements

Mice were given free access to running wheels. The number of wheel revolutions was recorded in 30 min intervals. Plotted values represent two weeks of measurements after two weeks of acclimatization. In separate experiments, Anipill capsules (Animal Monitoring) were implanted intraperitoneally under isoflurane anesthesia for body temperature measurements. After a recovery period of 2 weeks, body temperature was recorded in 15 min intervals.

Ghrelin and leptin sensitivity

Animals were acclimatized to handling before the experiment. Intraperitoneal injections were performed with 2 and 5 mg/kg body weight of rat ghrelin (Bachem H-4862) and rat leptin (R&D 498-OB-05M), respectively, in PBS vehicle. Vehicle control, ghrelin and leptin, respectively, were injected in subsequent experiments into the same animals. Food pellets were weighed and exchanged after injections. Ghrelin injections were done at 12:00. Food intake was measured 1, 2 and 3 hours after injection. Two consecutive leptin injections were made at 17:30 and at 07:30 on the next day. Food intake and body weight were measured 16 and 24 hours later.

Cell culture

The MHypoA-59 cell line (Bioconcept CLU468) was grown in monolayer cultures in regular DMEM (Sigma-Aldrich D 5796) supplemented with 5% fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT), 4.5 mg/ml glucose and 1% penicillin/streptomycin. Cells were maintained at 37°C with 5% CO2. Cells were grown to 50% confluence before infection. PGC-1α knock-down was induced using adenoviral vectors expressing specific short hairpin RNA (shRNA) against PGC-1α or scrambled control shRNA. Both viruses expressed EGFP to allow infection efficiency monitoring. Two days after infection, regular growth medium was exchanged with fresh regular growth medium or with low glucose DMEM (1 mg/ml, Sigma-Aldrich D 6046) without FBS to induce cell starvation. After 4 hours, the medium was exchanged with low glucose DMEM supplemented with 5% FBS to mimic refeeding. Cells were harvested 4h after starvation and 1 h after refeeding. Cells exposed to normal growth medium were used as a fed state.

ARC nucleus punch isolation and imaging

Mice were killed by CO2 inhalation. Mouse brains were harvested and directly frozen in 2-methylbutane (M32631). Brain tissue was embedded in optimal cutting temperature medium (OCT, Tissue-Tek 25608-930). For arcuate nucleus isolation 100-200 µm sections containing the region of interest were cut with a cryostat (Leica). Sections were placed in RNA later solution

(Qiagen 76104) and the hypothalamic region containing the ARC nucleus was isolated using a punch needle (Leica 39443001). For AgRP and POMC neuron imaging, 15µm sections containing the arcuate nucleus of AgRP- or POMC-EGFP-Cre and AgRP- or POMC-EGFP-Cre-PGC1α KO mice expressing EGFP in AgRP or POMC neurons were visualized with a Zeiss point scanning confocal microscope.

DNA/RNA extraction and PCR

For DNA extraction, ARC nuclei were put in DNA extraction buffer (50 mM Tris-HCL pH-8.0, 100 mM NaCl, 10 mM EDTA, 0.5 % Nonidet P-40, 20 mg/ml Proteinase K) and vortexed for 30 seconds. DNA was extracted in an overnight incubation at 55°C under a constant agitation at 400 rpm in an Eppendorf Thermomixer. On the next day, proteinase K was heat-deactivated for 10 min at 95°C. The presence of AgRP Cre/+, POMC Cre/+ allele and the deletion of PGC-1α was then assessed using the PCR primers listed in Supplemental Table 1

Total RNA from ARC and non-ARC nucleus punches was isolated using the RNeasy Micro Kit (Qiagen 74004). RNA quality and concentration were measured with an Agilent Bioanalyzer (Agilent 2100 Bioanalyzer, Agilent Technologies). 50 ng of RNA were used for reverse transcription using the SuperScript II reverse transcriptase (Invitrogen 18064-014). Total RNA from mHypoA-59 cell and whole hypothalamus was extracted using the TRI reagent (Sigma-Aldrich T9424) according to the manufacturer's instructions. RNA concentration and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific). 1ug of RNA was used for cDNA synthesis as described above.

Quantitative real-time PCR

The level of relative mRNA was quantified by real-time PCR on a StepOnePlus system (Applied Biosystems) using Fast SYBR green PCR master mix (Applied Biosystems 4385612). Relative quantification was performed with the ΔΔCT method using the TATA binding protein (TBP) as housekeeping control. All primers used have similar PCR efficiency. TBP levels were

similar between genotypes in a given experimental condition. Primers sequences are listed in Supplemental Table S1.

Statistical analysis

Data were analyzed with Student's t test or with two-way ANOVA (GraphPad Prism software). Bonferroni post-test were used to do multiple comparison analysis following two-way ANOVA. All data are plotted as mean ± SEM.

D. Results

ARC nucleus specific deletion of PGC-1α in AgRP- and POMC-PGC1α KO mice

To investigate the role of PGC-1α in the ARC nucleus, animals with specific ablations of PGC-1α expression in AgRP or POMC neurons were generated by crossing PGC-1αloxP/loxP with transgenic AgRPCre/+ or POMCCre/+ mice, respectively. The presence of the Cre transgene under the control of either the AgRP or the POMC promoter was identified by PCR (Fig. 1a) in punches targeting the ARC nucleus. In both animal models, excision of the floxed PGC-1α allele was detected in areas isolated from the ARC nucleus, but not from other hypothalamic regions (Fig. 1b).

PGC-1α ablation in AgRP but not in POMC neurons promotes fat storage and reduced food intake

To assess the role of PGC-1α in AgRP and POMC neurons in whole body energy homeostasis, we evaluated body composition, food intake and glucose tolerance in both AgRP- and POMC-PGC1α KO mice. Of these, neither animals with PGC-1α deletion in AgRP or in POMC neurons did show differences in total body mass (Fig. 2a and b). Nevertheless, a significant shift in body composition from lean to fat mass was observed in AgRP-PGC1α KO mice compared to their control littermates (Fig. 2b and Fig. S1a). In association with elevated fat mass, higher triglycerides levels were detected in AgRP-PGC1α KO mice (Fig. 2e), while we observed no significant alteration of blood glucose levels (Fig. 2f) or glucose tolerance (Fig. 3a and c) even though a trends towards higher blood glucose was seen in both tests. Surprisingly, despite the increase in adiposity, PGC-1α deletion in AgRP neurons was not associated with increased food intake. On the contrary, food intake was reduced during nighttime in AgRP-PGC1α KO mice (Fig. 4a). None of these parameters, including body composition, food intake or glucose tolerance, were affected by PGC-1α deletion in POMC neurons (Fig. 2b and d, Fig. S1b, Fig. 3b and d and Fig. 4b).

PGC-1α deletion in AgRP, but not in POMC neurons, affects RER values, spontaneous locomotor activity and body temperature.

To further evaluate the metabolic phenotype of both AgRP- and POMC-PGC1α KO animals we used a Comprehensive Laboratory Animal Monitoring System (CLAMS). In line with the absence of changes in body composition and food intake, our CLAMS analysis did not reveal any changes in VO2 consumption (Fig. 4d), energy substrate utilization (Fig. 4f) or locomotor activity (Fig. 4h) in fed POMC-PGC1α mice. Similarly, VO2 levels (Fig. 4c) were unchanged in AgRP-PGC1α KO mice. We however observed a significant reduction in the respiratory exchange ratio (RER) in the AgRP-PGC1α KO mice during nighttime (Fig. 4e). In addition, a trend towards overall reduction of locomotor activity was noted in the AgRP-PGC1α KO animals (Fig. 4g). To further assess spontaneous locomotor activity in the absence of AgRP specific PGC-1α expression, voluntary wheel running was quantified over a period of 14 days. As for CLAMS in-cage movements, AgRP-PGC1α KO mice showed a trend towards reduced wheel-running activity (Fig. 5a). Interestingly, reduced RER values and locomotor activity at night were also associated with significantly lower body temperature levels in these mice in the absence of running wheels (Fig. 5b).

PGC-1α ablation in AgRP neurons affects leptin sensitivity

Because AgRP-PGC1α KO animals showed reduced food intake, we hypothesized that PGC-1α could be important for the response of AgRP neurons to hormones that regulate food intake. To test this, we first studied the effect of peripheral injection of ghrelin, an orexigenic peptide that is secreted upon starvation and that promotes food intake via AgRP neuronal activation **(Andrews 2011)**. A strong increase of food intake 1, 2 and 3 hours after ghrelin injections in both ctr and AgRP-PGC1α KO animals was observed (Fig. 6a), indicating that PGC-1α deletion in AgRP neurons did not impair ghrelin signaling.

We next evaluated the response of AgRP-PGC1α KO animals to leptin, a hormone known to inhibit AgRP and activate POMC neurons to reduce food intake **(Stanley, Wynne et al. 2005)**. In line with our previous observation, food intake over a 24-h time period was significantly

lower in AgRP-PGC1α KO mice compared to control mice upon vehicle injection (Fig. 6b). Interestingly, leptin injections significantly lowered food intake and body mass changes (Fig. 6b and c) in control animals but not in AgRP-PGC1α KO mice, thus indicating impaired leptin signaling upon specific ablation of PGC-1α in AgRP neurons.

PGC-1α in AgRP neurons controls fasting-induced AgRP expression

In light of the modulation of basal energy homeostasis and leptin response in AgRP-PGC1α KO animals, we then decided to assess their ability to adapt energy intake and expenditure to metabolic challenges such as a 24-h fasting followed by a 24-h refeeding period. As in ad libitum feeding conditions, the amount of food consumed after a 24-h fast was significantly reduced in AgRP-PGC1α KO compared to ctr mice, most notably in the second part of the re-feeding period (Fig. 7a). Of note, while RER was not changed in AgRP-PGC1α KO mice upon fasting, spontaneous activity again exhibited a trend towards lower values in the refed AgRP-PGC1α KO animals (Fig. 7b and c).

Since feeding activity is regulated by different neuropeptides in the hypothalamus, we measured the expression of the AgRP, NPY and POMC genes in fasted and fed conditions. In fed animals, no changes in orexigenic or anorexigenic gene expression levels were detected in the absence of PGC-1α (Fig. 7d). In stark contrast, the induction of AgRP gene expression by fasting was significantly blunted in AgRP-PGC1α KO mice (Fig. 7e). Of note, while fed and fasting NPY expression levels were similar between genotypes, a trend towards higher POMC expression was also observed in mice lacking PGC-1α in AgRP neurons (Fig. 7e).

To test if the reduction of AgRP-induction was due to a direct or an indirect effect of PGC-1α deletion in AgRP neurons, PGC-1α was knocked down in hypothalamic immortalized cells using siRNA-based approaches (Fig. S2). Similar to our in vivo results, PGC-1α reduction did not alter AgRP expression in fed hypothalamic cells. Conversely, a significantly reduced transcriptional induction of the AgRP gene was found under starvation conditions in cells with a

knockdown of PGC-1α (Fig. 7f). Taken together, our in vivo and in vitro data indicate that PGC-1α regulates the levels of AgRP expression in response to fasting.

E. Discussion

The ARC nucleus is crucial for the maintenance of whole-body energy balance. PGC-1α is one of the key regulators of cellular energy homeostasis and strongly affects mitochondrial biogenesis and oxidative metabolism. In addition, PGC-1α regulates various metabolic processes in peripheral organs such as brown-adipose tissue thermogenesis **(Puigserver, Wu et al. 1998)**, hepatic gluconeogenesis **(Puigserver, Rhee et al. 2003)** and endurance-training adaptation of skeletal muscle **(Lin, Wu et al. 2002, Handschin, Chin et al. 2007)**. Interestingly, PGC-1α expression is important in hippocampus for dendritic spines maintenance **(Jager, Handschin et al. 2007)**, in hypothalamic cells to protect against high fat diet-induced pathological changes **(Morselli, Fuente-Martin et al. 2014)** and exhibits daily oscillations in the hypothalamus **(Gerhart-Hines, Rodgers et al. 2007)**. Moreover, brain-specific PGC-1α deletion in mice induces a hypermetabolic state **(Ma, Li et al. 2010)**, pointing towards a key role for PGC-1α in the neuronal network controlling energy balance.

Absence of PGC-1α in POMC neurons does not alter energy balance

We thus further examined the contribution of central PGC-1α to the regulation of peripheral metabolism by generating mice with specific deletion of PGC-1α in AgRP and POMC neurons. POMC-PGC1α KO mice displayed no difference in feeding behavior, locomotor activity, basal metabolism, energy substrate utilization or glucose tolerance. Moreover, we did not observe any significant change in fasted or ghrelin-treated POMC-PGC1α KO animals (data not shown). In our experimental conditions, PGC-1α might thus simply be dispensable for POMC neuron, which are less sensitive to change in food intake than AgRP neurons **(Henry, Sugino et al. 2015)**. Therefore, different physiological contexts might have to be identified in order to discover a role for PGC-1α in POMC neurons. For example, the neuropeptides that are secreted from POMC neurons are not only important to induce satiety, but are also involved in regulating sexual behavior (α-melanocyte-stimulating hormone), stress-related release of hormones from the adrenal gland (adrenocorticotropic hormone) or endogenous opioid effects, e.g. in strenuous exercise (β-endorphin) **(Solomon 1999)**. Accordingly, a role for PGC-1α in regulating POMC neuronal function in other contexts, e.g. stress induced by exercise or other stimuli,

cannot be excluded based on the current data and thus should be the subject of future studies. Similarly, we cannot rule out that PGC-1α ablation could be compensated by PGC-1β in POMC neurons as both PGC1 coactivators are active and share redundant functions in different cell types **(Wareski, Vaarmann et al. 2009, Egger, Samardzija et al. 2012)**. Hence, specific ablation of PGC-1β and double knockout mice for both coactivators in POMC neurons should provide further insights into the specific and redundant effects of PGC-1α and -1β in this neuronal population. Previous studies revealed that multiple lineages of hypothalamic neurons express POMC **(Padilla, Carmody et al. 2010)**. In addition, pre- and postnatal ablation of AgRP neurons leads to different feeding behavior phenotypes **(Luquet, Perez et al. 2005)** that may be influenced by developmental compensation in central pathways that regulate food intake. Therefore, a comparative study with tamoxifen-inducible AgRP- and POMC-cre mouse models developed by Elmquist and colleagues **(Berglund, Liu et al. 2013, Wang, Liu et al. 2014)** to allow the deletion of PGC-1α in adult neurons might be of interest to assess the different behavioral phenotypes.

PGC-1α deletion in AgRP neurons impairs whole body energy homeostasis

In contrast to the POMC neuron-specific ablation, our results show that specific deletion of PGC-1α in AgRP cells results in significant changes in whole-body energy homeostasis. We found that AgRP-PGC1α KO mice display increased body fat in association with elevated circulating triglycerides levels. The increased adiposity could be a consequence of lower energy expenditure, as indicated by the decrease in basal body temperature and spontaneous locomotion in these mice. Previous studies showed that AgRP neurons rely on the expression of specific genes to regulate locomotor activity **(Mesaros, Koralov et al. 2008, Ren, Orozco et al. 2012, Huang, Lee et al. 2013)**. PGC-1α could be thus part of the hypothalamic network modulating spontaneous locomotion. Incidentally, the behavioral phenotype of AgRP-PGC-1α KO mice is dramatically different from the global and the brain-specific knockouts. For example, the trend towards hypoactivity in AgRP-PGC1α KO mice is diametrically opposite to the hyperactivity of the global and brain-specific knockout animals where neuronal degeneration in the striatum is thought to trigger a Huntington's-like phenotype **(Lin, Wu et al. 2004, Ma, Li et**

al. 2010). Similarly, increased adiposity in AgRP-PGC1α KO animals contrasts with the decreased fat mass observed in global and brain-specific knockout mice. Thus, collectively, the results obtained in animals with an AgRP specific deletion of PGC-1α now allow us to demonstrate that PGC-1α in AgRP neurons is intimately involved in the regulation of whole body energy homeostasis. This particular function of PGC-1α in AgRP neurons was likely masked by confounding alterations in brain-specific and global knockout animals. The cause of these differences remains to be established and probably reflects specific functions in different neuronal populations, e.g. those involved in the control of locomotion and energy balance. Of similar surprise, we found that in spite of an increased adiposity, AgRP-PGC1α KO mice displayed a lower RER, implying higher lipid oxidation, as well as hypophagia. Even though reduced locomotor activity and decreased body temperature could contribute to the observed phenotype, further investigation should be attempted to elucidate these seemingly paradoxical findings. Intriguingly, our findings recapitulated many aspects of the paradoxical phenotype of AgRP-neuron-ablated mice that display increased adiposity and increased feeding efficiency in spite of enhanced lipid utilization as well as a reduced feeding response upon fasting **(Joly-Amado, Denis et al. 2012)**. Mice with ablated AgRP neurons also showed a reduced fat gain upon HFD feeding **(Joly-Amado, Denis et al. 2012)**, reminiscent of the AgRP-PGC1α KO animals where a HFD normalized the differences in body weight between the genotypes (Fig. S3a and b). In contrast however, the reduction in RER was observed during the whole dark period in the AgRP-PGC1α KO mice and correlated with the change in locomotor activity are different from the early drop in RER in the AgRP-neuron-ablated mice **(Joly-Amado, Denis et al. 2012)** (Fig. S3c and d). Collectively, even though the absence of PGC-1α did not affect the integrity of AgRP neurons (Fig. S3e), the numerous similarities between the two mouse models suggest that increased adiposity of mice lacking PGC-1α in AgRP neurons might also be due to other mechanisms than impaired caloric consumption. Further studies should thus assess the regulation exerted by the sympathetic nervous system on lipid storage, synthesis and utilization in AgRP-PGC1α KO animals analogous to the previous characterization of AgRP-neuron-ablated mice **(Joly-Amado, Denis et al. 2012)**. Moreover, since recent studies have shown that AgRP neurons are also important for non-feeding behavioral responses linked to motivation and stereotypic behaviors **(Dietrich, Bober et al. 2012, Betley, Xu et al. 2015, Dietrich, Zimmer et al.**

2015), the decreased spontaneous locomotion of the AgRP-PGC1α KO mice might also be unrelated to the reduced feeding and body mass changes but emerge from a modulation of anxiety and non-food associated behavioral phenotypes.

PGC-1α is important for response of AgRP neurons to food and energy challenges

We demonstrated that AgRP-PGC1α KO mice exhibit reduced food intake in the fed and in the fasted state. This occurs together with lower AgRP induction in fasted animals, in line with results obtained in global and brain-specific PGC-1α knockout animals **(Ma, Li et al. 2010)**. We moreover demonstrate in a hypothalamus-derived cell line that PGC-1α directly influences AgRP expression in a fasted-like state. Interestingly, PGC-1α has been shown to colocalize with AgGP neurons **(Draper, Kirigiti et al. 2010)** and its expression is elevated in the hypothalamus **(Ma, Li et al. 2010)** and AgRP cell line upon fasting (Fig. S1), further supporting a direct role for PGC-1α in AgRP neurons for their response to fasting. Therefore, importantly, our work now also suggests that the alteration of AgRP expression regulation in fasted global and brain-specific knockout animals is driven by the specific deletion of PGC-1α in AgRP neurons. Intriguingly, we observed that leptin injection does not reduce food intake in AgRP-PGC-1α KO animals. This absence of effect could be due to enhanced endogenous leptin signaling in the absence of PGC-1α, in line with the reduced food intake that we observed in the fed state and upon vehicle injection. Alternatively, increased fat mass is often associated with higher leptin production which could also lead to altered central signaling **(El-Haschimi, Pierroz et al. 2000, Lin, Thomas et al. 2000, Lee, Reed et al. 2001)**. Collectively, these results suggest that PGC-1α, in AgPR neurons might participate in the integration of the collective output of peripheral hormonal signals through AMPK **(Claret, Smith et al. 2007)**, SIRT1 **(Dietrich, Antunes et al. 2010)** and signaling cascades **(Andrews, Liu et al. 2008, Dieguez, Vazquez et al. 2011, Shadel and Horvath 2015)**, and engage various transcription factors, including those of the FoxO family **(Kitamura, Feng et al. 2006, Ren, Orozco et al. 2012)** as in other cell types **(Puigserver, Rhee et al. 2003, Rodgers, Lerin et al. 2005, Jager, Handschin et al. 2007)**. Moreover, PGC-1α-dependent remodeling of mitochondrial number and dynamics, known to be crucial for AgRP neurons control of energy balance **(Dietrich, Liu et al. 2013, Schneeberger, Dietrich et al. 2013,**

Nasrallah and Horvath 2014), could also contribute to the consequence of PGC-1α ablation in these neurons on food intake, locomotor activity and body temperature. However, the exact molecular mechanisms that are engaged by PGC-1α in AgRP neurons remain to be determined.

Conclusion

In summary, we now demonstrate that PGC-1α is directly involved in the regulation of feeding, activity and body temperature by modulating the activity of AgRP neurons. Notably, these changes are associated with an increase in fat and a decrease in lean mass. Importantly, for the first time, the role of PGC-1α in the regulation of appetite can be dissociated from confounding effects of knockout of PGC-1α in peripheral tissues and in other areas of the brain. Finally, this study identifies PGC-1α as potential integrator of various signaling pathways in AgRP neurons and thereby sheds additional light on the regulation of whole body energy balance by hypothalamic neurons, which are attractive targets for therapeutic approaches in metabolic diseases **(Donato 2012)**.

F. Figures

Figure 1

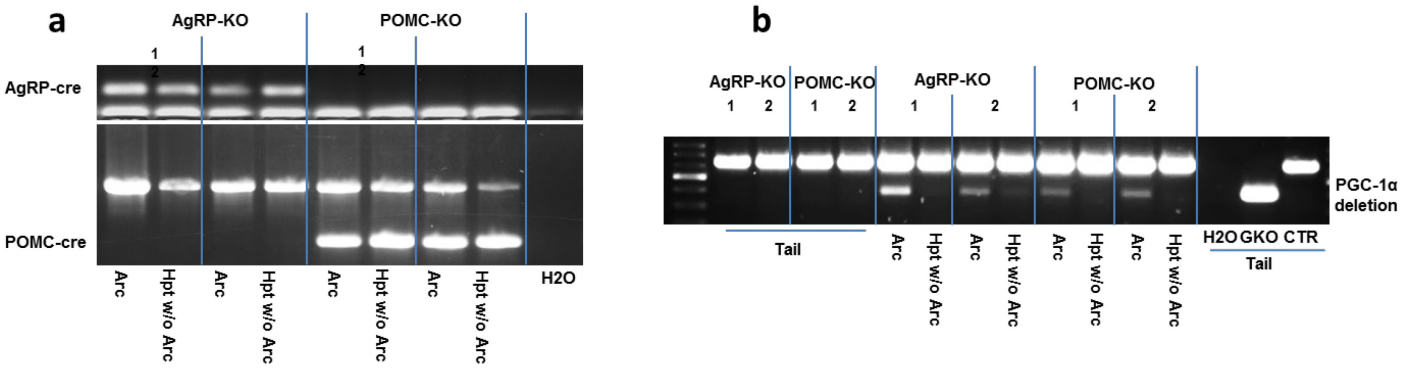


Figure 1: Specific deletion of PGC-1α in the ARC nucleus of AgRP and POMC mice.(a) Detection of AgRP-Cre and POMC-Cre expression in punches targeting the ARC nucleus. Genotyping PCR with specific primers was used to detect the presence of the AgRP-cre or POMC-cre allele in isolated hypothalamic region. (**b**) Genotyping PCR using specific primers showing the specific deletion of PGC-1α in the ARC.

Figure 2

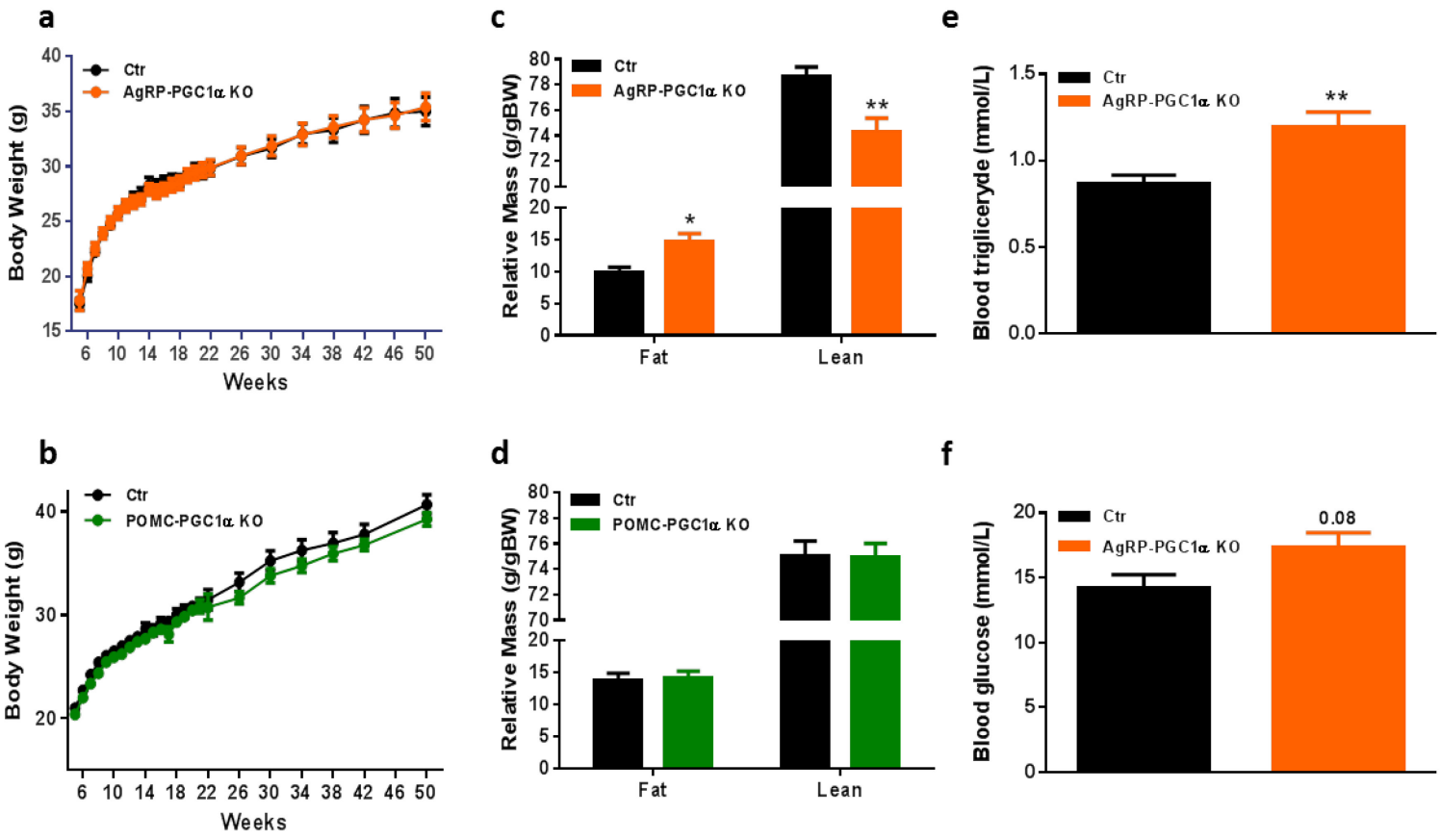

a
Ctr
AgRP-PGC1α KO
Body Weight (g)
Weeks
b
Ctr
POMC-PGC1α KO
Body Weight (g)
Weeks
c
Ctr
AgRP-PGC1α KO
Relative Mass (g/gBW)
Fat
Lean
*
**
d
Ctr
POMC-PGC1α KO
Relative Mass (g/gBW)
Fat
Lean
e
Ctr
AgRP-PGC1α KO
Blood triglyceryde (mmol/L)
**
f
Ctr
AgRP-PGC1α KO
Blood glucose (mmol/L)
0.08

Figure 2: PGC-1α deletion in AgRP, but not in POMC neurons promotes fat storage. (a and b) Body weight curves (n = 10-14) **(c and d)** body composition (n = 7-8) and **(e and f)** blood triglycerides and glucose levels (n=5-7) in AgRP-, POMC-PGC1α KO and WT mice. Values and error bars represent the mean ± SEM. *p<0.05; **p<0.01.

Figure 3

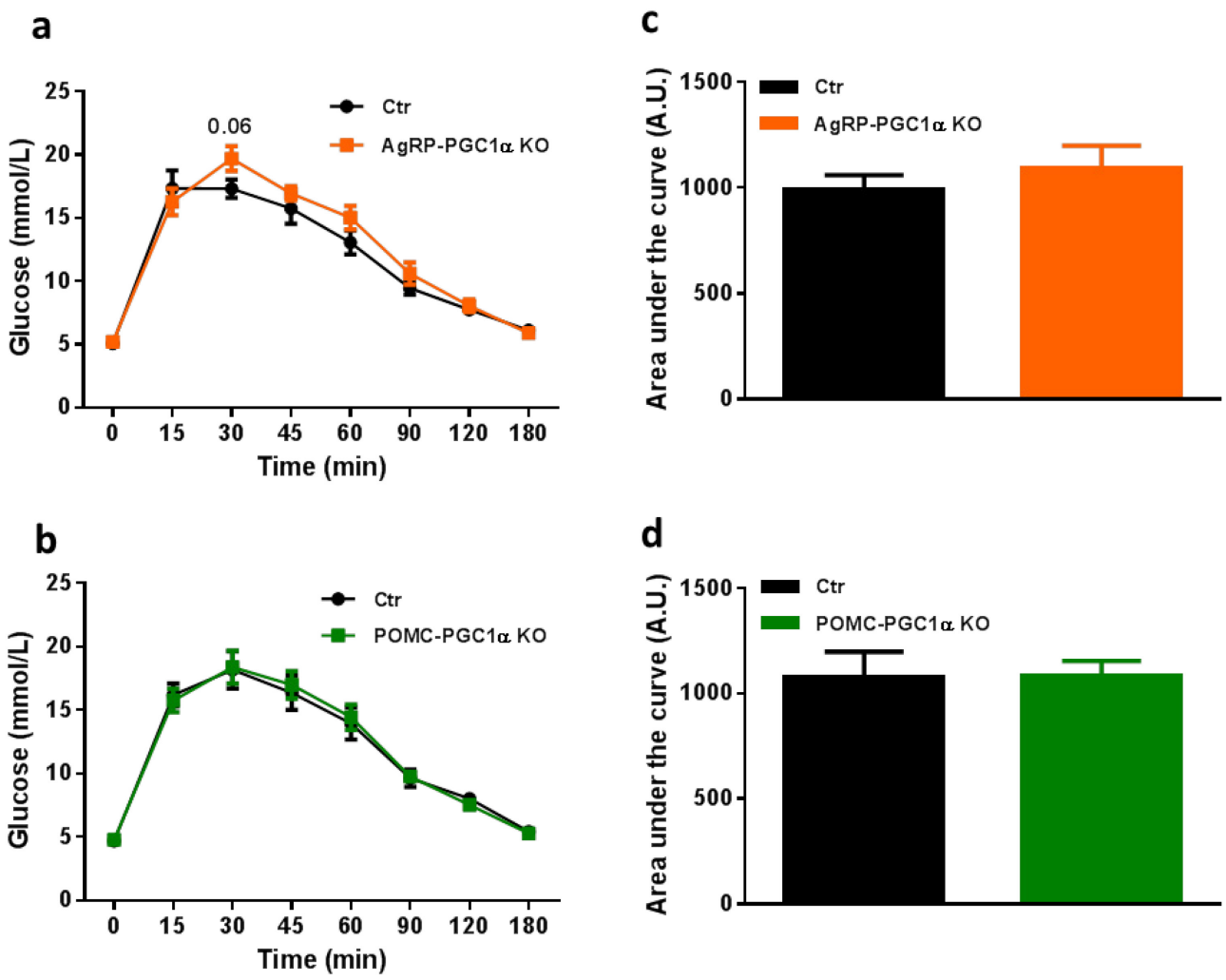

a
Glucose (mmol/L)
25
20
15
10
5
0
0.06
Ctr
AgRP-PGC1α KO
0
15
30
45
60
90
120
180
Time (min)
c
Area under the curve (A.U.)
1500
1000
500
0
Ctr
AgRP-PGC1α KO
b
Glucose (mmol/L)
Ctr
POMC-PGC1α KO
Time (min)
d
Area under the curve (A.U.)
Ctr
POMC-PGC1α KO

Figure 3: Glucose homeostasis is not altered in AgRP- and POMC-PGC1α KO mice. (a and b) Blood glucose curve during glucose tolerance test and **(c and d)** calculated area under the curves in AgRP-, POMC-PGC1α KO and WT mice (n = 7-8). Values and error bars represent the mean ± SEM. *p<0.05.

Figure 4

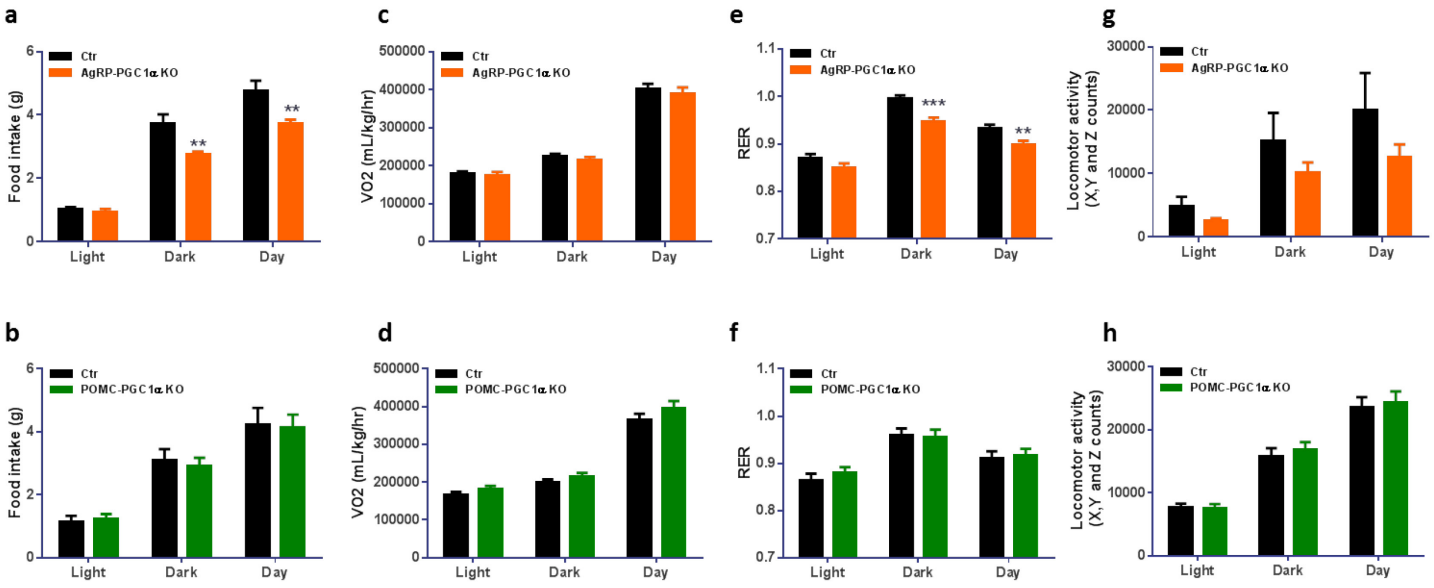

a
Ctr
AgRP-PGC1α KO
Food intake (g)
Light
Dark
Day
b
Ctr
POMC-PGC1α KO
Food intake (g)
c
VO2 (mL/kg/hr)
d
e
RER
f
g
Locomotor activity (X,Y and Z counts)
h

Figure 4 PGC-1α is required by AgRP but not POMC neurons to control basal metabolism. (a and b) Food intake, **(c and d)** oxygen consumption **(e and f)** respiratory exchange ratio and **(g and h)** spontaneous locomotion of AgRP-, POMC-PGC1α KO and WT mice (n = 7-8). Values and error bars represent the mean ± SEM. **p<0.01; ***p<0.001.

Figure 5

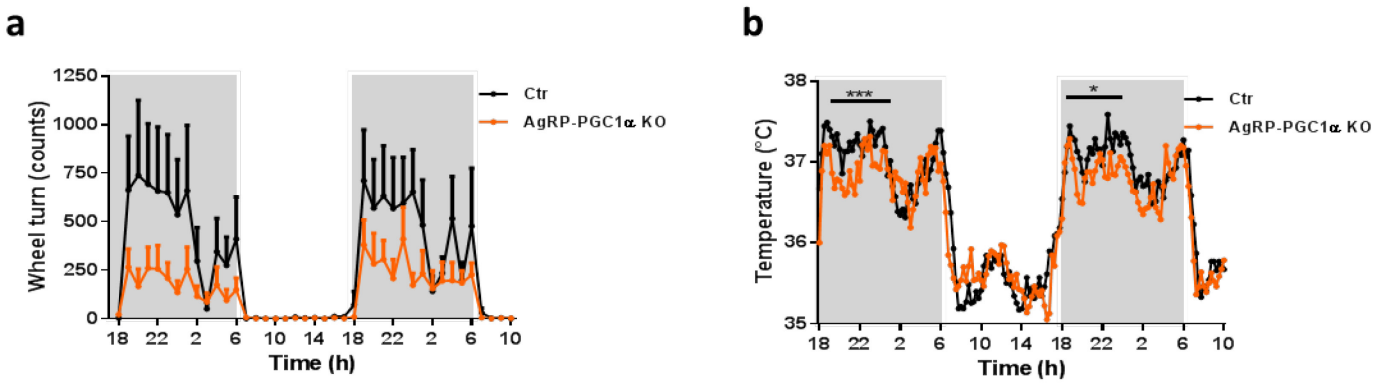

a
Wheel turn (counts)
1250
1000
750
500
250
0
18 22 2 6 10 14 18 22 2 6 10
Time (h)
Ctr
AgRP-PGC1α KO
b
Temperature (°C)
38
37
36
35
***
*
18 22 2 6 10 14 18 22 2 6 10
Time (h)
Ctr
AgRP-PGC1α KO

Figure 5 PGC-1α deletion in AgRP neurons reduces energy expenditure. (a) Voluntary activity measured by running wheel revolutions in AgRP-PGC1α KO and WT mice (n = 5-6). Values represent 2 weeks of measurements. **(b)** Basal body temperature measured during 48-h in AgRP-PGC1α KO and WT mice (n = 7-8) in the absence of running wheel. Values and error bars represent the mean ± SEM. *p<0.05; ***p<0.001.

Figure 6

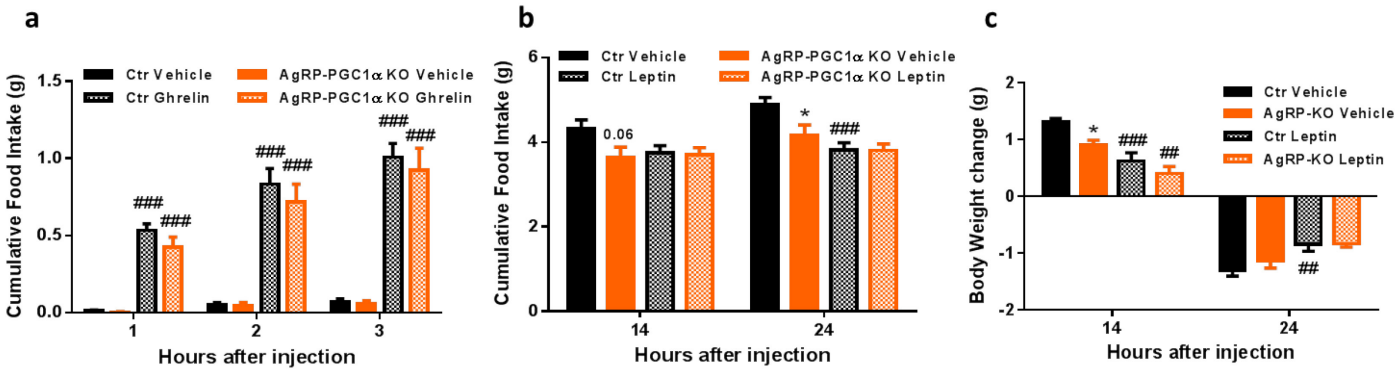

a
Ctr Vehicle
Ctr Ghrelin
AgRP-PGC1α KO Vehicle
AgRP-PGC1α KO Ghrelin
Cumulative Food Intake (g)
Hours after injection
b
Ctr Vehicle
Ctr Leptin
AgRP-PGC1α KO Vehicle
AgRP-PGC1α KO Leptin
0.06
Cumulative Food Intake (g)
Hours after injection
c
Ctr Vehicle
AgRP-KO Vehicle
Ctr Leptin
AgRP-KO Leptin
Body Weight change (g)
Hours after injection

Figure 6 PGC-1α deletion in AgRP neurons alters response to leptin. (a) Food intake AgRP-PGC1α KO and WT 1, 2 and 3 hours after ghrelin or vehicle injection (n = 8). **(b and c)** Feeding response and body weight changes of 8 weeks old AgRP-PGC1α KO and WT individually housed mice 16 and 24 hours after leptin or vehicle injection (n = 8). A second injection was performed 16 hours later. Values and error bars represent the mean ± SEM. *p<0.05 indicates statistically significant differences between genotypes, ## p<0.01; ### p<0.001 indicate statistically significant differences between vehicle and ghrelin or leptin injections.

Figure 7

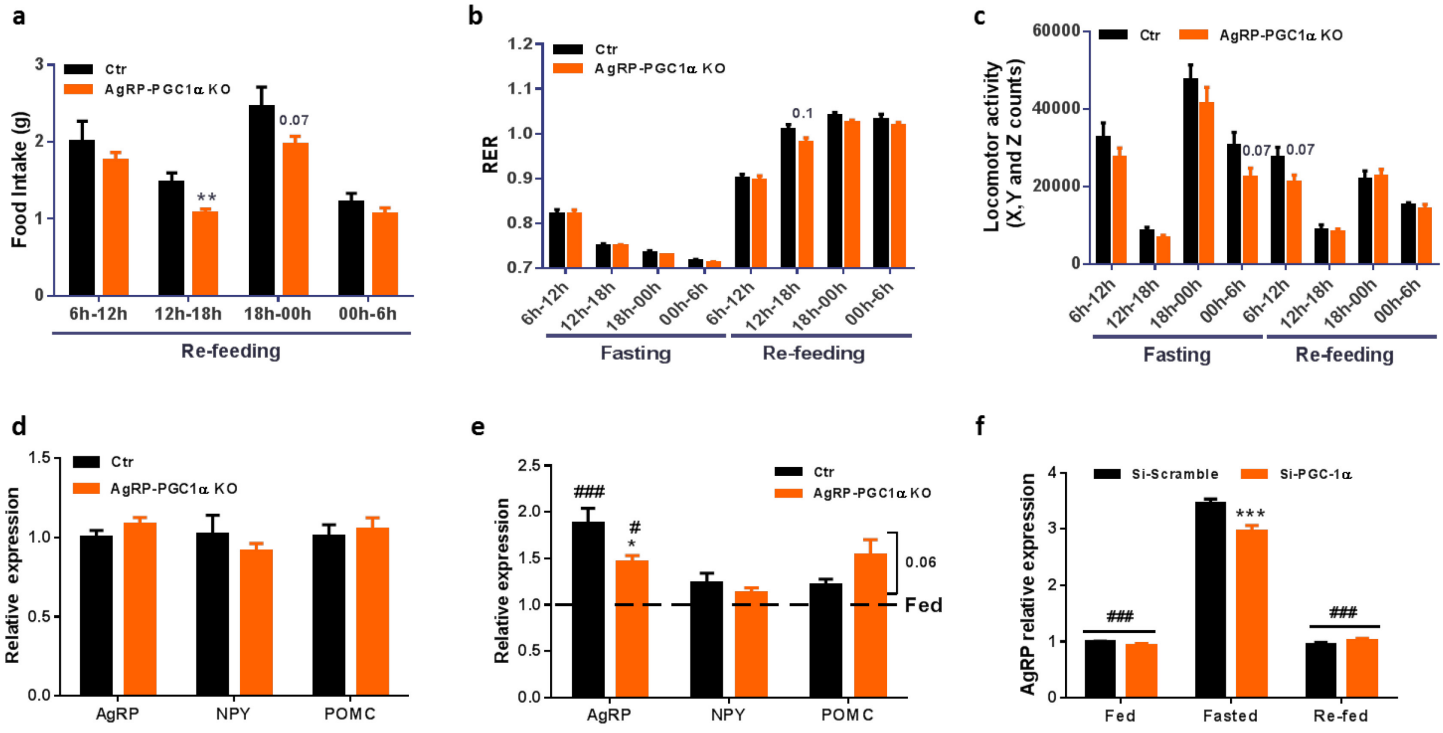

a
Ctr
AgRP-PGC1α KO
Food Intake (g)
0.07
**
6h-12h
12h-18h
18h-00h
00h-6h
Re-feeding
b
Ctr
AgRP-PGC1α KO
RER
0.1
6h-12h
12h-18h
18h-00h
00h-6h
Fasting
Re-feeding
c
Ctr
AgRP-PGC1α KO
Locomotor activity (X, Y and Z counts)
0.07
0.07
Fasting
Re-feeding
d
Ctr
AgRP-PGC1α KO
Relative expression
AgRP
NPY
POMC
e
Ctr
AgRP-PGC1α KO
Relative expression
###
#
*
0.06
Fed
AgRP
NPY
POMC
f
Si-Scramble
Si-PGC-1α
AgRP relative expression
***
###
###
Fed
Fasted
Re-fed

Figure 7 PGC-1α deletion in AgRP neurons impairs energy homeostasis and hypothalamic signaling in response to fasting. (a) Food intake, **(b)** respiratory exchange ratio and **(c)** spontaneous locomotion in AgRP-PGC1α KO and WT mice measured with CLAMS during fasting and refeeding (n = 7-8). **(d and e)** AgRP, NPY and POMC mRNA levels in the hypothalamus of AgRP-PGC1α KO and WT in fed and overnight fasted mice measured by qPCR (n = 4-8). Data are normalized by mRNA values of fed animals. **(f)** AgRP mRNA level in fed, 4-hours starved and 1-hour refed hypothalamic cells measured by qPCR (n = 3). Values and error bars represent the mean ± SEM. *p<0.05; ***p<0.001 indicate statistically significant differences between genotypes, ## p<0.01; #### p<0.001 indicate statistically significant differences between fed and fasted conditions.

Table S1

Gene name	Forward primer	Reverse primer	Common Primer
AgRP-cre	GCTTCTTCAATGCCTTTTGC	GTGTGTGGTTCCAGCATGAC	AGGAACTGCTTCCTTCACGA
POMC-cre	TGGCTCAATGTCCTTCCTGG	CACATAAGCTGCATCGTTAA	GAGATATCTTTAACCCTGATC
PGC-1α-WT	ACCTGTCTTTGCCTATGATTC	CCAGTTTCTTCATTGGTGTG	
PGC-1α-KO	TCCAGTAGGCAGAGATTTATGAC	CCAACTGTCTATAATTCCAGTTC	
AgRP	AACCTCTGTAGTCGCACCTAGC	AAACCGTCCCATCCTTTATTCT	
POMC	GAGCTGATGACCTCTAGCCTCT	ATCAGAGCCGACTGTGAAATCT	
NPY	TGTTTGGGCATTCTGGCTGA	TAGTGTCGCAGAGCGGAGTA	
PGC-1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG	
TBP	TGCTGTTGGTGATTGTTGGT	CTGGCTTGTGTGGGAAAGAT	

Figure S1

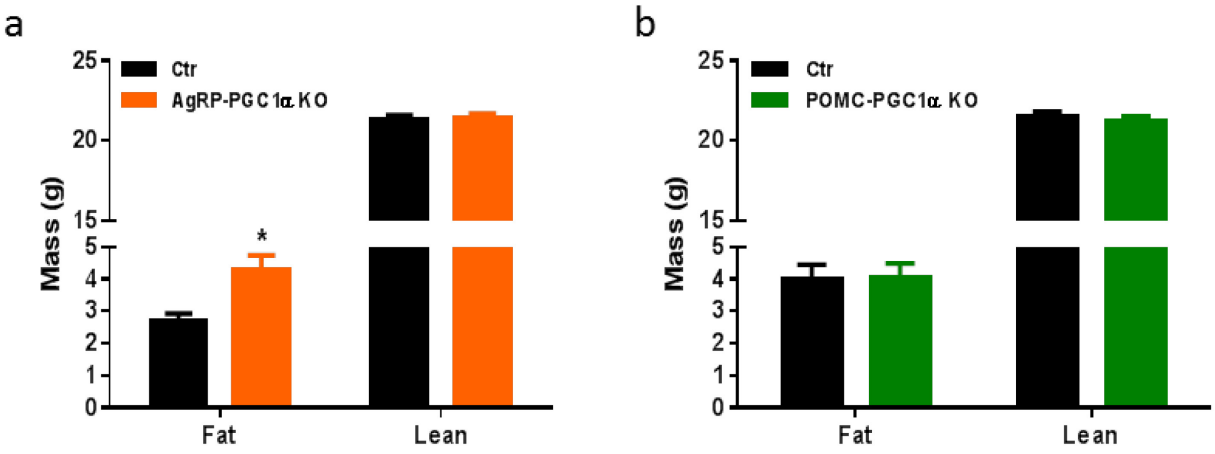

a
Ctr
AgRP-PGC1α KO
Mass (g)
25
20
15
5
4
3
2
1
0
*
Fat
Lean
b
Ctr
POMC-PGC1α KO
Mass (g)
25
20
15
5
4
3
2
1
0
Fat
Lean

Figure S1 Raw body mass values (a and b) Fat and lean mass absolute values of AgRP- and POMC-PGC1α KO and ctr mice (n=7-8) corresponding to relative body composition shown in figure 2. Values and error bars represent the mean ± SEM. *p<0.05

Figure S2

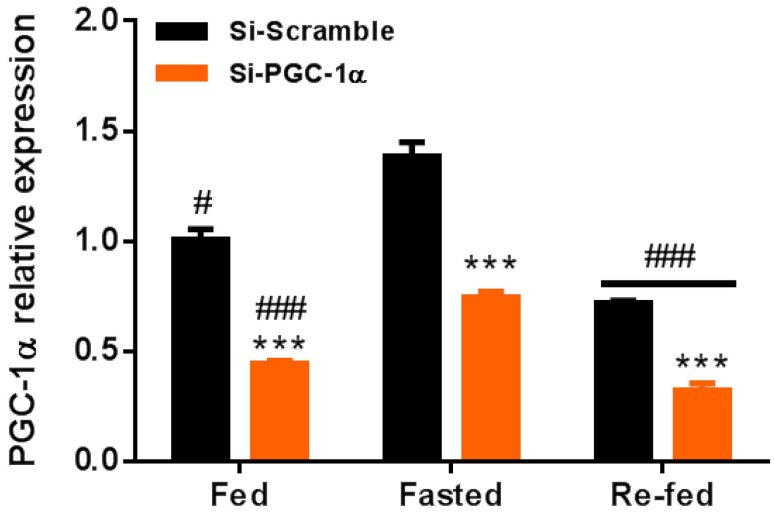

PGC-1α relative expression
2.0
1.5
1.0
0.5
0.0
Si-Scramble
Si-PGC-1α
#
###
***
***
###
***
Fed
Fasted
Re-fed

Figure S2 PGC-1α deletion in AgRP expressing hypothalamic cell. PGC-1α mRNA level in fed, 4-h starved and 1-h refed hypothalamic cells measured by qPCR (n = 3). Values and error bars represent the mean ± SEM. ***p<0.001 indicates statistically significant differences between si-PGC-1α and si-scrambled control viruses. # p<0.05; ### p<0.001 indicate statistically significant differences between fed and fasted conditions.

Figure S3

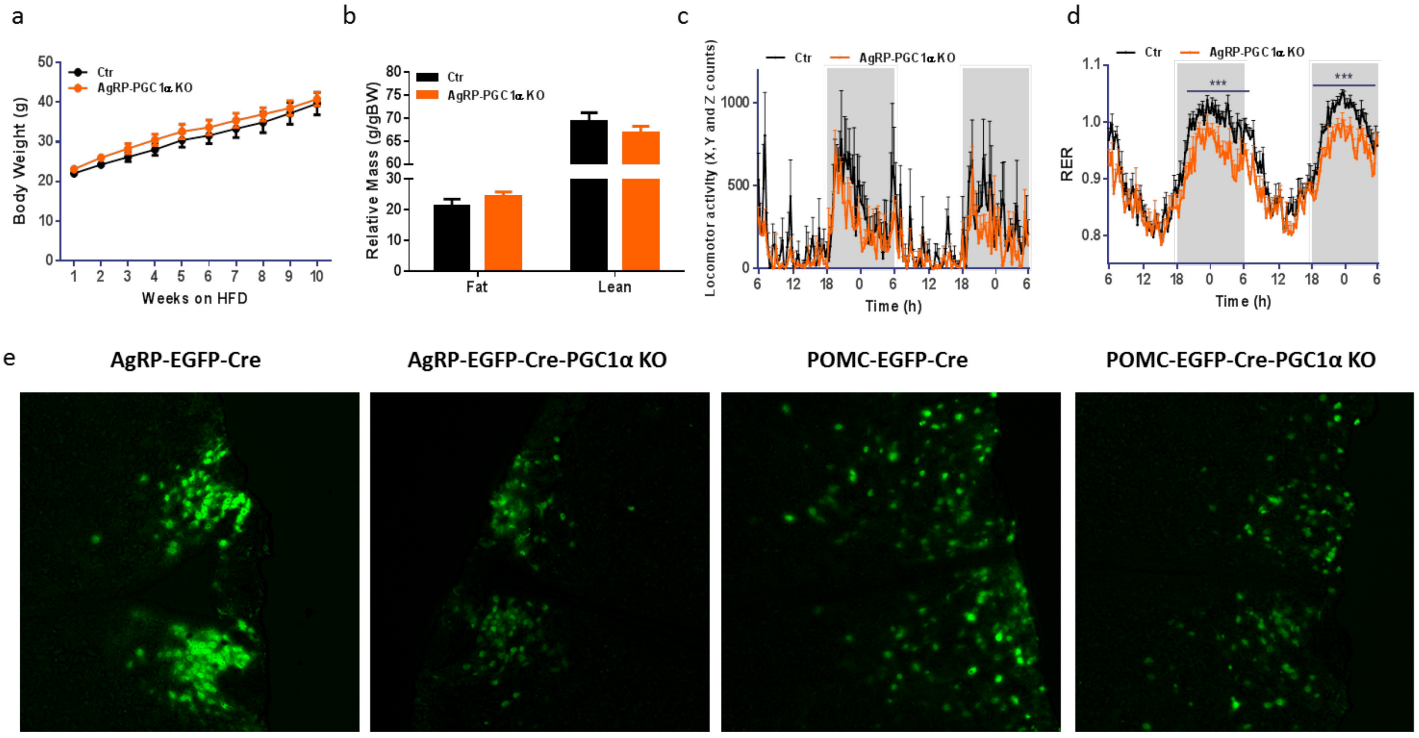

a
b
c
d
Ctr
AgRP-PGC1α KO
Body Weight (g)
Weeks on HFD
Relative Mass (g/gBW)
Fat
Lean
Locomotor activity (X, Y and Z counts)
Time (h)
RER
***
e
AgRP-EGFP-Cre
AgRP-EGFP-Cre-PGC1α KO
POMC-EGFP-Cre
POMC-EGFP-Cre-PGC1α KO

Figure S3 PGC-1α deletion in AgRP-/POMC-neurons. (a and b) Body weight curve on high fat diet (HFD) and relative body composition after 8 weeks of HFD in AgRP-PGC1α KO and ctr mice (n=4-6). **(c and d)** Time course representation of AgRP-PGC1α KO and ctr mice locomotor activity and RER shown in figure 4 (n=7-8). **(e)** Expression of EGFP under AgRP/POMC promoters in AGRP/POMC neurons of ctr and AgRP/POMC-PGC-1α KO mice. Values and error bars represent the mean ± SEM. ***p<0.001

G. References

Andrews, Z. B. (2011). "Central mechanisms involved in the orexigenic actions of ghrelin." Peptides **32**(11): 2248-2255.

Andrews, Z. B., Z. W. Liu, N. Walllingford, D. M. Erion, E. Borok, J. M. Friedman, M. H. Tschop, M. Shanabrough, G. Cline, G. I. Shulman, A. Coppola, X. B. Gao, T. L. Horvath and S. Diano (2008). "UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals." Nature **454**(7206): 846-851.

Berglund, E. D., C. Liu, J. W. Sohn, T. Liu, M. H. Kim, C. E. Lee, C. R. Vianna, K. W. Williams, Y. Xu and J. K. Elmquist (2013). "Serotonin 2C receptors in pro-opiomelanocortin neurons regulate energy and glucose homeostasis." The Journal of clinical investigation **123**(12): 5061-5070.

Betley, J. N., S. Xu, Z. F. Cao, R. Gong, C. J. Magnus, Y. Yu and S. M. Sternson (2015). "Neurons for hunger and thirst transmit a negative-valence teaching signal." Nature **521**(7551): 180-185.

Claret, M., M. A. Smith, R. L. Batterham, C. Selman, A. I. Choudhury, L. G. Fryer, M. Clements, H. Al-Qassab, H. Heffron, A. W. Xu, J. R. Speakman, G. S. Barsh, B. Viollet, S. Vaulont, M. L. Ashford, D. Carling and D. J. Withers (2007). "AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons." The Journal of clinical investigation **117**(8): 2325-2336.

Coll, A. P., I. S. Farooqi and S. O'Rahilly (2007). "The hormonal control of food intake." Cell **129**(2): 251-262.

Cone, R. D., M. A. Cowley, A. A. Butler, W. Fan, D. L. Marks and M. J. Low (2001). "The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis." International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity **25 Suppl 5**: S63-67.

Coppari, R., G. Ramadori and J. K. Elmquist (2009). "The role of transcriptional regulators in central control of appetite and body weight." Nature clinical practice. Endocrinology & metabolism **5**(3): 160-166.

Dieguez, C., M. J. Vazquez, A. Romero, M. Lopez and R. Nogueiras (2011). "Hypothalamic control of lipid metabolism: focus on leptin, ghrelin and melanocortins." Neuroendocrinology **94**(1): 1-11.

Dietrich, M. O., C. Antunes, G. Geliang, Z. W. Liu, E. Borok, Y. Nie, A. W. Xu, D. O. Souza, Q. Gao, S. Diano, X. B. Gao and T. L. Horvath (2010). "Agrp neurons mediate Sirt1's action on the melanocortin system and energy balance: roles for Sirt1 in neuronal firing and synaptic plasticity." The Journal of neuroscience : the official journal of the Society for Neuroscience **30**(35): 11815-11825.

Dietrich, M. O., J. Bober, J. G. Ferreira, L. A. Tellez, Y. S. Mineur, D. O. Souza, X. B. Gao, M. R. Picciotto, I. Araujo, Z. W. Liu and T. L. Horvath (2012). "AgRP neurons regulate development of dopamine neuronal plasticity and nonfood-associated behaviors." Nature neuroscience **15**(8): 1108-1110.

Dietrich, M. O., Z. W. Liu and T. L. Horvath (2013). "Mitochondrial dynamics controlled by mitofusins regulate Agrp neuronal activity and diet-induced obesity." Cell **155**(1): 188-199.

Dietrich, M. O., M. R. Zimmer, J. Bober and T. L. Horvath (2015). "Hypothalamic Agrp neurons drive stereotypic behaviors beyond feeding." Cell **160**(6): 1222-1232.

Donato, J., Jr. (2012). "The central nervous system as a promising target to treat diabetes mellitus." Current topics in medicinal chemistry **12**(19): 2070-2081.

Draper, S., M. Kirigiti, M. Glavas, B. Grayson, C. N. Chong, B. Jiang, M. S. Smith, L. M. Zeltser and K. L. Grove (2010). "Differential gene expression between neuropeptide Y expressing neurons of the dorsomedial nucleus of the hypothalamus and the arcuate nucleus: microarray analysis study." Brain research **1350**: 139-150.

Egger, A., M. Samardzija, V. Sothilingam, N. Tanimoto, C. Lange, S. Salatino, L. Fang, M. Garcia-Garrido, S. Beck, M. J. Okoniewski, A. Neutzner, M. W. Seeliger, C. Grimm and C. Handschin (2012). "PGC-1alpha determines light damage susceptibility of the murine retina." PloS one **7**(2): e31272.

El-Haschimi, K., D. D. Pierroz, S. M. Hileman, C. Bjorbaek and J. S. Flier (2000). "Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity." J Clin Invest **105**(12): 1827-1832.

Gerhart-Hines, Z., J. T. Rodgers, O. Bare, C. Lerin, S. H. Kim, R. Mostoslavsky, F. W. Alt, Z. Wu and P. Puigserver (2007). "Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha." EMBO J **26**(7): 1913-1923.

Handschin, C., S. Chin, P. Li, F. Liu, E. Maratos-Flier, N. K. Lebrasseur, Z. Yan and B. M. Spiegelman (2007). "Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals." J Biol Chem **282**(41): 30014-30021.

Henry, F. E., K. Sugino, A. Tozer, T. Branco and S. M. Sternson (2015). "Cell type-specific transcriptomics of hypothalamic energy-sensing neuron responses to weight-loss." eLife **4**.

Huang, H., S. H. Lee, C. P. Ye, I. S. Lima, B. C. Oh, B. B. Lowell, J. M. Zabolotny and Y. B. Kim (2013). "ROCK1 in AgRP Neurons Regulates Energy Expenditure and Locomotor Activity in Male Mice." Endocrinology **154**(10): 3660-3670.

Jager, S., C. Handschin, J. St-Pierre and B. M. Spiegelman (2007). "AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha." Proc Natl Acad Sci U S A **104**(29): 12017-12022.

Joly-Amado, A., C. Cansell, R. G. Denis, A. S. Delbes, J. Castel, S. Martinez and S. Luquet (2014). "The hypothalamic arcuate nucleus and the control of peripheral substrates." Best practice & research. Clinical endocrinology & metabolism **28**(5): 725-737.

Joly-Amado, A., R. G. Denis, J. Castel, A. Lacombe, C. Cansell, C. Rouch, N. Kassis, J. Dairou, P. D. Cani, R. Ventura-Clapier, A. Prola, M. Flamment, F. Foufelle, C. Magnan and S. Luquet (2012). "Hypothalamic AgRP-neurons control peripheral substrate utilization and nutrient partitioning." EMBO J **31**(22): 4276-4288.

Kitamura, T., Y. Feng, Y. I. Kitamura, S. C. Chua, Jr., A. W. Xu, G. S. Barsh, L. Rossetti and D. Accili (2006). "Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake." Nature medicine **12**(5): 534-540.

Lee, J. H., D. R. Reed and R. A. Price (2001). "Leptin resistance is associated with extreme obesity and aggregates in families." Int J Obesity **25**(10): 1471-1473.

Lin, J., H. Wu, P. T. Tarr, C. Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby and B. M. Spiegelman (2002). "Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres." Nature **418**(6899): 797-801.

Lin, J., P. H. Wu, P. T. Tarr, K. S. Lindenberg, J. St-Pierre, C. Y. Zhang, V. K. Mootha, S. Jager, C. R. Vianna, R. M. Reznick, L. Cui, M. Manieri, M. X. Donovan, Z. Wu, M. P. Cooper, M. C. Fan, L. M. Rohas, A. M. Zavacki, S. Cinti, G. I. Shulman, B. B. Lowell, D. Krainc and B. M. Spiegelman (2004). "Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice." Cell **119**(1): 121-135.

Lin, S., T. C. Thomas, L. H. Storlien and X. F. Huang (2000). "Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice." Int J Obesity **24**(5): 639-646.

Luquet, S., F. A. Perez, T. S. Hnasko and R. D. Palmiter (2005). "NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates." Science **310**(5748): 683-685.

Ma, D., S. Li, E. K. Lucas, R. M. Cowell and J. D. Lin (2010). "Neuronal inactivation of peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) protects mice from diet-induced obesity and leads to degenerative lesions." The Journal of biological chemistry **285**(50): 39087-39095.

Martinez-Redondo, V., A. T. Pettersson and J. L. Ruas (2015). "The hitchhiker's guide to PGC-1alpha isoform structure and biological functions." Diabetologia **58**(9): 1969-1977.

Mesaros, A., S. B. Koralov, E. Rother, F. T. Wunderlich, M. B. Ernst, G. S. Barsh, K. Rajewsky and J. C. Bruning (2008). "Activation of Stat3 signaling in AgRP neurons promotes locomotor activity." Cell Metab **7**(3): 236-248.

Morselli, E., E. Fuente-Martin, B. Finan, M. Kim, A. Frank, C. Garcia-Caceres, C. R. Navas, R. Gordillo, M. Neinast, S. P. Kalainayakan, D. L. Li, Y. Gao, C. X. Yi, L. Hahner, B. F. Palmer, M. H. Tschop and D. J. Clegg (2014). "Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha." Cell Rep **9**(2): 633-645.

Mountjoy, P. D. and G. A. Rutter (2007). "Glucose sensing by hypothalamic neurones and pancreatic islet cells: AMPle evidence for common mechanisms?" Experimental physiology **92**(2): 311-319.

Nasrallah, C. M. and T. L. Horvath (2014). "Mitochondrial dynamics in the central regulation of metabolism." Nature reviews. Endocrinology **10**(11): 650-658.

Neary, N. M., C. J. Small and S. R. Bloom (2003). "Gut and mind." Gut **52**(7): 918-921.

Padilla, S. L., J. S. Carmody and L. M. Zeltser (2010). "Pomc-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits." Nature medicine **16**(4): 403-405.

Puigserver, P., J. Rhee, J. Donovan, C. J. Walkey, J. C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili and B. M. Spiegelman (2003). "Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction." Nature **423**(6939): 550-555.

Puigserver, P., Z. Wu, C. W. Park, R. Graves, M. Wright and B. M. Spiegelman (1998). "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis." Cell **92**(6): 829-839.

Ren, H., I. J. Orozco, Y. Su, S. Suyama, R. Gutierrez-Juarez, T. L. Horvath, S. L. Wardlaw, L. Plum, O. Arancio and D. Accili (2012). "FoxO1 target Gpr17 activates AgRP neurons to regulate food intake." Cell **149**(6): 1314-1326.

Rodgers, J. T., C. Lerin, W. Haas, S. P. Gygi, B. M. Spiegelman and P. Puigserver (2005). "Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1." Nature **434**(7029): 113-118.

Ruan, H. B., M. O. Dietrich, Z. W. Liu, M. R. Zimmer, M. D. Li, J. P. Singh, K. Zhang, R. Yin, J. Wu, T. L. Horvath and X. Yang (2014). "O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat." Cell **159**(2): 306-317.

Sandoval, D., D. Cota and R. J. Seeley (2008). "The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation." Annual review of physiology **70**: 513-535.

Schneeberger, M., M. O. Dietrich, D. Sebastian, M. Imbernon, C. Castano, A. Garcia, Y. Esteban, A. Gonzalez-Franquesa, I. C. Rodriguez, A. Bortolozzi, P. M. Garcia-Roves, R. Gomis, R. Nogueiras, T. L. Horvath, A. Zorzano and M. Claret (2013). "Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance." Cell **155**(1): 172-187.

Shadel, G. S. and T. L. Horvath (2015). "Mitochondrial ROS Signaling in Organismal Homeostasis." Cell **163**(3): 560-569.

Solomon, S. (1999). "POMC-derived peptides and their biological action." Ann N Y Acad Sci **885**: 22-40.

Stanley, S., K. Wynne, B. McGowan and S. Bloom (2005). "Hormonal regulation of food intake." Physiological reviews **85**(4): 1131-1158.

Varela, L. and T. L. Horvath (2012). "Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis." EMBO Rep **13**(12): 1079-1086.

Wang, Q., C. Liu, A. Uchida, J. C. Chuang, A. Walker, T. Liu, S. Osborne-Lawrence, B. L. Mason, C. Mosher, E. D. Berglund, J. K. Elmquist and J. M. Zigman (2014). "Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin." Molecular metabolism **3**(1): 64-72.

Wareski, P., A. Vaarmann, V. Choubey, D. Safiulina, J. Liiv, M. Kuum and A. Kaasik (2009). "PGC-1{alpha} and PGC-1{beta} regulate mitochondrial density in neurons." The Journal of biological chemistry **284**(32): 21379-21385.

Woods, S. C. (2009). "The control of food intake: behavioral versus molecular perspectives." Cell metabolism **9**(6): 489-498.

III. Manuscript 2: Modulation of skeletal muscle aging through PGC-1α-controlled mitochondrial calcium metabolism and cell death

Jonathan F. Gill[1], Julien Delezie[1], Gesa Santos[1], Shawn McGuirk[2], Svenia Schnyder[1] Julie St-Pierre[2], and Christoph Handschin[1]

[1]Biozentrum, Division of Pharmacology/Neurobiology, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

[2]Department of Biochemistry, Rosalind and Morris Goodman Cancer Centre, McGill University, 3655 promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6

This manuscript is in submission

A. Abstract

Age-related impairment of muscle function severely impacts the health of a growing elderly population. While mitochondrial dysfunction correlates with muscle aging, the underlying pathological mechanisms remain poorly understood. Using mouse models with overexpressed or abolished peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) expression in skeletal muscle, we investigated the impact of modulation of this key mitochondrial function regulator on age-associated skeletal muscle plasticity. We show that in addition to preserving oxidative metabolism, PGC-1α also regulates mitochondrial calcium buffering and SR interaction during aging. As a consequence, the formation of tubular aggregates is reduced and age- and ceramide-induced muscle cell death blunted by PGC-1α in muscle. Collectively, our data highlight the important contribution of PGC-1α-dependent mitochondrial calcium buffering, ER stress and apoptosis to the aging process in this tissue.

B. Introduction

Muscle strength and mass progressively decline with age, leading to physical disability, and ultimately higher morbidity and mortality **(Szulc, Beck et al. 2005, Hirani, Blyth et al. 2015)**. Although the cause of muscle aging is multifactorial **(Walston 2012)**, reduced mitochondrial function is a commonly observed epiphenomenon associated with muscle deterioration during aging **(Short, Bigelow et al. 2005, Peterson, Johannsen et al. 2012)**. For example, a reduction in mitochondrial biogenesis **(Vina, Gomez-Cabrera et al. 2009)**, decreased mitochondrial mass **(Ji and Kang 2015)** and aberrant fission to fusion rates **(Iqbal, Ostojic et al. 2013)** concomitant with a mitochondrial turnover drop **(Gaziev, Abdullaev et al. 2014)** have been observed in muscle aging. Together with increased mitochondrial DNA damage, depolarized and swollen mitochondria **(Short, Bigelow et al. 2005)**, diminished rates of oxidative phosphorylation (OXPHOS) as well as Krebs cycle activity **(Short, Bigelow et al. 2005, Wenz, Rossi et al. 2009, Kang, Chung et al. 2013, Ji and Kang 2015)**, these pathological changes collectively lead to reduced mitochondrial respiration and lower ATP synthesis **(Short, Bigelow et al. 2005)**. Consequently, reduced ATP production **(Wanagat, Cao et al. 2001)**, impaired calcium homeostasis **(Doonan, Chandramoorthy et al. 2014)**, elevated apoptosis **(Wang and Youle 2009)** or increased levels of reactive oxygen species (ROS) **(Harman 2006)** represent key cellular alterations that might be triggered by age-associated mitochondrial impairments and that could all contribute to age-associated muscle dysfunction.

Importantly, mitochondria, together with the sarcoplasmic reticulum (SR), control cellular calcium homeostasis **(Marin, Encabo et al. 1999, Rizzuto, De Stefani et al. 2012)**. These two organelles communicate via contact sites, termed mitochondria-associated ER membranes (MAMs), focal hotspots for the synthesis and transfer of phospholipids, initiation of mitochondrial fission and mitophagy, signal transduction events, and exchange of calcium **(Marchi, Patergnani et al. 2014)**. Aging impairs mitochondria-SR association and mitochondrial calcium uptake **(Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**, which can contribute to a dysregulation of cellular calcium homeostasis **(Duchen 2000)**. All

three structures, mitochondria, MAMs and the SR, have been linked to the initiation of cell death in general **(Grimm 2012)** and increased apoptosis levels in old muscle **(Dirks and Leeuwenburgh 2002, Dirks and Leeuwenburgh 2004, Whitman, Wacker et al. 2005)**. For example, apoptosis can be initiated in mitochondria via caspase-dependent and -independent pathways **(Danial and Korsmeyer 2004)**, both of which are increased with age **(Danial and Korsmeyer 2004, Dirks and Leeuwenburgh 2004, Siu, Pistilli et al. 2005)**. In addition, the rise of mitochondrial and nuclear DNA damage due to increased level of ROS also contributes to age-related muscle apoptosis **(Harman 2006)**. Finally, endoplasmic reticulum stress is also a strong promoter of apoptotic events **(Szegezdi, Logue et al. 2006)**.

Important regulators of mitochondrial biogenesis and function, most notably the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), are reduced in skeletal muscle in the aging process and have been linked to the decrease in mitochondrial function **(Ling, Poulsen et al. 2004)**. Importantly, this deterioration can partly be restored by exercise **(Kang, Chung et al. 2013)**. Likewise, transgenic overexpression of PGC-1α reduces sarcopenia and other skeletal muscle dysfunctions in the mouse **(Wenz, Rossi et al. 2009)** and extends lifespan in the drosophila **(Rera, Bahadorani et al. 2011)**. Inversely, muscle-specific PGC-1α knockout animals suffer from exacerbated age-related glucose intolerance and systemic inflammation **(Sczelecki, Besse-Patin et al. 2014)**. It is however unclear how modulation of PGC-1α affects skeletal muscle function in aging. We now show that muscle PGC-1α regulates and dramatically improves mitochondrial and cellular calcium handling, SR stress, and prevents tubular aggregates formation as well as cell death in the old muscle.

C. Experimental procedures

Animals

Mice with muscle specific PGC-1α deletion (mKO-PGC-1α) or overexpression (mTg-PGC-1α) driven by the human alpha-skeletal actin and muscle creatine kinase promoters, respectively, were previously described **(Lin, Wu et al. 2002, Handschin, Choi et al. 2007)**. C57/Bl6 wild-type (WT) mice were obtained from Janvier (Janvier sas, cs 4105, le genest St-isle f-53941, St Berthevin Cedex). Only male mice were studied at 3, 12 and 24 month-old (3Mo 12Mo and 24Mo). Animals were kept under a 12h/12h light-dark cycle with lights on from 06:00 to 18:00 in humidity-controlled rooms at 23°C. All animals had free access to regular chow diet (Provimi Kliba 3432) and water. All experiments were performed in accordance with the federal guidelines for animal experimentation and were approved by the Kantonales Veterinäramt of the Kanton Basel-Stadt.

Mouse muscle preparation

Mice were sacrificed by CO2 inhalation. Muscles were directly harvested and either snap-frozen in liquid nitrogen for protein and RNA extraction, frozen in cooled-isopentane and embedded in 7% tragacanth for cryosection staining, immediately used for determination of respiration or calcium uptake in isolated mitochondria or fixed for electron microcopy.

2.3 Genomic DNA/RNA extraction and qPCR

- Genomic DNA

For genomic DNA extraction, crushed gastrocnemius muscles were shaken overnight at 55° in 600 µl lysis buffer (10mM TrisHCl, 1mM EDTA, 0.1% SDS 5% Proteinase). Lysates were centrifuge at 8000g for 15 min at room temperature and supernatants were transferred in fresh tubes. Residual RNA was removed by incubation with RNase A (20mg/ml) for 30 min at 37°C under constant agitation. RNase A was inactivated for 10 min at 95°C and samples were cooled-

down to room temperature. One volume of Phenol/Chloroform/Isoamylalcohol 25:24:1 was added and samples were centrifuged at 8000g for 15 min at room temperature. The aqueous phase was transferred to a new tube before addition of one volume of chloroform. The centrifugation step was repeated and the new aqueous phase was transferred again to a new tube where one volume of isopropanol containing 0.3M of sodium acetate was added. Samples were gently mixed and placed at -20°C for 30 min. DNA was recovered by centrifugation at 8000g for 15 min at 4°C. DNA pellets were washed 2 times with 1ml of ice-cold 75% ethanol, dried during few minutes and finally resuspended in ddH2O. An amount of 0.1ug of gDNA was used for qPCR.

- RNA extraction and qPCR

Total RNA was isolated from powdered muscles using lysing matrix tubes (MP Biomedicals 6913-500) and TRI Reagent (Sigma-Aldrich T9424). Total RNA from cells using the Direct-zol RNA MiniPrep kit (Zymo Research R2050 according to the manufacturer's instructions). After treatment with RNase-free DNase (Invitrogen 18068-015), one microgram of RNA was used for reverse transcription using the SuperScript II reverse transcriptase (Invitrogen 18064-014).

The level of relative genomic DNA or mRNA was quantified by real-time PCR on a Light Cycler 480 system (Roche Diagnostics) using FastStart essential DNA probe master mix (Roche Diagnostics 06402682001). Relative quantification of mRNA for gene expression comparison was performed with the ΔΔCT method using the TATA binding protein (TBP) gene as reference. The quantification of mitochondrial DNA copy number was done using the same method by normalizing the average of COX1 ATP6 and ND1 DNA copy number by the average copy number of the nuclear genes beta globin and 34B6. Beta globin and TBP levels were similar between genotypes in a given experimental condition. Primers sequences are listed in supplemental table 1.

Histology

Tragacan-embedded muscles were cut to 8 µm sections with a cryostat (Leica, CM1950). H&E staining was performed as described in the DMD_M.1.2.007 SOP (http://www.treat-nmd.eu/downloads). For SDH staining, sections were incubated in SDH buffer (phosphatbuffer 0,035M, Na-Succinate 0.1M, teranitro blue trezolium salt 0.1%, phenazin methosulfat 0.1%), rinsed with water, fixed with 4% formalin and rinsed with water again before being mounted with CC/Mount (Sigma, C9368). For calsequestrin 1 labelling, cryosections were fixed 20 min with 4%PFA in PBS (Sigma-Aldrich, D8537) at room temperature. Sections were blocked in PBS supplemented with 0.4% Triton X-100 (Sigma-Aldrich, 93426), 3% Goat Serum (Sigma-Aldrich, G9023), 1% bovine serum albumin (BSA) (Sigma-Aldrich, A9418). Sections were blocked for 30 min at room temperature and incubated with primary and secondary antibodies for one hour at room temperature. 3 washes of 5 min in PBS were performed before and after antibodies incubation. Primary and secondary antibodies were diluted in blocking solution. Calsequestrin 1 (ThermoScientific, MA3-913) and laminin (Sigma, L9393) antibodies dilutions were 1/250 and 1/5000 respectively. AlexaFluor 488 (Life Technology, A-11008) and 647 (A-21242) secondary antibodies dilutions were 1/500 and 1/250 respectively. Labelled sections were mounted with ProLong Gold Antifade reagent with dapi (Life Technologies, P36931). Images of calsequestrin 1 immunolabellings and H&E stainings were taken at the imaging core facility of the Biozentrum with the FEI MORE microscope using a 20x or 40X magnification lens with the same acquisition settings. Numbers of tubular aggregates were quantified in all fibers of each stained muscles using ImageJ software with unaltered images. Representative images were however adjusted for brightness and contrast.

Protein extraction and Western Blot

Total proteins were extracted from quadriceps muscles as described in **(Perez-Schindler, Summermatter et al. 2013)**. Total proteins from C2C12 cells were extracted after an ice-cold PBS wash following the same procedure. Equal amounts of protein were separated on mini-TGX 4-20% stain free pre-cast gel (Biorad, 4568096). Proteins were labelled with trihalo compounds

of the stain-free gel by exposing the gel to UV for 1 min. Gel and nitrocellulose membranes were equilibrated for 5 min in transfer buffer and protein were transferred on the nitrocellulose membrane during 1h under a constant voltage of 100V. Membranes were then blocked 1 h at room temperature with 5% milk or BSA diluted in TBS-T and washed 2 times 5 min with TBS-T. Proteins of interest were then labelled overnight at 4°C with primary antibodies diluted in TBS-T containing 0.02% sodium azide and either 3% milk or 3% BSA. Membranes were washed three times 5 min with TBS-T. Membranes were then incubated 1 h at room temperature with peroxidase-conjugated secondary antibodies diluted in TBS-T containing 3% milk or BSA. Membranes were then washed 3 times for a total of 15 min. Antibody binding was revealed using enhanced chemiluminescence HRP substrate detection kits and imaged using a fusion FX imager. Proteins of interest were normalized against total protein content determined using the trihalo compounds labeling. Two reference samples were loaded in the different gels for inter-gel normalization. Quantification of proteins was done with fusion FX software. Blocking and antibodies solutions, antibodies information and dilutions and ECL detection kits are described for each protein of interest in supplemental table 2. All primary antibodies were diluted at 1/1000 except Calsequestrin 1 antibody that was diluted at 1/3000. All secondary antibodies were diluted at 1/10000.

Transmission electronic microscopy

Samples were prepared for transmission electronic microscopy as in **(Arnold, Gill et al. 2014)**. Volume density of mitochondria was calculated according to methods previously described by Weibel and digitally adapted to Adobe Photoshop **(Baranska, Baran et al. 1997)**, in micrographs taken from transversal sections. Volume density is classically defined as the ratio of test points residing within mitochondria and the total amount of test points within the field of view. Mitochondria were identified and outlined manually and, using the Measurement tool in Photoshop, the number of pixels in each image contained in each mitochondrion were counted and compared to the total number of pixels in the image. In total, 5 images were quantified per block of stained muscle tissue, for a total of 5 blocks per mouse.

Mitochondrial respiration assay

Quadriceps muscles were quickly rinsed in PBS and in PBS with 10 mM EDTA and finely minced in Petri dishes filled with 2ml of isolation buffer 1 (EDTA 10 mM, D-mannitol 215 mM, sucrose (0.075M), free-fatty acid BSA (Sigma-Aldrich) 0.1%, HEPES 2 mM pH 7.4 in distilled water). Muscle solutions were transferred in Potter-Elvehjem grinders for homogenization with manual pestle. Homogenates were centrifuged for 10 min at 700g. Supernatants were transferred and centrifuge for 10 min at 10500g. Pellets were resuspended in 500µl of isolation buffer 2 (EGTA 3 mM, D-mannitol 215 mM, sucrose 0.075M, free-fatty acid BSA (Sigma-Aldrich) 0.1%, HEPES 2 mM pH 7.4 in distilled water), centrifuged again at 10500g for 10 min and finally resuspended in 100 µl of isolation buffer 2. Mitochondrial protein concentrations were determined by Bradford assay. Mitochondrial proteins were diluted in a 37°C warm mitochondrial assay buffer (MgCl2 5 mM, D-Mannitol 220 mM, KH2PO4 10mM, EGTA 1 mM, free-fatty acid BSA 0.2%, HEPES 2 mM, sucrose 70 mM pH 7.0 in distilled water) and 1µg was loaded in a 96-well plate and centrifuged at 2000g for 20 min. After centrifugation 135 µl of mitochondrial assay buffer completed with 20mM succinate and 2µM rotenone were gently added to each well and the plate was warmed up to 37°C for 10 min. The plate was then loaded into a calibrated Seahorse XF96 Extracellular Flux Analyzer for mitochondrial respiration assay using the mito stress test kit (Seahorse Bioscience, # 103015-100). Mitochondrial respiration assay consisted in equilibration, 1 min mixing, 3 min pause, 1 min mixing, 3 min waiting, 0.5 min mix, 3 min measure, 1 min mixing, 3 min measure, 0.5 min mixing, injection of 4 mM ADP, 1 min mixing, 3 min measure, 1 min mixing, injection of 3.125 µM Oligomycin, 0.5 min mixing, 3 min measure, 1 min mixing, injection of 4 µM FCCP, 0.5 min mixing, 3 min measure, 1 min mixing, injection of 4 µM Antimycin, 0.5 min mixing, 3 min measure. All steps before plate loading were performed at 4°C.

Mitochondrial calcium uptake assay

Quadriceps, tibialis anterior (TA) and EDL muscles of both legs from one mouse were pooled together, rinsed 2 times with PBS and minced finely with scissors in 0.5 ml of

mitochondrial isotonic buffer (Mannitol 225 mM, Sucrose 75 mM, MOPS 5 mM, EGTA 0.5 mM, Taurine 2 mM, pH 7.25). The resulting muscle homogenates were incubated 3 min in 10ml of mitochondrial isotonic buffer containing nargase at 0.1 mg/ml (Sigma-Aldrich, P8038) before the addition of BSA at 0.2%. Muscles solutions were then transferred in Potter-Elvehjem grinders and homogenized with manual pestles. Homogenates were centrifuged for 6 min at 1200g for 2 times, discarding fat and cellular debris between centrifugations. Obtained supernatants were centrifuged for 10 min at 9000g. Residual fat was discarded and mitochondrial pellets were washed with 15ml of mitochondrial isolation buffer before a second centrifugation at 9000g for 10 min. Final mitochondrial pellets were gently resuspended in a final volume of 150 µl of mitochondrial isotonic buffer. Mitochondrial protein concentrations were determined by Bradford method and 275 µg of mitochondria were centrifuged and gently resuspended in 100 µl of mitochondrial calcium assay buffer (KCl 120 mM, Tris 10 mM, MOPS 5 mM, K2HPO4 5 mM, pH 7.4). 100 µl of calcium buffer containing 5 µM of the fluorescent calcium indicator calcium geen 5N (molecular probes, C3737) was loaded in a 96-well black plate (Nunc) and baseline fluorescence was measured using a Tecan infinite M1000 multiplate reader. When the signal was stable, mitochondrial preparations were added and calcium green 5N fluorescence was continuously recorded during subsequent addition of calcium.

C2C12 growth, differentiation and viral infection

C2C12 myoblasts were cultured Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich D 5796), supplemented with 10% fetal bovine serum (HyClone Laboratories, Inc., Logan, UT), 4.5 mg/ml glucose and 1% penicillin/streptomycin and were incubated at 37 °C with 5% CO2. Myotube differentiation was achieved by incubation of 95% confluent myoblast in differentiation medium consisting of DMEM containing 2% horse serum, 4.5 mg/ml glucose and 1% penicillin/streptomycin during 4 days. PGC-1α overexpression experiments were done using adenoviral vectors expressing the green fluorescent protein (GFP) alone or bicistronic GFP-PGC-1α. GFP was used to monitor infection efficiency. Infection was initiated in 50% confluent myoblast or in myotubes after 4 days of differentiation. Knock-down of estrogen related

receptor α (ERRα) combined with PGC-1α overexpression was achieved in C2C12 myoblasts by infecting cells with adenoviral vectors containing specific short hairpin RNA (shRNA) sequences against ERRα (shERRα) or Lacz (shLacz) simultaneously with viruses used to study PGC-1α upregulation.

For gene and protein expression experiments, 2 days after infection, myoblast or myotubes were treated during 8 hours with 100 µM ceramide (Sigma-Aldrich 01912) or 0.1% DMSO diluted in growth or differentiation medium respectively. Pictures of myotubes after the treatment were taken using Leica DMI4000B microscope. For cell death assay, myoblast were treated with 0.1% DMSO or with 50 and 100 µM ceramide during 8 hours. Treatment medium was then exchanged for growth medium containing 5 µM of propidium ioide. After an incubation of 30 min at 37°C with 5% CO2, propidium ioide incorporation reflecting cell death was measured using a tecan infinite M1000 multiplate reader.

Treadmill experiment and spontaneous locomotor activity

To assess locomotor performance, mice ran in an open treadmill (Columbus instrument). Mice were acclimatized to the treadmill for 5 min at 8 m/min followed by 5 min at 10 m/min, at an incline of 5° during two consecutive days. After one resting day, an exhaustion test was performed at an inclination of 5° and a starting running speed of 4.8 m/min which was then increased by 1.6 m/min every 3 min until a maximum speed of 29 m/min. Maximal running speed were recorded when exhaustion was reached. Blood lactate levels were measured from the tail vein before and 10 min after the endurance test with a lactate plus meter (Nova biomedical).

Spontaneous locomotor activity was recorded by counting number of beam breaks in an indirect calorimetric system (CLAMS, Columbus Instruments) in 15 min intervals. Data were analyzed after one day of acclimatization for 2 days.

Statistical analysis

Data were analyzed with two-way ANOVA (GraphPad Prism software). Sidak post-tests were used to do multiple comparison analysis following two-way ANOVA. All data are plotted as mean ± SEM.

D. Results

Muscle PGC-1α overexpression prevents aged-related mitochondrial and muscle dysfunction

To assess the impact of aging on muscle PGC-1α and mitochondrial function, we first measured PGC-1α mRNA levels in different muscles of young (3 month-old) and aged (24 month-old) WT, mKO-PGC-1α and mTg-PGC-1α mice. We observed that aging reduced PGC-1α gene expression by approximately 90% in soleus, 50% in TA and 25% in gastrocnemius muscles of WT animals (Fig. 1a).

OXPHOS transcripts were not affected by aging, but strongly controlled by gain- and loss-of-function of PGC-1α (Fig. S1a). Importantly however, despite the lack of a discernible effect on transcript levels, aging dramatically reduced mitochondrial protein content in WT muscles (Fig. 1b). Absence of PGC-1α in muscles of young mKO-PGC-1α animals mimicked while muscle-specific PGC-1α overexpression fully prevented this reduction of mitochondrial OXPHOS proteins in aged muscle tissues.

Consistent with the diminished OXPHOS protein levels, mitochondrial respiration, ATP production and mitochondrial maximal respiration capacities were remarkably reduced with age in muscles of WT mice, but fully preserved in mTg-PGC-1α mice (Fig. 1c). Similarly, while mitochondria biogenesis gene expression and mitochondrial mass were not altered by age or PGC-1α deletion, there were both significantly increased by PGC-1α overexpression (Fig. 1d and S1b and c).

Correlating with their preserved mitochondrial function, elevated levels of muscle PGC-1α protected mTg-PGC-1α mice against the age-related decrease in spontaneous locomotor activity and maximal running capacity (Fig. 1e and f). Blood lactate measurements indicated that old WT and young mKO-PGC-1α animals exhibit higher lactate production post-exercise, while this shift was severely blunted in the mTg-PGC-1α mice (Fig. 1g). An impairment in lipid

oxidation was further suggested by the downregulation of the muscle expression of genes involved in mitochondrial fatty acid β-oxidation, such as carnitine palmitoyltransferase 1B (Cpt1b), medium-chain acyl-CoA dehydrogenase (Mcad), and uncoupling protein 3 (Ucp3) in aged vs. young WT and young mKO-PGC-1α mice. The expression of these genes was markedly upregulated in young and maintained in old mice overexpressing PGC-1α.

PGC-1α modulates alterations of mitochondrial dynamics and SR association in the aging muscle

To a large extent, healthy mitochondrial function is ensured by mitochondrial fission and fusion. Dysregulation of mitochondrial dynamics in aging was indicated by decreased and increased gene expression of fusion and fission genes, respectively, in WT muscles (Fig. 2a). Mimicking the aging phenotype, muscle mitofusin 1 gene expression was reduced in young mKO-PGC-1α animals relative to age-matched WT mice. In contrast, PGC-1α overexpression upregulated the expression of genes related to both mitochondrial fusion and fission. Mechanistically, our results in C2C12 cells show that overexpression of PGC-1α induces the expression of the mitofusin genes as well as dynamin-related protein 1 (Drp1), fission 1 (Fis1) and optic atrophy 1 (Opa1) only in the presence of ERRα, demonstrating that PGC-1α effects are dependent on Estrogen Related Receptor α (ERRα) expression (Fig. 2c and d). In contrast, PGC-1α-dependent induction of BCL2/Adenovirus E1B 19kDa Interacting Protein 3 (BNIP3) was not affected by the knockdown of ERRα (Fig. 2c).

In addition to regulating mitochondrial dynamics, mitofusin proteins also constitute essential elements of the MAM, linking the SR and the mitochondrial compartments. In parallel with reduced expression of mitofusins during aging, other MAM proteins such as GRP75 and voltage-dependent anion channel (VDAC) were likewise downregulated at the transcript and protein level with age (Fig. 2a and b). As for Mfn1, young mKO-PGC-1α mice already exhibited the age-related VDAC protein reduction observed in WT mice, and muscle PGC-1α overexpression abolished the decreased expression of both VDAC and GRP75 during aging. As

shown for the mitochondrial dynamic gene expression, our cell experiments indicate that the modulation of the MAM transcription by PGC-1α is ERRα dependent (Fig. 2d and e).

PGC-1α improves mitochondrial calcium buffering during aging

Intriguingly, not only OXPHOS, but also mitochondrial calcium buffering capacities are reduced in aging due to lower mitochondrial calcium uptake and impaired mitochondrial-SR association **(Fraysse, Desaphy et al. 2006)**. While it has been demonstrated that PGC-1α affects SR-controlled calcium homeostasis in skeletal muscle **(Summermatter, Thurnheer et al. 2012)**, the functional link between PGC-1α and mitochondrial calcium handling in young or old muscle is poorly understood. We therefore evaluated mitochondrial calcium buffering capacity in our mouse models. In WT muscles, aging significantly reduced the expression of genes involved in mitochondrial calcium uptake and calcium transfer from the SR, including leucine zipper and EF-hand containing transmembrane protein 1 (Letm1) and inositol 1,4,5-trisphosphate (IP3) receptor type1 (IP3R1) (Fig. 3a). Except for IP3R1, mRNA levels of mitochondrial calcium buffering genes in muscles of old mKO-PGC-1α mice were similar to levels found in old WT mice. PGC-1α upregulation abolished age-associated downregulation of IP3R1 and Letm1 and significantly upregulated all genes related to mitochondrial calcium buffering in the muscle of old animals. Importantly, VDAC is also a key protein regulating the entry of calcium and metabolites into the mitochondria in addition to its role in maintaining MAM integrity. Increased expression of both VDAC protein and mitochondrial calcium buffer genes by PGC-1α was not only observed in mouse muscle in vivo (Fig. 2b), but also in C2C12 cells, and is dependent on ERRα activity (Fig. 2d and e and Fig.3b).

To complement the analysis of transcript and protein levels, we then evaluated calcium uptake in isolated mitochondria from muscles of young mTg-PGC-1α and WT mice (Fig. 3d). We demonstrated that after injection of 130 and 150 mM of calcium, mitochondrial calcium uptake was elevated by 100% in mTg-PGC-1α mitochondria compared to WT (Fig. 3e). It is important to note that a total amount of 275 µg of mitochondria were used for calcium uptake

measurements, representing 100% of mitochondria from WT muscles but only 13% of mitochondria from muscles overexpressing PGC-1α (Fig. 3f). The increased calcium buffering capacity of the individual mTg-PGC-1α mitochondria is thus multiplied by the elevated number of total mitochondria to reflect the overall higher mitochondrial calcium buffering capacity during aging in this mouse model.

PGC-1α prevents ER stress and tubular aggregate formation in the aging muscle

Controlled calcium transfer from the ER to mitochondria is important to alleviate endoplasmic reticulum stress (ER stress) and reduce cell death **(Malhotra and Kaufman 2011)**. To test whether the modulation in mitochondrial-SR interaction and mitochondrial calcium buffering affected the SR, we studied ER stress in our conditions. Measurements of the expression of genes and proteins responding to ER stress, such as the ER stress marker XBP1 and the chaperon protein BIP, indicated that ER stress increases with age in WT and mKO-PGC-1α muscles (Fig. 4a and b). PGC-1α overexpression reduced XBP1 mRNA levels and fully abolished BIP protein upregulation during aging. Interestingly, PGC-1α muscle deletion led to an increased activation of the calcium stress marker caspase 12, which was exacerbated with age (Fig. 4b). Consistently, poly-ubiquitination of proteins, indicating proteasomal degradation of potentially misfolded proteins, was dramatically increased in mKO-PGC-1α muscles with age and reduced in both young and old muscles of mTg-PGC-1α mice, respectively (Fig. S2a). Finally, the in vivo reduction of XBP1 mRNA levels and protein poly-ubiquitination by PGC-1α were recapitulated in C2C12 cells (Fig. S2b and c).

Functional dysregulation of SR function is linked to the development of tubular aggregates of SR membranes in muscle fibers of old mice **(Agbulut, Destombes et al. 2000)**. H&E staining of the TA muscle revealed the presence of eosin-labeled structures in old WT and mKO-PGC-1α animals (Fig 4c) that were confirmed to be tubular aggregates by CSQ1 protein staining (Fig. 4d) and electron microscopy (Fig. S3a). While the age-dependent increase in WT muscles was striking, TA muscles of old mKO-PGC-1α mice exhibited a further 2 fold-elevation in

tubular aggregates compared to age-matched WT mice (Fig 4e). Strikingly, we could not detect any tubular aggregates in fast muscles of aged mTg-PGC-1α mice (Fig. 4c and e and Fig. S3a). Moreover, soleus muscles (Fig. S3b) or SDH-positive fibers of other muscle beds in old WT and mKO-PGC-1α mice were likewise devoid of tubular aggregates, suggesting a link to the metabolic fiber type (Fig. S3c). Abnormal calcium homeostasis and accumulation of calsequestrin 1 (CSQ1) and other SR proteins in skeletal muscle have been linked to the formation of tubular aggregates **(Chevessier, Marty et al. 2004, Bohm, Chevessier et al. 2013)**. Accordingly, in line with the absence of tubular aggregate formation in muscle of old mTg-PGC-1α mice, elevation of muscle PGC-1α levels fully prevented the age-related increase of CSQ1 protein observed in WT mice (Fig 4f). Cellular experiments furthermore revealed that PGC-1α acutely regulates CSQ1 protein in a cell autonomous-manner (Fig 4g). Of note, in addition to the tubular aggregates, electronic microscopy also revealed other abnormal structures in muscles fibers of aged mKO-PGC-1α and WT mice (Fig. S3d) that were absent from any of the young animals and from old mTg-PGC-1α mice.

PGC-1α alleviates cell death during aging

Dysregulated mitochondrial function, ER stress, mitochondrial-SR interaction and calcium homeostasis have all been linked to increased cell death **(Shiraishi, Tatsumi et al. 2001, Chen, Won et al. 2002, Gomez-Cabrera, Sanchis-Gomar et al. 2012)**. We therefore assessed whether age-associated apoptosis in skeletal muscle was affected by modulation of PGC-1α. P53, a major regulator of cell death induction upon DNA damage and stress conditions was massively upregulated during aging of WT and mKO-PGC-1α muscles, but not in muscle overexpressing PGC-1α (Fig. 5a). Compared to aged WT animals, muscles of old mKO-PGC-1α and mTg-PGC-1α mice exhibited a trends toward a 2-fold increased ($p=0.06$) and a 2-fold decrease ($p=0.05$), respectively, in P53 protein levels. Interestingly, mRNA levels of insulin like growth factor binding protein 5 (IGFBP5) and the p53 targets p21 and PUMA followed a similar pattern of expression (Fig. 5b). The expression of the cell survival-related gene X-linked inhibitor of apoptosis protein (XIAP) was down-regulated with age in WT and mKO-PGC-1α muscles and Cyclin A transcript expression was lower in old mKO-PGC-1α muscles relative to age-matched

WT muscles. PGC-1α significantly elevated the expression of all pro-survival genes in young, and XIAP mRNA levels in old mTg-PGC-1α mice (Fig. 5b). Caspase 3 cleavage, a main marker of cell death, was increased with age in mKO-PGC-1α mice and significantly higher in muscles of old mKO-PGC-1α mice relative to muscles of age-matched WT animals (Fig. 5c). Additionally, in old muscle tissues, the smallest cleavage product of caspase 3 was reduced by PGC-1α overexpression. Taken together, our findings suggest a protective function of muscle PGC-1α against age-induced muscle cell death.

PGC-1α protects against ceramide-induced cell death

To evaluate the direct impact of PGC-1α on cell death initiation, we used ceramide to promote cell death in C2C12 cells expressing endogenous and elevated PGC-1α levels. Ceramide acts as a second messenger for apoptosis linked to ER stress, mitochondrial impairment and dysregulation of calcium homeostasis **(Jarvis, Grant et al. 1996)**. We observed that PGC-1α markedly protected C2C12 cells against cell death induced by ceramide treatment as evaluated by microscopy and by a propidium ioide-based cell death assay (Fig. 6a and b). Similar findings have previously been reported in HeLa cells **(Bianchi, Vandecasteele et al. 2006)**. Furthermore, PGC-1α overexpression abolished the induction of p53 and the cleavage of caspase 3 proteins upon ceramide treatment, as well as the expression of the DNA damage marker phospho-H2A Histone Family Member X (pH2AX) and the ceramide-associated decrease in phosphorylation of the phosphor-Prepro-Retinoblastoma-Associated Protein (ppRB) protein in muscle cells (Fig 6c). Moreover, PGC-1α upregulation abrogated ceramide-dependent changes of gene expression related to cell death and cell survival (Fig 6d). The protective effect of PGC-1α against the deleterious outcome of ceramide exposure in muscle cells suggests an important role for this gene in the prevention of cellular death.

E. Discussion

The deterioration of mitochondrial function and PGC-1α expression in the aging skeletal muscle has been well documented **(Ling, Poulsen et al. 2004, Short, Bigelow et al. 2005, Vina, Gomez-Cabrera et al. 2009, Ghosh, Lertwattanarak et al. 2011, Johnson, Robinson et al. 2013, Kang, Chung et al. 2013)**. PGC-1α is a key regulator of mitochondrial biogenesis, dynamics and function. Moreover, the effects of this transcriptional coactivator extend to other organelles and cell structures, e.g. the unfolded protein response after exercise **(Wu, Ruas et al. 2011)** or SR-controlled calcium homeostasis **(Summermatter, Thurnheer et al. 2012)**. We now show that PGC-1α collectively controls the function and interaction between mitochondria and the SR, and thereby prevents the decline during aging. Even though the SR is the main buffering site for intramyocellular calcium, mitochondrial calcium uptake is not only important for electron transport chain function, but also contributes to resting calcium levels **(Rizzuto, De Stefani et al. 2012)**. An age-related decline in mitochondrial calcium buffering capacity therefore likely contributes to the rise of intracellular calcium level in the aging muscle **(Fraysse, Desaphy et al. 2006, Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**. The greatly increased calcium buffering capacity of mitochondria in the PGC-1α muscle-specific transgenic animals could therefore alleviate much of the stress exerted by increased intracellular calcium in the old muscle. Moreover, improved mitochondrial function and ATP production help to maintain calcium re-uptake into the SR via the ATP-dependent calcium pumps in this animal model **(Allen, Lamb et al. 2008)**. At the same time, the reduction in polyubiquinated proteins and several genes encoding ER stress mediators indicate a PGC-1α-dependent reduction of the SR burden. PGC-1α-controlled abrogation of the accumulation of both age-related Csq1 protein and tubular aggregates further illustrates the preserved calcium homeostasis and muscle protection from ER stress development by PGC-1α during aging.

Due to their calcium loading capacity **(Salviati, Pierobon-Bormioli et al. 1985)**, tubular aggregates are alternatively thought to be a compensatory mechanism to counteract age-associated cellular calcium increase, rather than a consequence of calcium metabolism

dysregulation. However, these aggregates are always observed in contexts of pathologically impaired muscle function **(Chevessier, Bauche-Godard et al. 2005, Ghosh, Narayanappa et al. 2010)**. Tubular aggregates are predominantly composed of densely small packed tubules arising from sarcoplasmic reticulum **(Chevessier, Marty et al. 2004)**. We report that PGC-1α fully protects muscle from tubular aggregate formation during muscle aging, while ablation of the PGC-1α gene in muscle markedly exacerbates tubular aggregate occurrence. Tubular aggregates are associated with both natural and premature aging in mouse **(Ludatscher, Silbermann et al. 1983, Agbulut, Destombes et al. 2000, Zhou, Freeman et al. 2013)**. In humans, tubular aggregates have been observed in several myopathies including peripheral neuropathy, amyotrophic lateral sclerosis or myotonic dystrophy **(Chevessier, Bauche-Godard et al. 2005, Ghosh, Narayanappa et al. 2010, Schiaffino 2012)**, and are the main symptom that accompanies myalgia in tubular aggregate myopathy **(Schiaffino 2012)**. Interestingly, mice with muscle PGC-1α deletion also display tubular aggregates upon muscle denervation which are absent in WT mice **(Vainshtein, Desjardins et al. 2015)**. A link between mitochondrial calcium handling and tubular aggregate formation was furthermore suggested by results obtained in young MCU1 knockout mice, which recapitulate some of the phenotype of old and PGC-1α knockout muscle, including mitochondrial dysfunctions, increased blood lactate levels, impaired muscle function and the formation of tubular aggregates **(Liu, Liu et al. 2016)**. A central role for PGC-1α in this context is finally implied by our SDH staining and electronic microscopic data as well as various other studies that revealed a strong preference of tubular aggregate formation for glycolytic fibers **(Chariot, Benbrik et al. 1993, Funk, Ceuterick-de Groote et al. 2013)**. The potent effect of PGC-1α on driving an oxidative muscle fiber shift could thus furthermore contribute to the inhibition of tubular aggregate formation. It thus would be interesting to study whether increased muscle PGC-1α prevents tubular aggregates formation in mice prematurely displaying tubular aggregates and associated myopathies **(Schubert, Sotgia et al. 2007, Zhou, Freeman et al. 2013)**.

Mitochondrial dysfunction, ATP depletion and ER stress, as observed in old WT and the mKO-PGC-1α mice, are strong promoters of cell death **(Lemasters, Qian et al. 1999, Shiraishi, Tatsumi et al. 2001, Malhotra and Kaufman 2011)**. For example, increased expression of

caspase-12 is furthermore linked to elevated apoptosis during muscle aging **(Dirks and Leeuwenburgh 2004)**. The protective effect of muscle PGC-1α on mitochondrial and SR function could thus secondarily result in reduced cell death initiation. Importantly however, the ex vivo experiments using ceramide, a pro-apoptotic stimulus that acts through induction of ER stress and mitochondrial dysfunction **(Arora, Jones et al. 1997, Liu, Xia et al. 2014)**, indicate a potent role for PGC-1α to directly decrease the induction of apoptosis. An anti-apoptotic function of PGC-1α has previously been suggested in different cell types, including the retina **(Egger, Samardzija et al. 2012)**, vascular endothelial cells **(Valle, Alvarez-Barrientos et al. 2005)**, neurons **(Luo, Zhu et al. 2009)** or skeletal muscle **(Adhihetty, Uguccioni et al. 2009, Wenz, Rossi et al. 2009)**. Our findings now provide direct evidence of a broader PGC-1α-controlled program that links functional mitochondria, SR and calcium handling to cell death regulation in muscle, which becomes compromised with decreased PGC-1α expression during aging, and ultimately leads to higher rates of myofiber apoptosis **(Wenz, Rossi et al. 2009)**. Of note, our findings on P53 and pH2AX suggest an even broader role for muscle PGC-1α in constraining DNA damage. Similar findings have been reported in vascular endothelial cells while other studies have postulated a telomere-P53-PGC-1α signaling axis to be involved in apoptosis and senescence **(Sahin, Colla et al. 2011, Xiong, Patrushev et al. 2015)**. Future studies will thus indicate whether cellular senescence is likewise affected by PGC-1α expression in old skeletal muscle.

In summary, we now describe how modulation of PGC-1α levels in young and old skeletal muscle has broad ranging consequences on the maintenance of mitochondrial and SR functions, including the coordinated control of calcium homeostasis. Intriguingly, this control is exerted in a context-specific manner: for example, while PGC-1α upregulates the unfolded protein response to deal with the acute consequences of exercise **(Wu, Ruas et al. 2011)**, increased muscle PGC-1α in old muscle alleviates the aging-related burden on the SR. Accordingly, mKO-PGC-1α mice in many regards exhibit a premature aging phenotype while preventing the decrease of muscle PGC-1α expression alleviates many age-related pathological changes. Our findings now provide an explanation for this observation, which has already been phenotypically described in the respective animal models **(Wenz, Rossi et al. 2009, Sczelecki,**

Besse-Patin et al. 2014). More importantly, since exercise is a powerful intervention to ameliorate health span in old individuals, and physical activity exerts many of the health beneficial effects through control of PGC-1α levels in this context **(Kang, Chung et al. 2013)**, our findings reveal novel avenues that could be exploited to reduce muscle weakness, frailty and other pathological alterations associated with aging.

F. Figures

Figure 1

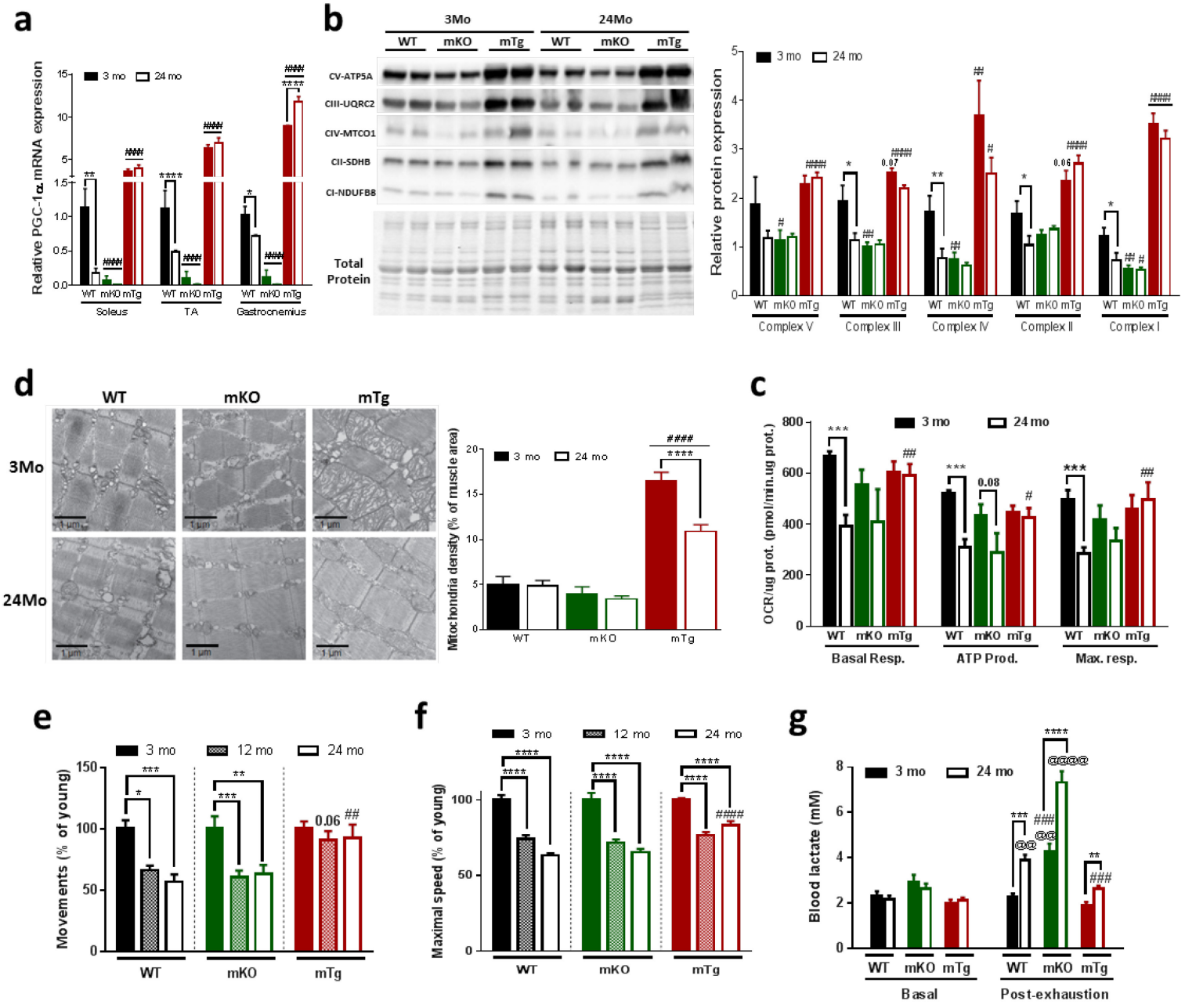
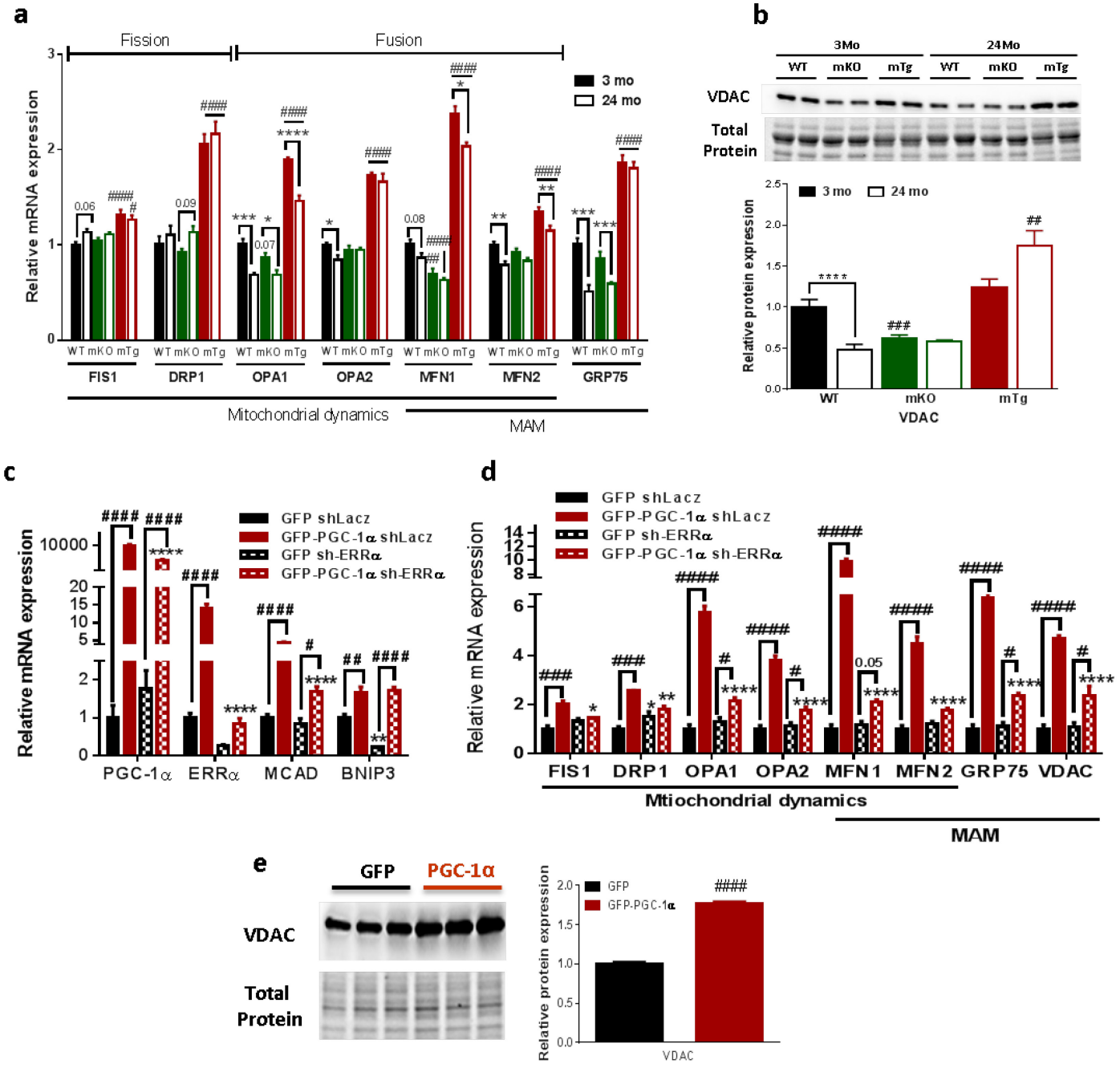


Figure 1: mPGC-1α prevents mitochondrial dysfunction and dysregulation and muscle disorders during aging (a) Relative PGC-1α mRNA levels in different muscle (n=6). **(b)** Relative protein levels of OXPHOS genes (n=6). **(c)** Mitochondrial respiration ATP production and maximal respiratory capacity (n=3-6). **(d)** Quantification and electronic microscopic pictures representative of mitochondrial mass **(e).** Age-related reduction in spontaneous locomotor activity measured by the CLAMS system. Values represent 48h of recording (n=8-9) **(f)** Age-related reduction in mice maximal running speed during treadmill exhaustion test (n=10-12) **(g)** Blood lactate levels before and after exercise (n=8-10). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals, @ P < 0.05; @@ P < 0.01; @@@@p<0.0001 indicat statistically significant differences between pre- and post-exercise.

Figure 2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Fission
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Relative mRNA expression
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GRP75
Mitochondrial dynamics
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b
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mKO
mTg
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GFP-PGC-1α shLacz
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VDAC
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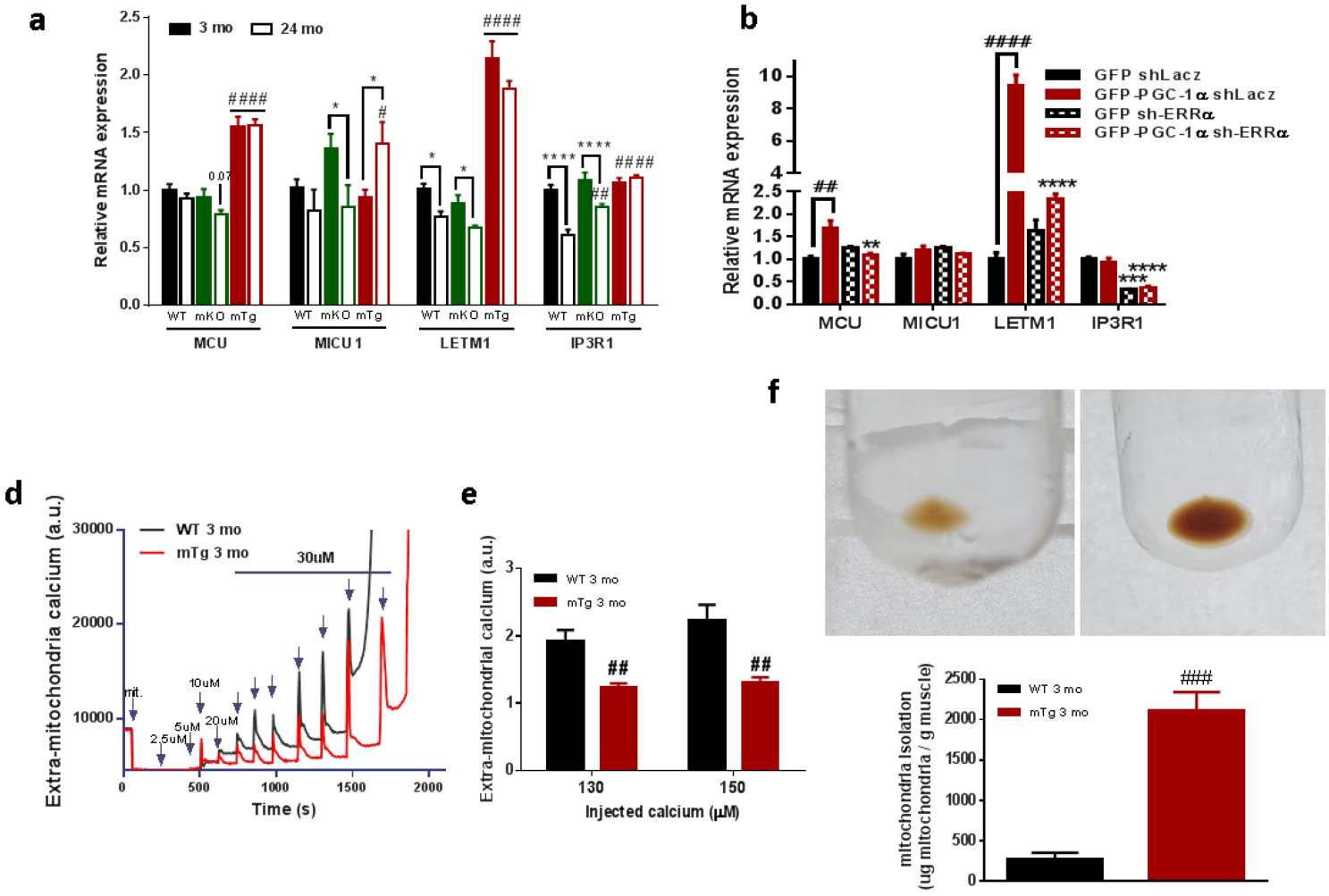
GFP
PGC-1α
VDAC
Total Protein
Relative protein expression
GFP
GFP-PGC-1α
VDAC

Figure 2: mPGC-1α improves mitochondrial dynamics and SR association in old muscle and C2C12 cell in a ERRα dependent manner (a and b) Relative muscle mRNA and protein levels of genes related to mitochondrial dynamics and SR-association (n=6). **(c-e)** Relative C2C12 cell mRNA and protein levels of PGC-1α and ERRα targets and of genes related to mitochondrial dynamics and SR-association (n=3 independent experiments with 3 technical replicates). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype or between cells with endogenous and overexpressed ERRα levels, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals or between cells with endogenous and overexpressed PGC-1α levels.

Figure 3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Relative mRNA expression
WT mKO mTg
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GFP sh-ERRα
GFP-PGC-1α sh-ERRα
Relative mRNA expression
MCU
MICU1
LETM1
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mTg 3 mo
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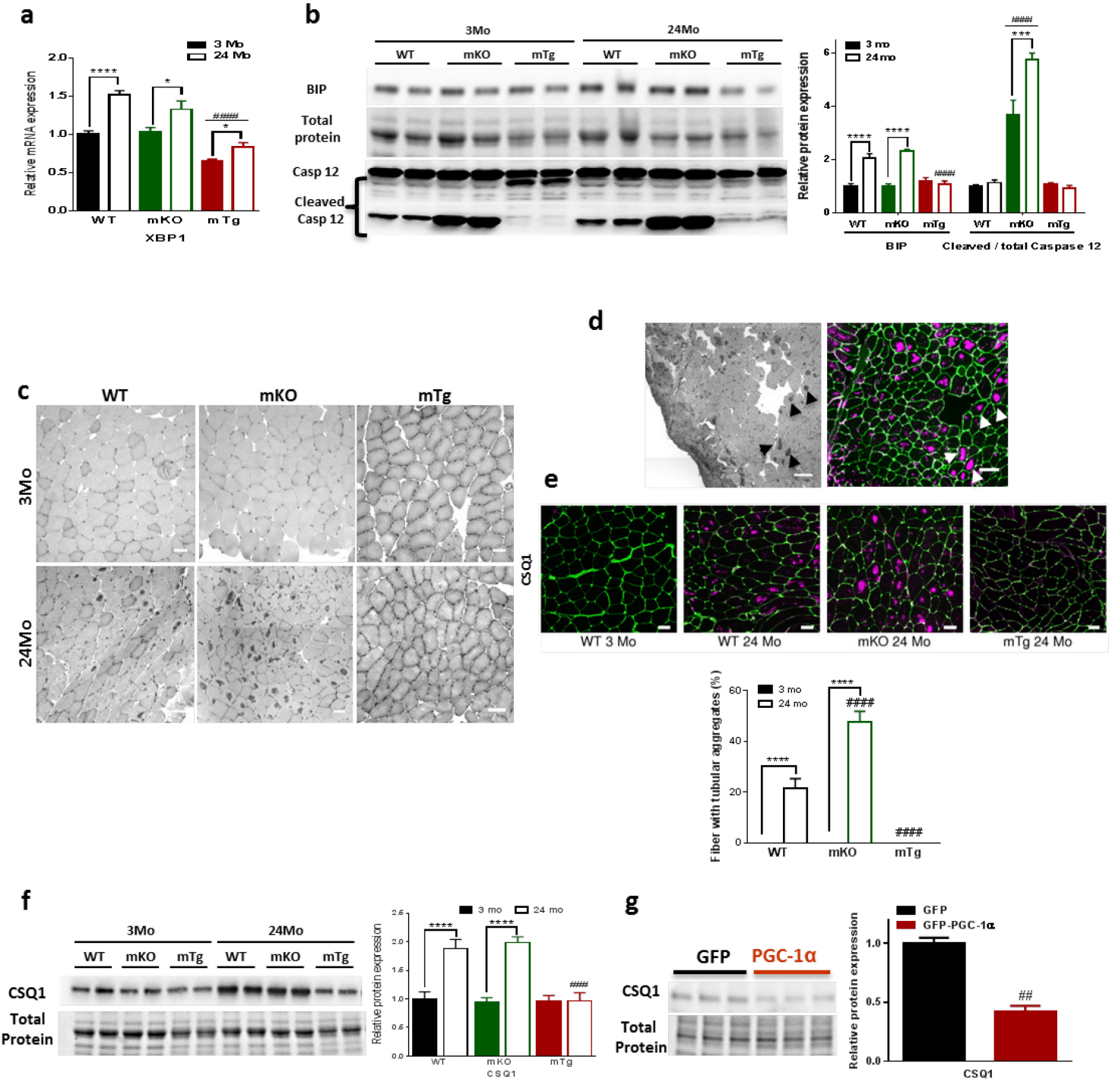
Extra-mitochondria calcium (a.u.)
Time (s)
e
WT 3 mo
mTg 3 mo
Extra-mitochondrial calcium (a.u.)
Injected calcium (μM)
f
WT 3 mo
mTg 3 mo
mitochondria Isolation
(ug mitochondria / g muscle)

Figure 3: mPGC-1α ameliorates mitochondrial calcium buffering (a) Relative muscle mRNA levels of mitochondrial calcium buffering genes (n=6). **(b)** Relative C2C12 myoblasts mRNA levels of mitochondrial calcium buffering gene (n=3 independent experiments with 3 technical replicates). **(d and e)** Representative cytosolic calcium traces upon calcium injection and quantification of relative cytosolic calcium levels after 130 and 150 µm calcium has been injected (n=4; 2 independent experiments with 2 muscles of each genotype used in each experiments). **(f)** Representative pictures of mitochondrial pellets after mitochondria isolation from hind limb muscles and measure of isolated mitochondrial quantity. Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype or between cells with endogenous and overexpressed ERRα levels, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals or between cells with endogenous and overexpressed PGC-1α levels.

Figure 4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3 Mo
24 Mo
Relative mRNA expression
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3Mo
24Mo
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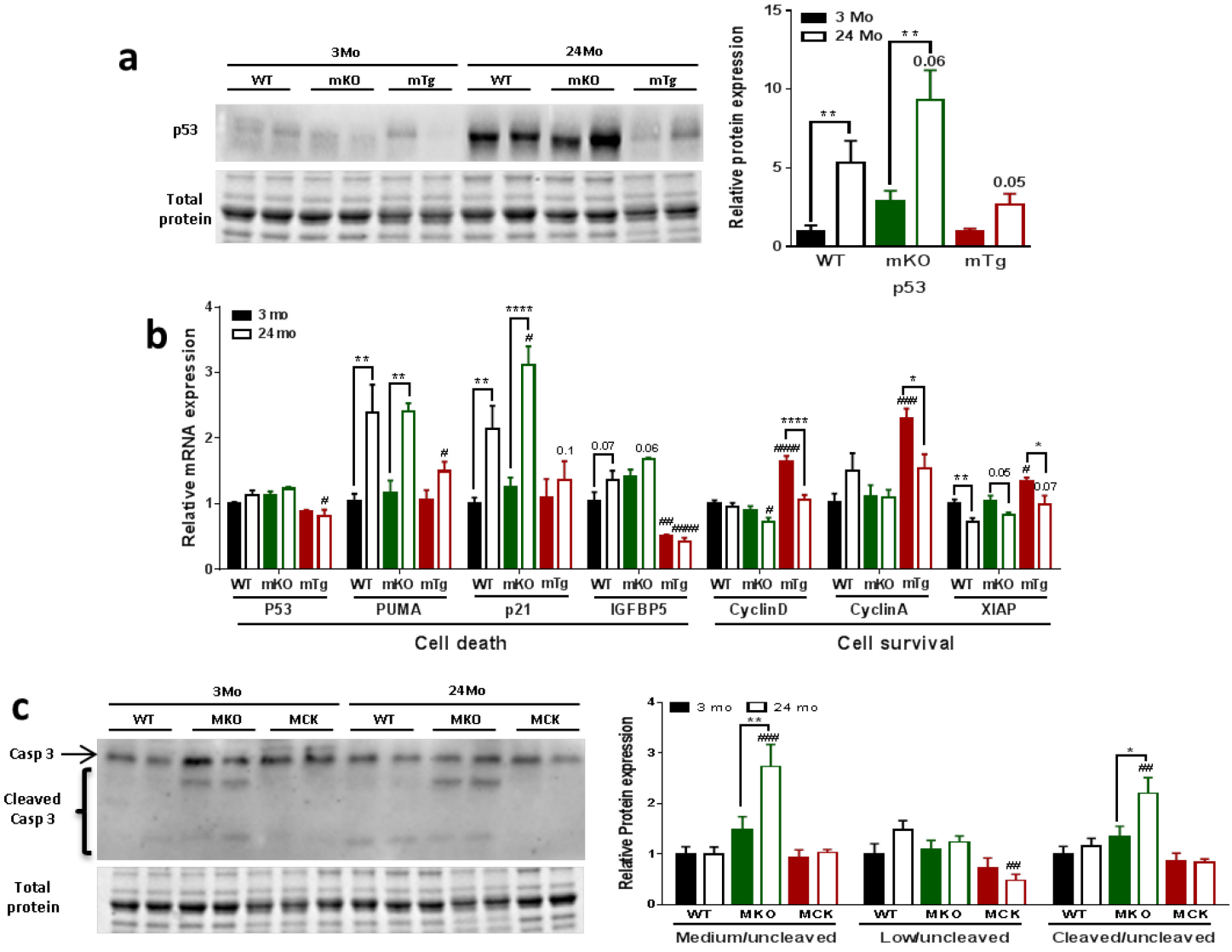
mKO
mTg
BIP
Total protein
Casp 12
Cleaved Casp 12
Relative protein expression
BIP
Cleaved / total Caspase 12
c
WT
mKO
mTg
3Mo
24Mo
d
e
CSQ1
WT 3 Mo
WT 24 Mo
mKO 24 Mo
mTg 24 Mo
Fiber with tubular aggregates (%)
f
CSQ1
Total Protein
g
GFP
PGC-1α
GFP-PGC-1α

Figure 4: mPGC-1α reduces ER stress during aging and fully prevents age-related tubular aggregate formation (a and b) Relative muscle mRNA and protein levels of ER stress related genes (n=6). **(c)** Representative pictures of H&E stained tibialis anterior cryosection including aggregates, scale bars represent 50 µm **(d).** Colocalization of H&E labelled aggregates (left picture) with calsequestrin 1 staining (right picture) indicated by arrows, scale bars represent 100 µm. **(e)** Representative pictures of tubular aggregates stained with calsequestrin 1 antibody scale bars represent 50 µm and quantification of the percentage of fibers containing tubular aggregates (n=6). **(f and g)** Calsquestrin 1 protein levels in young and old animals and in C2C12 myotubes. Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals or between cells with endogenous and overexpressed PGC-1α levels.

Figure 5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3Mo
24Mo
WT
mKO
mTg
p53
Total protein
3 Mo
24 Mo
Relative protein expression
p53
b
3 mo
24 mo
Relative mRNA expression
P53
PUMA
p21
IGFBP5
CyclinD
CyclinA
XIAP
Cell death
Cell survival
c
3Mo
24Mo
WT
MKO
MCK
Casp 3
Cleaved Casp 3
Total protein
Relative Protein expression
Medium/uncleaved
Low/uncleaved
Cleaved/uncleaved

Figure 5: mPGC-1α improves age-related muscle cell death (a-c) Relative muscle mRNA and protein levels of cell death and survival related genes (n=6). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals.

Figure 6

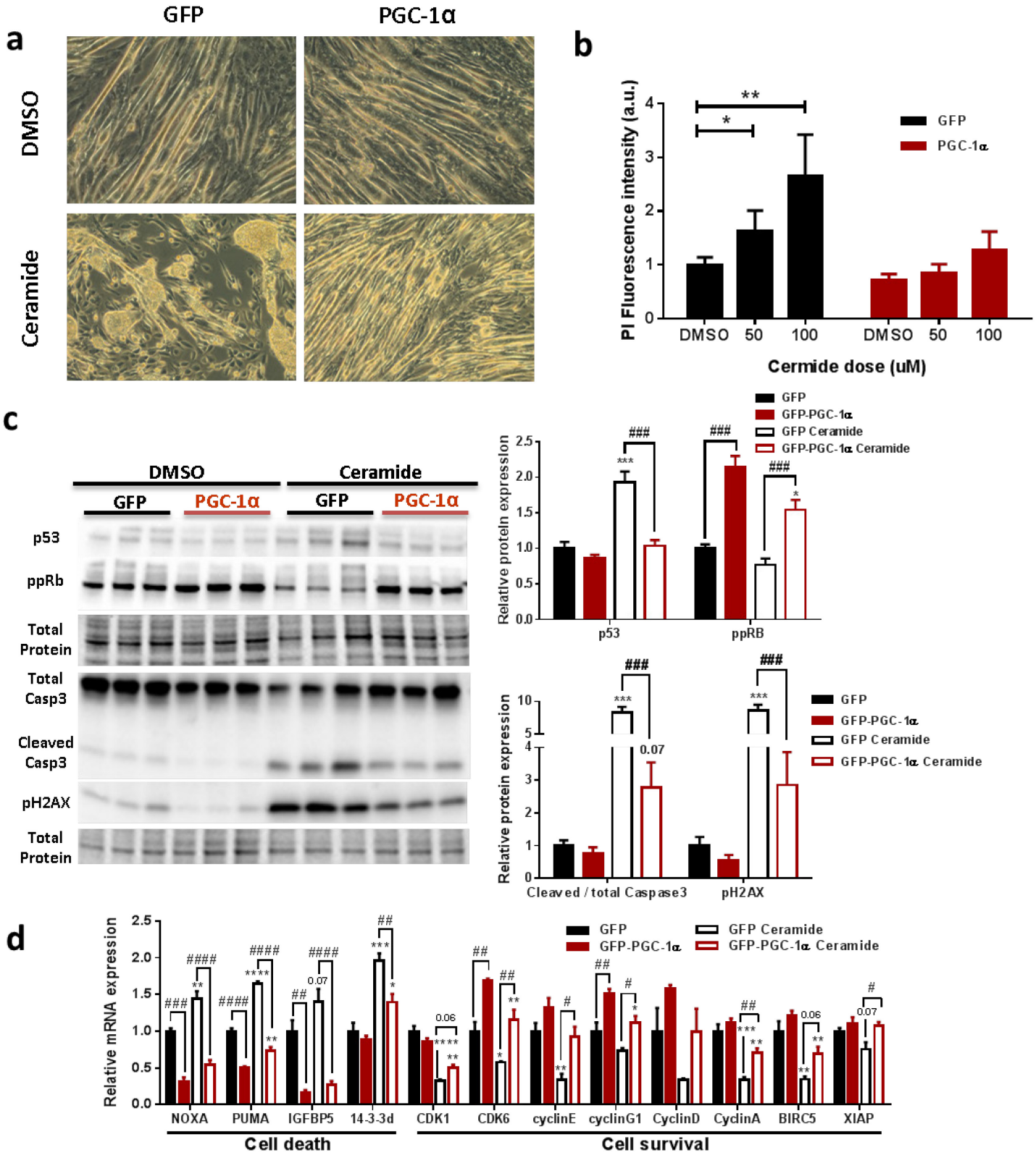
a
GFP
PGC-1α
DMSO
Ceramide
b
PI Fluorescence intensity (a.u.)
GFP
PGC-1α
DMSO 50 100 DMSO 50 100
Cermide dose (uM)
c
DMSO
Ceramide
GFP
PGC-1α
GFP
PGC-1α
p53
ppRb
Total Protein
Total Casp3
Cleaved Casp3
pH2AX
Total Protein
GFP
GFP-PGC-1α
GFP Ceramide
GFP-PGC-1α Ceramide
Relative protein expression
p53
ppRB
Cleaved / total Caspase3
pH2AX
d
Relative mRNA expression
NOXA
PUMA
IGFBP5
14-3-3d
CDK1
CDK6
cyclinE
cyclinG1
CyclinD
CyclinA
BIRC5
XIAP
Cell death
Cell survival

Figure 6: PGC-1α protects from ceramide-induced cell death (a) Representative picture of C2C12 myotubes expressing endogenous or increased PGC-1α levels after ceramide or DMSO treatment. **(b)** Relative propidium ioide incorporation in C2C12 myoblasts. **(c)** Relative C2C12 protein levels of P53 and ppRB in C2C12 myotubes and Caspase 3 and pH2AX in C2C12 myoblasts. **(d)** Relative C2C12 myotubes mRNA levels of cell death and cell survival markers (n=3 independent experiments with 3 technical replicates). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between cells treated with DMSO and ceramide, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between cells with endogenous and overexpressed PGC-1α levels.

Figure S1

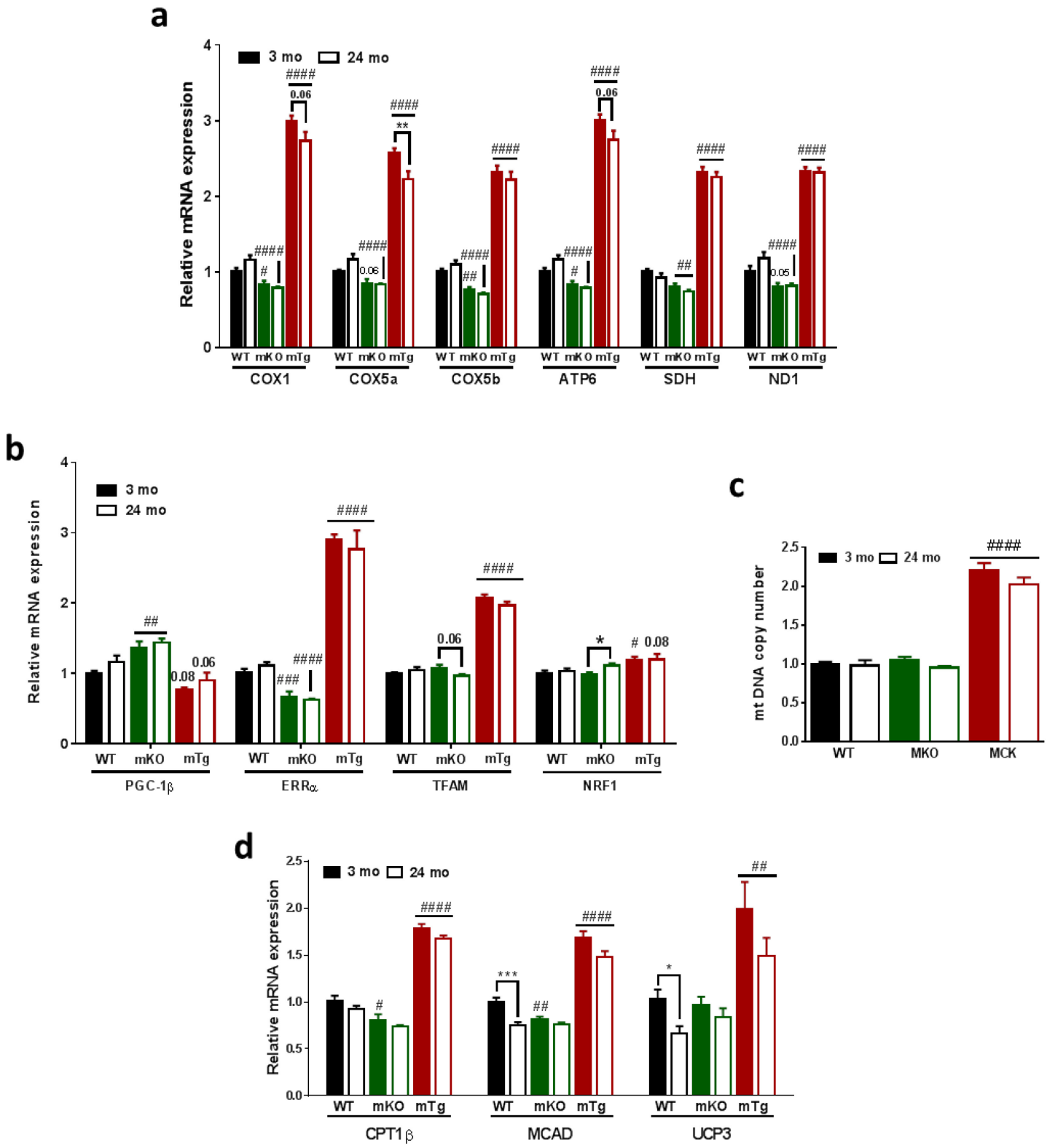
a
Relative mRNA expression
3 mo
24 mo
WT mKO mTg
COX1
COX5a
COX5b
ATP6
SDH
ND1
b
Relative mRNA expression
PGC-1β
ERRα
TFAM
NRF1
c
mt DNA copy number
WT
MKO
MCK
d
Relative mRNA expression
CPT1β
MCAD
UCP3

Figure S1: PGC-1α improves mitochondrial biogenesis and gene expression of mitochondrial metabolism (a) Relative muscle mRNA levels of mitochondrial genes (n=6). **(b)** Relative muscle mRNA levels of mitochondrial biogenesis genes (n=6). **(c)** Relative mitochondrial DNA copy number (n=6). **(d)** Relative muscle mRNA levels of fatty acid oxidation genes (n=6). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals.

Figure S2

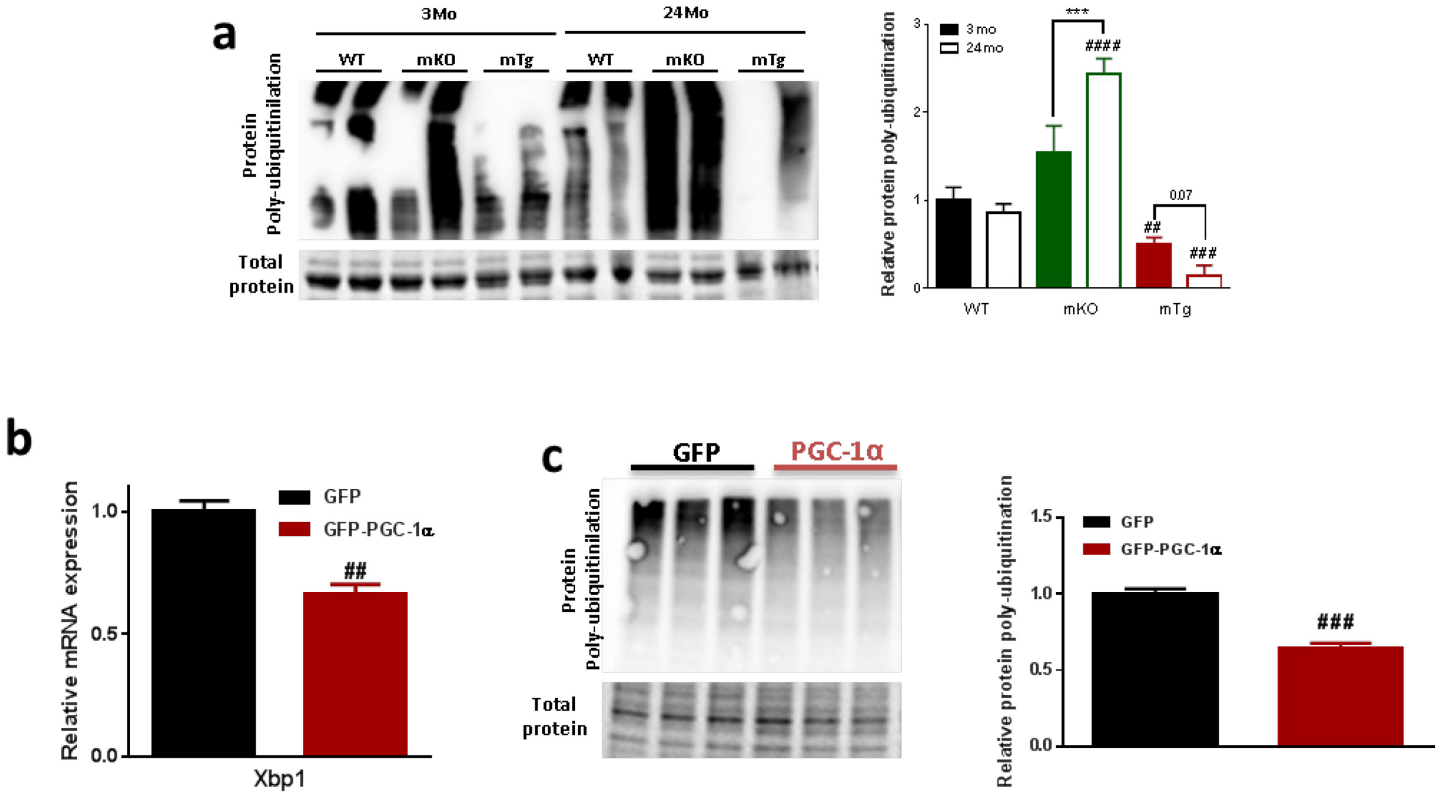
a
3Mo
24Mo
WT
mKO
mTg
WT
mKO
mTg
Protein Poly-ubiquitinilation
Total protein
Relative protein poly-ubiquitination
3 mo
24 mo
***
####
0.07
##
###
WT
mKO
mTg
b
Relative mRNA expression
GFP
GFP-PGC-1α
##
Xbp1
c
GFP
PGC-1α
Protein Poly-ubiquitinilation
Total protein
Relative protein poly-ubiquitination
GFP
GFP-PGC-1α
###

Figure S2: PGC-1α lowers ER stress markers in muscle during aging and in C2C12 cells (a) Relative muscle protein poly-ubiquitinilation during aging (n=6). **(b and c)** Relative myotube xbp1 gene expression and protein poly-ubiquitinilation (n=3 independent experiments with 3 technical replicates). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals or between cells with endogenous and overexpressed PGC-1α levels.

Figure S3

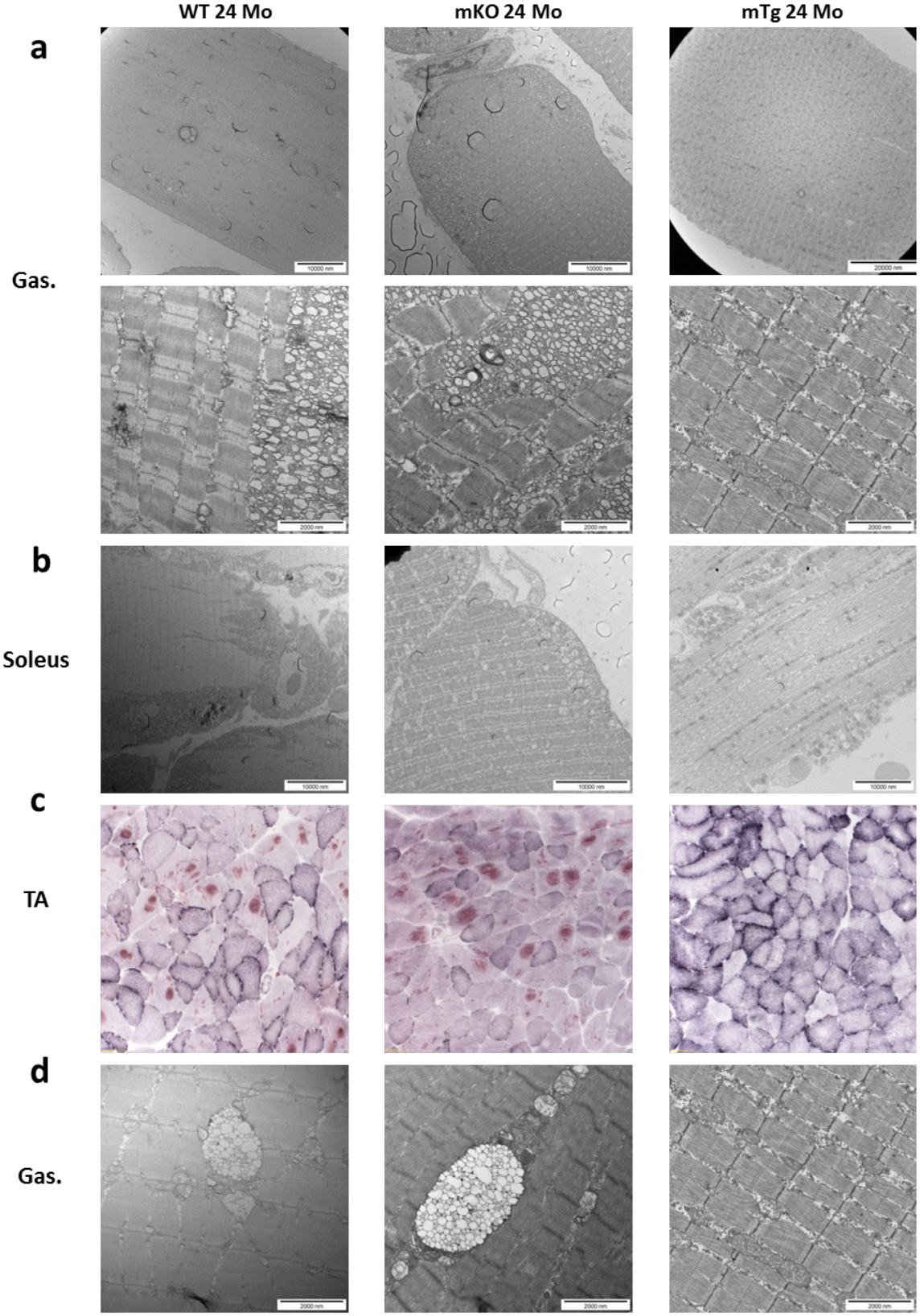

Figure S3: Tubular aggregates form preferentially in fast fibers and PGC-1α prevents the formation of abnormal structures (a-b) Representative electronic microscopic pictures of gastrocnemius and soleus muscles of old animals. The presence of tubular aggregates is indicated by arrows. **(c)** Representative pictures of SDH staining in the tibialis anterior of old muscles. **(d)** Representative electronic microscopic pictures of abnormal structures in gastrocnemius muscles of old animals .

Supplemental table 1

name	forward primer	reverse primer
βglobin	GAAGCGATTCTAGGGAGCAG	GGAGCAGCGATTCTGAGTAGA
14-3-3d	CGAAGACTAGGAGGAGGCAG	CTCTCCATGACTGCGAGGAT
36B4	ACTGGTCTAGGACCCGAGAAG	TCAATGGTGCCTCTGGAGATT
ATP6	AGTATGAGCTGGAGCCGTAATTACA	TGGAAGGAAGTGGGCAAGTG
BIRC5	GAGGCTGGCTTCATCCACTG	CTTTTTGCTTGTTGTTGGTCTCC
BNIP3	AAATTAAAGGGTGCGTGCGG	CAAAGTGGGGTTCGTGGGTA
CDK1	AGGTACTTACGGTGTGGTGTAT	CTCGCTTTCAAGTCTGATCTTCT
CDK6	GGCGTACCCACAGAAACCATA	AGGTAAGGGCCATCTGAAAACT
COX1	TGCTAGCCGCAGGCATTACT	GCGGGATCAAAGAAAGTTGTG
COX5a	CGCCGCTGTCTGTTCCAT	AAACTCCTCATCTGTCTCGTGTGA
COX5b	CTTCAGGCACCAAGGAAGAC	TTCACAGATGCAGCCCACTA
CPT1β	ATCATGTATCGCCGCAAACT	CCATCTGGTAGGAGCACATGG
CSQ1	ACTCAGAGAAGGATGCAGCT	CTCTACAGGGTCTTCTAGGA
CSQ2	AGCTTGTGGAGTTTGTGAAG	GGATTGTCAGTGTTGTCCC
CyclinA	GCCTTCACTCATTGCTGGAG	TGTTGTGCCAATGACTCAGG
CyclinD	GTTCATTTCCAACCCACCCTC	AGAAAGTGCGTTGTGCGGTAG
cyclinE	CCCTCTGACCATTGTGTCCT	TCGCACCACTGATAACCTGA
cyclinG1	GTTCACGACACCTTGCCATT	AGAAGGTCAAATCTCGGCCA
DRP1	GCGCTGATCCCGCGTCAT	CCGCACCCACTGTGTTGA
ERRα	CGGTGTGGCATCCTGTGA	CTCCCCTGGATGGTCCTCTT
FBOX32	AAAGCCCTCTCTTGGTTCTGACT	GAGAAGAGGTGCAGGGACTGA
FIS1	GCCCCTGCTACTGGACCAT	CCCTGAAAGCCTCACACTAAGG
GDF11	ACAGAGCAACTGGGGAATCG	AGTGTTCATCGCAGTCCAGG
GRP75	TGACCAAAGACAACATGGCG	TAGCTTTCTGACACGGAGCA
IP3R1	GCCTTGCTAGAGAAGAACGC	CATTGCAGCCTGGGTTATCC
IGFBP5	ATACAACCCAGAACGCCAGCT	ACCTGGGCTATGCACTTGATG
LETM1	CTCTGAGGCTGTGAAGGACA	CACCCTTCAGACCTTCCAGT
MCAD	AACACTTACTATGCCTCGATTGCA	CCATAGCCTCCGAAAATCTGAA
MCU	AAAGGAGCCAAAAAGTCACG	AACGGCGTGAGTTACAAACA
MFN1	CTGCTTCCTGAGTGTCGAGG	GCATGGGCCAGCTGATTAAC
MFN2	GGTCAGGGGTATCAGCGAAG	TTGTCCCAGAGCATGGCATT
MICU1	ACACCCTCAAGTCTGGCTTAT	TTCCCATCTTTGAAGTGCTTCTT
MSTN	GCTGGCCCAGTGGATCTAAA	GCCCCTCTTTTTCCACATTTT
Murf1	AGGCAGCCACCCGATGT	TCACACGTGAGACAGTAGATGTTGA
NCX	CTTCCCTGTTTGTGCTCCTGT	AGAAGCCCTTTATGTGGCAGTA
ND1	TCTGCCAGCCTGACCCATA	GGGCCCGGTTTGTTTCTG
NOXA	ACTGTGGTTCTGGCGCAGAT	TGAGCACACTCGTCCTTCAAGT
NRF1	TCCCCCGAGGACACTTCTT	ATCAGCTGCCGTGGAGTTG
OPA1	CTTGCCAGTTTAGCTCCCGA	CAATTTGGGACCTGCAGTGAA
OPA2	CCCAGCTCAGAAGACCTTGC	CCAGGTGAACCTGCAGTGAA
p21	GACACCACTGGAGGGTGACT	GGATTAGGGCTTCCTCTTGG
P53	GGGACAGCCAACTCTGTTATG	CTGTCTTCCAGATACTCGGGA
PGC-1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
PGC-1β	CCATGCTGTTGATGTTCCAC	GACGACTGACAGCACTTGGA
PUMA	ATGGCGGACGACCTCAAC	AGTCCCATGAAGAGATTGTACATGAC
SDH	GCTGGTGTGGATGTCACTAAGG	CCCACCCATGTTGTAATGCA
SERCA1	AGCCAGTGATGGAGAACTCG	CACCACCAACCAGATGTCAG
SERCA2	GAGAACGCTCACACAAAGACC	CAATTCGTTGGAGCCCCAT
TBP	ATATAATCCCAAGCGATTTGC	GTCCGTGGCTCTCTTATTCTC
TFAM	GGTCGCATCCCCTCGTCTA	GGATAGCTACCCATGCTGGAAA
TRIADIN	ATGACTGAGATCACTGCTGAAGG	ATGTTGTCACAATGTCTTCGGT
UCP3	TTTTGCGGACCTCCTCACTT	TGGATCTGCAGACGGACCTT
XBP1	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG
XIAP	GCTTGGCGCGAAAAGGTGG	TTGCACGGTGTCTCCTTCAC

Supplemental table 2

		Primary antibodies			
Labelling	Bloking solution	Antibody name	Product #	Company	Solution
Calsequestrin 1	milk	Calsequestrin Antibody (VIIID12)	MA3-913	Thermoscientific	BSA
pH2AX	BSA	Anti-gamma H2A.X (phospho S139) antibody	ab11174	Abcam	BSA
pprB	milk	phospho-RB(ser780)(C84F6) Rabbit mAB	3590	Cell signaling	BSA
Caspase 3	milk	Caspase-3 Antibody	9662	Cell signaling	BSA
P53	milk	p53 (1C12) Mouse mAb	2524	Cell signaling	milk
VDAC	BSA	VDAC (D73D12) Rabbit mAb	4661	Cell signaling	BSA
Oxphos protein	BSA	Total OXPHOS Rodent WB Antibody Cocktail	ab110413	Abcam	BSA
poly-ubiquitinilation	BSA	Mono- and polyubiquitinylated conjugates monoclonal antibody	Enzo	BML-PW8810-0500	BSA
BIP	BSA	Anti-GRP78 BiP antibody	ab21685	Abcam	BSA
Caspase 12	BSA	Caspase-12 Antibody	2202	Cell signaling	BSA

	Secondary antibodies				
Labelling	Antibody name	Product #	Company	Solution	ECL
Calsequestrin 1	Gt anti-ms	115-035-146	Jackson immunoresearch	milk	West Dura
pH2AX	Goat anti-mouse	115-035-146	Jackson immunoresearch	BSA	West Femto
pprB	Swine anti-rb	P0399	Dako	milk	West Dura
Caspase 3	Swine anti-rb	P0399	Dako	milk	West Femto
P53	Goat anti-mouse	115-035-146	Jackson immunoresearch	milk	West Dura
VDAC	Swine anti-rb	P0399	Dako	BSA	West Dura
Oxphos protein	Rb anti-ms	P0260	Dako	BSA	West Dura
poly-ubiquitinilation	Rb anti-ms	P0260	Dako	BSA	West Dura
BIP	Swine anti-rb	P0399	Dako	BSA	West Dura
Caspase 12	Swine anti-rb	P0399	Dako	BSA	West Dura
	SuperSignal™ West Dura Extended Duration Substrate			Thermoscientific #34076	
	SuperSignal™ West Femto Maximum Sensitivity Substrate			Thermoscientific #34095	

G. References

Adhihetty, P. J., G. Uguccioni, L. Leick, J. Hidalgo, H. Pilegaard and D. A. Hood (2009). "The role of PGC-1alpha on mitochondrial function and apoptotic susceptibility in muscle." American journal of physiology. Cell physiology 297(1): C217-225.

Allen, D. G., G. D. Lamb and H. Westerblad (2008). "Skeletal muscle fatigue: cellular mechanisms." Physiological reviews **88**(1): 287-332.

Arnold, A. S., J. Gill, M. Christe, R. Ruiz, S. McGuirk, J. St-Pierre, L. Tabares and C. Handschin (2014). "Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1alpha." Nature communications **5**: 3569.

Arora, A. S., B. J. Jones, T. C. Patel, S. F. Bronk and G. J. Gores (1997). "Ceramide induces hepatocyte cell death through disruption of mitochondrial function in the rat." Hepatology **25**(4): 958-963.

Baranska, W., W. Baran, P. Skopinski and H. Ziemba (1997). "Morphometric analysis of satellite cells in rat skeletal muscles: soleus and extensor digitorum longus." Acta Anat (Basel) **160**(2): 88-94.

Bianchi, K., G. Vandecasteele, C. Carli, A. Romagnoli, G. Szabadkai and R. Rizzuto (2006). "Regulation of Ca2+ signalling and Ca2+-mediated cell death by the transcriptional coactivator PGC-1alpha." Cell death and differentiation **13**(4): 586-596.

Bohm, J., F. Chevessier, A. Maues De Paula, C. Koch, S. Attarian, C. Feger, D. Hantai, P. Laforet, K. Ghorab, J. M. Vallat, M. Fardeau, D. Figarella-Branger, J. Pouget, N. B. Romero, M. Koch, C. Ebel, N. Levy, M. Krahn, B. Eymard, M. Bartoli and J. Laporte (2013). "Constitutive activation of the calcium sensor STIM1 causes tubular-aggregate myopathy." American journal of human genetics **92**(2): 271-278.

Chariot, P., E. Benbrik, A. Schaeffer and R. Gherardi (1993). "Tubular aggregates and partial cytochrome c oxidase deficiency in skeletal muscle of patients with AIDS treated with zidovudine." Acta neuropathologica **85**(4): 431-436.

Chen, M., D. J. Won, S. Krajewski and R. A. Gottlieb (2002). "Calpain and mitochondria in ischemia/reperfusion injury." The Journal of biological chemistry **277**(32): 29181-29186.

Chevessier, F., S. Bauche-Godard, J. P. Leroy, J. Koenig, M. Paturneau-Jouas, B. Eymard, D. Hantai and M. Verdiere-Sahuque (2005). "The origin of tubular aggregates in human myopathies." Journal of Pathology **207**(3): 313-323.

Chevessier, F., I. Marty, M. Paturneau-Jouas, D. Hantai and M. Verdiere-Sahuque (2004). "Tubular aggregates are from whole sarcoplasmic reticulum origin: alterations in calcium binding protein expression in mouse skeletal muscle during aging." Neuromuscular disorders : NMD **14**(3): 208-216.

Danial, N. N. and S. J. Korsmeyer (2004). "Cell death: Critical control points." Cell **116**(2): 205-219.

Dirks, A. and C. Leeuwenburgh (2002). "Apoptosis in skeletal muscle with aging." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **282**(2): R519-R527.

Dirks, A. J. and C. Leeuwenburgh (2004). "Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12." Free Radical Biology and Medicine **36**(1): 27-39.

Doonan, P. J., H. C. Chandramoorthy, N. E. Hoffman, X. Q. Zhang, C. Cardenas, S. Shanmughapriya, S. Rajan, S. Vallem, X. W. Chen, J. K. Foskett, J. Y. Cheung, S. R. Houser and M. Madesh (2014). "LETM1-dependent mitochondrial Ca2+ flux modulates cellular bioenergetics and proliferation." Faseb Journal **28**(11): 4936-4949.

Duchen, M. R. (2000). "Mitochondria and calcium: from cell signalling to cell death." The Journal of physiology **529 Pt 1**: 57-68.

Egger, A., M. Samardzija, V. Sothilingam, N. Tanimoto, C. Lange, S. Salatino, L. Fang, M. Garcia-Garrido, S. Beck, M. J. Okoniewski, A. Neutzner, M. W. Seeliger, C. Grimm and C. Handschin (2012). "PGC-1alpha determines light damage susceptibility of the murine retina." PloS one **7**(2): e31272.

Fraysse, B., J. F. Desaphy, J. F. Rolland, S. Pierno, A. Liantonio, V. Giannuzzi, C. Camerino, M. P. Didonna, D. Cocchi, A. De Luca and D. Conte Camerino (2006). "Fiber type-related changes in rat skeletal muscle calcium homeostasis during aging and restoration by growth hormone." Neurobiology of disease **21**(2): 372-380.

Funk, F., C. Ceuterick-de Groote, J. J. Martin, A. Meinhardt, A. L. Taratuto, J. De Bleecker, R. Van Coster, B. De Paepe, U. Schara, M. Vorgerd, M. Hausler, S. Koppi, M. Maschke, P. De Jonghe, L. Van Maldergem, S. Noel, C. W. Zimmermann, S. Wirth, S. Isenmann, R. Stadler, J. M. Schroder, J. B. Schulz, J. Weis and K. G. Claeys (2013). "Morphological spectrum and clinical features of myopathies with tubular aggregates." Histology and histopathology **28**(8): 1041-1054.

Gaziev, A. I., S. Abdullaev and A. Podlutsky (2014). "Mitochondrial function and mitochondrial DNA maintenance with advancing age." Biogerontology **15**(5): 417-438.

Ghosh, A., G. Narayanappa, A. B. Taly, Y. T. Chickbasavaiya, A. Mahadevan, V. Santosh, N. Atchayaram, I. Mohapatra and S. K. Shankar (2010). "Tubular aggregate myopathy: A phenotypic spectrum and morphological study." Neurology India **58**(5): 747-751.

Ghosh, S., R. Lertwattanarak, N. Lefort, M. Molina-Carrion, J. Joya-Galeana, B. P. Bowen, J. Garduno-Garcia Jde, M. Abdul-Ghani, A. Richardson, R. A. DeFronzo, L. Mandarino, H. Van Remmen and N. Musi (2011). "Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance." Diabetes **60**(8): 2051-2060.

Gomez-Cabrera, M. C., F. Sanchis-Gomar, R. Garcia-Valles, H. Pareja-Galeano, J. Gambini, C. Borras and J. Vina (2012). "Mitochondria as sources and targets of damage in cellular aging." Clinical chemistry and laboratory medicine : CCLM / FESCC **50**(8): 1287-1295.

Grimm, S. (2012). "The ER-mitochondria interface: the social network of cell death." Biochim Biophys Acta **1823**(2): 327-334.

Handschin, C., C. S. Choi, S. Chin, S. Kim, D. Kawamori, A. J. Kurpad, N. Neubauer, J. Hu, V. K. Mootha, Y. B. Kim, R. N. Kulkarni, G. I. Shulman and B. M. Spiegelman (2007). "Abnormal glucose homeostasis in

skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk." The Journal of clinical investigation **117**(11): 3463-3474.

Harman, D. (2006). "Free Radical Theory of Aging: An Update Increasing the Functional Life Span." Understanding and Modulating Aging **1067**: 10-21.

Hirani, V., F. Blyth, V. Naganathan, D. G. Le Couteur, M. J. Seibel, L. M. Waite, D. J. Handelsman and R. G. Cumming (2015). "Sarcopenia Is Associated With Incident Disability, Institutionalization, and Mortality in Community-Dwelling Older Men: The Concord Health and Ageing in Men Project." Journal of the American Medical Directors Association **16**(7): 607-613.

Iqbal, S., O. Ostojic, K. Singh, A. M. Joseph and D. A. Hood (2013). "Expression of Mitochondrial Fission and Fusion Regulatory Proteins in Skeletal Muscle during Chronic Use and Disuse." Muscle & Nerve **48**(6): 963-970.

Jarvis, W. D., S. Grant and R. N. Kolesnick (1996). "Ceramide and the induction of apoptosis." Clinical cancer research : an official journal of the American Association for Cancer Research **2**(1): 1-6.

Ji, L. L. and C. Kang (2015). "Role of PGC-1alpha in sarcopenia: etiology and potential intervention - a mini-review." Gerontology **61**(2): 139-148.

Johnson, M. L., M. M. Robinson and K. S. Nair (2013). "Skeletal muscle aging and the mitochondrion." Trends in endocrinology and metabolism: TEM **24**(5): 247-256.

Lemasters, J. J., T. Qian, C. A. Bradham, D. A. Brenner, W. E. Cascio, L. C. Trost, Y. Nishimura, A. L. Nieminen and B. Herman (1999). "Mitochondrial dysfunction in the pathogenesis of necrotic and apoptotic cell death." J Bioenerg Biomembr **31**(4): 305-319.

Lin, J., H. Wu, P. T. Tarr, C. Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby and B. M. Spiegelman (2002). "Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres." Nature **418**(6899): 797-801.

Ling, C., P. Poulsen, E. Carlsson, M. Ridderstrale, P. Almgren, J. Wojtaszewski, H. Beck-Nielsen, L. Groop and A. Vaag (2004). "Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins." The Journal of clinical investigation **114**(10): 1518-1526.

Liu, J. C., J. Liu, K. M. Holmstrom, S. Menazza, R. J. Parks, M. M. Fergusson, Z. X. Yu, D. A. Springer, C. Halsey, C. Liu, E. Murphy and T. Finkel (2016). "MICU1 Serves as a Molecular Gatekeeper to Prevent In Vivo Mitochondrial Calcium Overload." Cell Rep **16**(6): 1561-1573.

Liu, Z., Y. Xia, B. Li, H. Xu, C. Wang, Y. Liu, Y. Li, C. Li, N. Gao and L. Li (2014). "Induction of ER stress-mediated apoptosis by ceramide via disruption of ER Ca(2+) homeostasis in human adenoid cystic carcinoma cells." Cell Biosci **4**: 71.

Ludatscher, R., M. Silbermann, D. Gershon and A. Reznick (1983). "The effects of enforced running on the gastrocnemius muscle in aging mice: an ultrastructural study." Experimental gerontology **18**(2): 113-123.

Luo, Y., W. Zhu, J. Jia, C. Zhang and Y. Xu (2009). "NMDA receptor dependent PGC-1alpha up-regulation protects the cortical neuron against oxygen-glucose deprivation/reperfusion injury." Journal of molecular neuroscience : MN **39**(1-2): 262-268.

Malhotra, J. D. and R. J. Kaufman (2011). "ER stress and its functional link to mitochondria: role in cell survival and death." Cold Spring Harb Perspect Biol **3**(9): a004424.

Marchi, S., S. Patergnani and P. Pinton (2014). "The endoplasmic reticulum-mitochondria connection: one touch, multiple functions." Biochim Biophys Acta **1837**(4): 461-469.

Marin, J., A. Encabo, A. Briones, E. C. Garcia-Cohen and M. J. Alonso (1999). "Mechanisms involved in the cellular calcium homeostasis in vascular smooth muscle: calcium pumps." Life Sci **64**(5): 279-303.

Perez-Schindler, J., S. Summermatter, G. Santos, F. Zorzato and C. Handschin (2013). "The transcriptional coactivator PGC-1alpha is dispensable for chronic overload-induced skeletal muscle hypertrophy and metabolic remodeling." Proceedings of the National Academy of Sciences of the United States of America **110**(50): 20314-20319.

Peterson, C. M., D. L. Johannsen and E. Ravussin (2012). "Skeletal muscle mitochondria and aging: a review." Journal of aging research **2012**: 194821.

Rera, M., S. Bahadorani, J. Cho, C. L. Koehler, M. Ulgherait, J. H. Hur, W. S. Ansari, T. Lo, Jr., D. L. Jones and D. W. Walker (2011). "Modulation of longevity and tissue homeostasis by the Drosophila PGC-1 homolog." Cell Metab **14**(5): 623-634.

Rizzuto, R., D. De Stefani, A. Raffaello and C. Mammucari (2012). "Mitochondria as sensors and regulators of calcium signalling." Nat Rev Mol Cell Biol **13**(9): 566-578.

Sahin, E., S. Colla, M. Liesa, J. Moslehi, F. L. Muller, M. Guo, M. Cooper, D. Kotton, A. J. Fabian, C. Walkey, R. S. Maser, G. Tonon, F. Foerster, R. Xiong, Y. A. Wang, S. A. Shukla, M. Jaskelioff, E. S. Martin, T. P. Heffernan, A. Protopopov, E. Ivanova, J. E. Mahoney, M. Kost-Alimova, S. R. Perry, R. Bronson, R. Liao, R. Mulligan, O. S. Shirihai, L. Chin and R. A. DePinho (2011). "Telomere dysfunction induces metabolic and mitochondrial compromise." Nature **470**(7334): 359-365.

Salviati, G., S. Pierobon-Bormioli, R. Betto, E. Damiani, C. Angelini, S. P. Ringel, S. Salvatori and A. Margreth (1985). "Tubular aggregates: sarcoplasmic reticulum origin, calcium storage ability, and functional implications." Muscle & nerve **8**(4): 299-306.

Schiaffino, S. (2012). "Tubular aggregates in skeletal muscle: just a special type of protein aggregates?" Neuromuscular disorders : NMD **22**(3): 199-207.

Schubert, W., F. Sotgia, A. W. Cohen, F. Capozza, G. Bonuccelli, C. Bruno, C. Minetti, E. Bonilla, S. Dimauro and M. P. Lisanti (2007). "Caveolin-1(-/-)- and caveolin-2(-/-)-deficient mice both display numerous skeletal muscle abnormalities, with tubular aggregate formation." The American journal of pathology **170**(1): 316-333.

Sczelecki, S., A. Besse-Patin, A. Abboud, S. Kleiner, D. Laznik-Bogoslavski, C. D. Wrann, J. L. Ruas, B. Haibe-Kains and J. L. Estall (2014). "Loss of Pgc-1alpha expression in aging mouse muscle potentiates glucose

intolerance and systemic inflammation." American journal of physiology. Endocrinology and metabolism **306**(2): E157-167.

Shiraishi, J., T. Tatsumi, N. Keira, K. Akashi, A. Mano, S. Yamanaka, S. Matoba, J. Asayama, T. Yaoi, S. Fushiki, H. Fliss and M. Nakagawa (2001). "Important role of energy-dependent mitochondrial pathways in cultured rat cardiac myocyte apoptosis." American journal of physiology. Heart and circulatory physiology **281**(4): H1637-1647.

Short, K. R., M. L. Bigelow, J. Kahl, R. Singh, J. Coenen-Schimke, S. Raghavakaimal and K. S. Nair (2005). "Decline in skeletal muscle mitochondrial function with aging in humans." Proceedings of the National Academy of Sciences of the United States of America **102**(15): 5618-5623.

Siu, P. M., E. E. Pistilli and S. E. Alway (2005). "Apoptotic responses to hindlimb suspension in gastrocnemius muscles from young adult and aged rats." American Journal of Physiology-Regulatory Integrative and Comparative Physiology **289**(4): R1015-R1026.

Summermatter, S., R. Thurnheer, G. Santos, B. Mosca, O. Baum, S. Treves, H. Hoppeler, F. Zorzato and C. Handschin (2012). "Remodeling of calcium handling in skeletal muscle through PGC-1alpha: impact on force, fatigability, and fiber type." American journal of physiology. Cell physiology **302**(1): C88-99.

Szegezdi, E., S. E. Logue, A. M. Gorman and A. Samali (2006). "Mediators of endoplasmic reticulum stress-induced apoptosis." EMBO Rep **7**(9): 880-885.

Szulc, P., T. J. Beck, F. Marchand and P. D. Delmas (2005). "Low skeletal muscle mass is associated with poor structural parameters of bone and impaired balance in elderly men--the MINOS study." Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research **20**(5): 721-729.

Vainshtein, A., E. M. Desjardins, A. Armani, M. Sandri and D. A. Hood (2015). "PGC-1alpha modulates denervation-induced mitophagy in skeletal muscle." Skeletal muscle **5**: 9.

Valle, I., A. Alvarez-Barrientos, E. Arza, S. Lamas and M. Monsalve (2005). "PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells." Cardiovascular research **66**(3): 562-573.

Vina, J., M. C. Gomez-Cabrera, C. Borras, T. Froio, F. Sanchis-Gomar, V. E. Martinez-Bello and F. V. Pallardo (2009). "Mitochondrial biogenesis in exercise and in ageing." Advanced Drug Delivery Reviews **61**(14): 1369-1374.

Walston, J. D. (2012). "Sarcopenia in older adults." Current opinion in rheumatology **24**(6): 623-627.

Wanagat, J., Z. J. Cao, P. Pathare and J. M. Aiken (2001). "Mitochondrial DNA deletion mutations colocalize with segmental electron transport system abnormalities, muscle fiber atrophy, fiber splitting, and oxidative damage in sarcopenia." Faseb Journal **15**(2): 322-332.

Wang, C. and R. J. Youle (2009). "The role of mitochondria in apoptosis*." Annual review of genetics **43**: 95-118.

Wenz, T., S. G. Rossi, R. L. Rotundo, B. M. Spiegelman and C. T. Moraes (2009). "Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging." Proceedings of the National Academy of Sciences of the United States of America **106**(48): 20405-20410.

Whitman, S. A., M. J. Wacker, S. R. Richmond and M. P. Godard (2005). "Contributions of the ubiquitin-proteasome pathway and apoptosis to human skeletal muscle wasting with age." Pflugers Archiv-European Journal of Physiology **450**(6): 437-446.

Wu, J., J. L. Ruas, J. L. Estall, K. A. Rasbach, J. H. Choi, L. Ye, P. Bostrom, H. M. Tyra, R. W. Crawford, K. P. Campbell, D. T. Rutkowski, R. J. Kaufman and B. M. Spiegelman (2011). "The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex." Cell metabolism **13**(2): 160-169.

Xiong, S., N. Patrushev, F. Forouzandeh, L. Hilenski and R. W. Alexander (2015). "PGC-1alpha Modulates Telomere Function and DNA Damage in Protecting against Aging-Related Chronic Diseases." Cell reports **12**(9): 1391-1399.

Zhou, J., T. A. Freeman, F. Ahmad, X. Shang, E. Mangano, E. Gao, J. Farber, Y. Wang, X. L. Ma, J. Woodgett, R. J. Vagnozzi, H. Lal and T. Force (2013). "GSK-3alpha is a central regulator of age-related pathologies in mice." The Journal of clinical investigation **123**(4): 1821-1832.

Uncategorized References

Agbulut, O., J. Destombes, D. Thiesson and G. Butler-Browne (2000). "Age-related appearance of tubular aggregates in the skeletal muscle of almost all male inbred mice." Histochemistry and cell biology **114**(6): 477-481.

Fernandez-Sanz, C., M. Ruiz-Meana, E. Miro-Casas, E. Nunez, J. Castellano, M. Loureiro, I. Barba, M. Poncelas, A. Rodriguez-Sinovas, J. Vazquez and D. Garcia-Dorado (2014). "Defective sarcoplasmic reticulum-mitochondria calcium exchange in aged mouse myocardium." Cell Death & Disease **5**.

Kang, C. H., E. Chung, G. Diffee and L. L. Ji (2013). "Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: Role of PGC-1 alpha." Experimental Gerontology **48**(11): 1343-1350.

Pietrangelo, L., A. D'Incecco, A. Ainbinder, A. Michelucci, H. Kern, R. T. Dirksen, S. Boncompagni and F. Protasi (2015). "Age-dependent uncoupling of mitochondria from Ca2+ release units in skeletal muscle." Oncotarget **6**(34): 35358-35371.

IV. Manuscript 3: Muscle PGC-1α potentiates exercise and blunts the aging-induced deterioration of muscle function and motor coordination

Jonathan F. Gill, Gesa Santos, Svenia Schnyder and Christoph Handschin

[1]Biozentrum, Division of Pharmacology/Neurobiology, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland

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A. Abstract

Sarcopenia, age-related loss of muscle mass and function results in a drastic decline in motor function and mobility in elderly individuals. Regular physical activity is the only efficient intervention to prevent and treat sarcopenia. However, the mechanisms that underlie the therapeutic effect of exercise in this context remain unclear. Muscle peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a key regulatory hub in endurance exercise adaptation with decreased expression in old muscle. We therefore assessed whether PGC-1α is required for the exercise-controlled plasticity of old muscle and studied the extent to which PGC-1α elevation can substitute for bona fide physical activity. Using mouse models, we showed that PGC-1α muscle specific genetic ablation mimicked its age-related decline and triggered pre-mature sarcopenia, while its overexpression mildly alleviated the metabolic syndrome in old mice. Surprisingly, we additionally found that muscle PGC-1α was not only involved in the endurance and mitochondrial remodeling of exercise, but also phenocopied and contributed to balance and motor coordination improvements observed in old, trained animals. Our data therefore suggest that many of the beneficial effects of exercise on muscle mass and function in aging are modulated by activation of PGC-1α.

B. Introduction

Aging is a progressive and inevitable biological process leading to a decay and dysfunction of organs. Skeletal muscle tissue is prominently affected by this decline, as characterized by a loss of strength and mass starting in the 4th decade in humans, a process known as sarcopenia **(Nair 2005)**. Age-related loss of muscle function is further exacerbated by reduced balance and motor coordination resulting in avoidance of physical ability, increased frailty, elevated number of falls and thus bone fractures **(Lord, Clark et al. 1991)**, highly predictive for hospitalization and morbidity **(Rolland, Czerwinski et al. 2008)**. In addition, muscle dysfunction is associated with many age-associated metabolic disorders such as insulin resistance, type 2 diabetes or hypertension, thereby increasing the risk of cardiovascular death **(Nair 2005)**. While multiple events are involved in muscle aging **(Marcell 2003)**, mitochondrial dysfunction has been strongly linked to sarcopenia **(Short, Bigelow et al. 2005)**.

Exercise training is the most efficient intervention to improve muscle function and whole body metabolism **(Egan and Zierath 2013)**, while physical inactivity increases the risk for many chronic diseases associated with aging **(Booth, Roberts et al. 2012)**. Physical activity accordingly helps to ameliorate age-related skeletal muscle dysfunctions **(Cartee, Hepple et al. 2016)**, not only by preserving muscle mass and strength but also balance, motor coordination and mobility **(Garatachea, Pareja-Galeano et al. 2015)**. Furthermore, exercise also reduces the age-related decline in insulin sensitivity **(Cartee, Hepple et al. 2016)**. Finally, physical activity maintains normal mitochondrial function in older adults **(Gouspillou, Sgarioto et al. 2014)**. Mitochondrial activity and other exercise-induced phenotypic changes of skeletal muscle are controlled by the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). For example, PGC-1α is a master regulator of mitochondrial biogenesis **(Wu, Puigserver et al. 1999, Schreiber, Emter et al. 2004)**, mitochondrial function **(Wu, Puigserver et al. 1999)**, mitochondrial dynamics **(Cannavino, Brocca et al. 2015)**, fatty acids oxidation **(Hoeks, Arany et al. 2012)** and anti-oxidative processes **(St-Pierre, Drori et al. 2006)**. Interestingly PGC-1α levels are increased by exercise in muscle **(Safdar, Little et al. 2011)** and PGC-1α is considered to be a key player in

mitochondrial changes induced by exercise **(Ventura-Clapier, Mettauer et al. 2007, Safdar, Little et al. 2011)**. In addition, PGC-1α improves muscle dysfunction of mice with a mitochondria myopathy **(Wenz, Diaz et al. 2008)** and extends health- and life-span of a mouse model of pre-mature aging arising from mitochondria defects **(Sahin, Colla et al. 2011)**. Interestingly, in line with mitochondrial decline, PGC-1α expression decreases during muscle aging in different species including humans **(Short, Bigelow et al. 2005, Vina, Gomez-Cabrera et al. 2009, Ghosh, Lertwattanarak et al. 2011, Kang, Chung et al. 2013)**. Moreover PGC-1α regulates many processes involved in age-related diseases **(Wenz 2011)** and improves different muscular disorders including sarcopenia, fiber atrophy **(Sandri, Lin et al. 2006)** or Duchenne muscular dystrophy **(Handschin, Kobayashi et al. 2007)**.

In this work, we investigated the impact of life-long PGC-1α modulation on sarcopenia, age-related motor dysfunctions and whole-body metabolism in 2-years old mice. While a number of effects of muscle-specific overexpression **(Wenz, Rossi et al. 2009)** and gene ablation of PGC-1α **(Sczelecki, Besse-Patin et al. 2014)** on muscle physiology in aging have been demonstrated, we now studied the beneficial effects of treadmill exercise during muscle aging and assessed whether these effects were mimicked and/or dependent on muscle PGC-1α expression. We observed that reduced and elevated PGC-1α levels accelerate and delay, respectively, distinct aspects of muscle aging. Moreover, we found that PGC-1α slightly improves whole-body metabolism and that many beneficial effects of exercise on mitochondria and muscle function are potentiated by and dependent on the transcriptional coactivator.

C. Experimental procedures

Animals

Mice with muscle ablation (mKO) and overexpression (mTg) of PGC-1α were described previously **(Lin, Wu et al. 2002, Handschin, Choi et al. 2007)**. C57/Bl6 wild-type (WT) mice were obtained from Janvier (Janvier sas, cs 4105, le genest St-isle f-53941, St Berthevin Cedex). Animals were fed ad libitum with regular chow diet (Provimi Kliba 3432) and kept under a 12h/12h light-dark cycle (06:00 to 18:00) at 23°C. Studies were performed with 3, 12 and 24 month-old male mice (3 mo 12 mo and 24 mo). At 21 months, groups of mice were trained on a treadmill during 12 weeks, 3 times per week, for 30 min. Maximal speed was determined prior to the beginning of the training by an exhaustion test. The exercise protocol started at 50% of maximal speed in the first week and was gradually increased each week to reach 80%. All experiments were performed in accordance with the federal guidelines for animal experimentation and were approved by the Kantonales Veterinäramt of the Kanton Basel-Stadt.

Mice phenotyping

For all behavioral experiments, mice were acclimatized to the room for 1h prior to the tests.

- Balance performance

Mice were placed on one side of an inclined 6mm diameter beam and were motivated to walk across the bar towards a red house used as a positive stimulus. A lamp was placed at the starting point as an adverse stimulus. Mice were acclimatized to the apparatus for one day with 3 beam crossing including 15 second of rest in the red house between trials. Time required to cross the beam and number of foot slips made during the crossing were recorded during the 3 following days with 3 trials per day.

- Motor coordination and planning

Mice were placed on a horizontally oriented, rotating cylinder (rod). For 3 consecutive days, mice were acclimatized 3x1 minute per day on the rotarod instrument set to rotate at 5 rpm. On the 5 following days, mice were tested with accelerating speed (5-68 rpm in 7 minutes with directional reversal). Mice were tested 3 times in a row with 10 min of rest between each trial. The time the mice spend on the rod before falling was recorded and averaged over the 5 days.

- Maximal and isometric muscle force

Maximal grip strength was measured using a grip strength meter (Chatillon). Five measurements of the maximal force were performed with 1 min recovery periods between the repetitions. The maximum value obtained was used for the analysis. Fatigue resistance and isometric force were measured by placing mice on an elevated inverted grid and measuring the maximum time until the mice released and felt.

- Endurance exercise capacity

Mice were acclimatized to treadmill running (Columbus Instruments) for two days. Acclimatization was done with an inclination of 5% at a speed of 8 m/min for 5 min followed by 5 min of 10 m/min. The exhaustion test was performed one day after acclimatization, starting at a speed of 4.8 m/min and a subsequent an increased by 1.6 m/min every 3 min until a speed of 29 m/min was reached. When mice reached exhaustion, the maximal running distance was recorded. Blood was drawn from the tail vein before and 10 min after the test and blood lactate levels were measured with a lactate plus meter (Nova biomedical).

Metabolism measurements

- Body composition

Body composition was assessed with an EchoMRI-100analyzer (EchoMRI Medical Systems). Fat and lean mass were adjusted to body weight.

- Glucose homeostasis

All mice were acclimatized to handling before the experiment. Mice were fasted for 16 h overnight before intraperitoneal injection of a bolus of 2 g (glucose)/kg (body weight). Blood glucose levels were recorded from the tail vein with a glucose meter (Accu-Chek, Roche Diagnostics) at 0, 15, 30, 45, 60, 90 and 120 min after glucose injection.

- Blood pressure

Blood pressure was measured with a tail cuff BP-2000 Blood Pressure Analysis System (Visitech Systems) on 5 consecutive days. 5 pre-measurements and 15 measurements were performed in a row. The average values of the 15 measurements over the 5 days were used for analysis.

Mouse muscle preparation

Body weight of the mice was measured before sacrifice by CO2 inhalation. Muscles were directly collected and weighed before being either snap-frozen in liquid nitrogen for protein and RNA extraction, embedded in 7% tragacanth using cooled-isopentane for cryosection staining or directly processed for mitochondrial respiration experiments.

Mitochondria DNA copy number and gene expression

- Genomic DNA

Powdered muscles were incubated overnight at 55° in lysis buffer (10mM TrisHCl, 1mM EDTA, 0.1% SDS 5% Proteinase). After a 15 min centrifugation step at 8000g, residual RNA of the

re-suspended lysate was removed with a RNase A treatment (20mg/ml) during 30 min at 37°C. RNase A was heat-inactivated at 95°C for 30 min. Samples were mixed with one volume of phenol/chloroform/isoamylalcohol (25:24:1) and centrifuged at 8000g for 15 min. The aqueous phase was transferred to a fresh tube and one volume of chloroform was added. After an identical centrifugation step, one volume of isopropanol containing 0.3M of sodium acetate was added. Samples were then kept at -20°C for 30 min. A centrifugation step was repeated at 4°C. DNA pellets were washed 2 times with 1ml of ice-cold 75% ethanol and finally recovered in ddH2O. 0.1ug of gDNA was analyzed by qPCR to measure mitochondrial DNA copy numbers.

- RNA extraction and qPCR

Lysing matrix tubes (MP Biomedicals 6913-500) and TRI Reagent (Sigma-Aldrich T9424) were used to isolate RNA from crushed muscle. NanoDrop 1000 spectrophotometer (Thermo Scientific) was used to measure RNA concentration and purity to ensure that the ratios of 260/280 and 260/230 were above 1.8. An Agilent Bioanalyzer (Agilent 2100 Bioanalyzer, Agilent Technologies) determined the level of RNA degradation. DNA was removed with RNase-free DNase (Invitrogen 18068-015) and one microgram of RNA was used for reverse transcription using SuperScript II reverse transcriptase (Invitrogen 18064-014).

Relative DNA copy number or mRNA levels were determined using a Light Cycler 480 system (Roche Diagnostics) with FastStart essential DNA probe master mix (Roche Diagnostics 06402682001). Relative mRNA quantification was done with the ΔΔCT method using the TATA binding protein (TBP) gene as reference. Mitochondrial DNA copy number was evaluated by normalizing the average of COX1 ATP6 and ND1 DNA copy number with the average copy number of the nuclear genes beta globin and 34B6. Beta globin, 34B6 and TBP levels were similar in all samples. Primer used: PGC-1α for 5'-AGCCGTGACCACTGACAACGAG-'3, PGC-1α rev 5'-GCTGCATGGTTCTGAGTGCTAAG-'3' ; TBP for 5'-ATATAATCCCAAGCGATTTGC-'3, TBP rev ; 5'-GTCCGTGGCTCTCTTATTCTC-'3; COX1 for 5'-TGCTAGCCGCAGGCATTACT-'3, COX1 rev 5'-GCGGGATCAAAGAAAGTTGTG-'3; ATP6 for 5'-AGTATGAGCTGGAGCCGTAATTACA-'3, ATP6 rev 5'-

TGGAAGGAAGTGGGCAAGTG-'3; ND1 for 5'-TCTGCCAGCCTGACCCATA-'3, ND1 rev 5'-GGGCCCGGTTTGTTTCTG-'3 ; 36B4 for 5'-ACTGGTCTAGGACCCGAGAAG-'3, 36B4 rev 5'-TCAATGGTGCCTCTGGAGATT-'3; βGlobin for 5'-GAAGCGATTCTAGGGAGCAG-'3, βGlobin rev 5'-GGAGCAGCGATTCTGAGTAGA-'3

Immunostaining

8 µm muscle cryosections were cut with a cryostat (Leica, CM1950). Sections were blocked for 30min at ambient temperature in PBS containing 0.4% Triton X-100 (Sigma, 93426) and 10% goat serum (Sigma, G9023). Fiber types were labeled with primary and secondary antibodies for one hour at room temperature in PBS with 10% goat serum (antibody dilutions and information are described in table 1). Incubation with secondary antibodies was performed in the dark. All secondary antibodies were from Life technologies. 3 washes of 5 min in PBS were performed before and after antibody incubation. Nuclei were stained using ProLong Gold Antifade mounting medium with Dapi (Life Technologies, P36931). Images were taken using the FEI MORE microscope with a 20x magnification lens. Fiber type percentages and diameters were quantified in whole muscle sections using the ImageJ and cell^P software (Olympus Soft Imaging Solution).

Table 1:

Primary antibodies				Secondary antibodies		
Product#	Company	Labelling	Dilution	Product#	Labelling	Dilution
BA-F8	DSHB	m IgG b2 MHC type 1	1:25	A-21242	AF647 IgG GaM	1:250
SC-71	DSHB	m IgG1 MHC type 2a	1:200	A-21124	AF568 IgG1 GaM	1:100
BF-F3	DSHB	m IgM MHC type 2b	1:100	A-21042	AF488 IgM GaM	1:400
L9393	Sigma	r IgM Laminin	1:5000	A-11008	AF488 IgG GaR	1:500

Protein levels

Total protein was recovered from quadriceps muscles as described in **(Perez-Schindler, Summermatter et al. 2013)**. Equal amounts of protein were migrated on mini-TGX 4-20% stain free pre-cast gel (Biorad, 4568096) and labelled with the in-gel trihalo compounds under UV for 1 min. Gel and nitrocellulose membranes were allowed to equilibrate for 5 min in transfer buffer. Protein transfer was performed at a constant voltage of 100V for 1h. Membranes were blocked for 1 h at room temperature with 5% BSA diluted in TBS-T and washed 2x for 5 min with TBS-T. Membranes were then incubated overnight at 4°C with primary antibodies (Abcam, ab110413, 1/1000) diluted in TBS-T containing 0.02% sodium azide and 3% BSA. Membranes were washed 3x for 5 min with TBS-T and incubated 1 h at room temperature with peroxidase-conjugated secondary antibodies (Dako, P0260, 1/10000) diluted in TBS-T plus 3% BSA. The same washing step was repeated before antibody binding was revealed using an enhanced chemiluminescence HRP substrate detection kit (Thermoscientific, 34076) and imaged using a Fusion FX imager. Total protein content was determined using the trihalo compounds labeling. Two reference samples were loaded in the different gels for inter-gel normalization. Proteins quantification was performed using the Fusion FX software.

Mitochondrial respiration

Isolated muscles were consecutively rinsed with PBS and PBS containing10 mM EDTA. Muscles were put in 2 ml of isolation buffer 1 (EDTA 10 mM, D-mannitol 215 mM, sucrose (0.075M), free-fatty acid BSA (Sigma-Aldrich) 0.1%, HEPES 2 mM pH 7.4 in distilled water) and thoroughly minced. Muscle solutions were homogenized with manual pestle in Potter-Elvehjem grinders and centrifuged for 10 min at 700g. The resulting supernatants were centrifuged for 10 min at 10500g and pellets were re-suspended in 500µl of isolation buffer 2 (EGTA 3 mM, D-mannitol 215 mM, sucrose 0.075M, free-fatty acid BSA (Sigma-Aldrich) 0.1%, HEPES 2 mM pH 7.4 in distilled water) before another centrifugation step at 10500g for 10 min. Mitochondria were finally re-suspended in 100 µl of isolation buffer 2 and protein concentrations were assessed by Bradford assay before being diluted in 37°C pre-warmed mitochondrial assay buffer

(MAS) (MgCl2 5 mM, D-Mannitol 220 mM, KH2PO4 10mM, EGTA 1 mM, free-fatty acid BSA 0.2%, HEPES 2 mM, sucrose 70 mM pH 7.0 in distilled water). 1µg of mitochondrial protein was loaded into a 96-well plate and centrifuged at 2000g for 20 min. 135 µl of mitochondrial assay buffer with 20mM succinate and 2µM rotenone were gently added to each well and the plate was warmed up to 37°C for 10 min. A calibrated Seahorse XF96 Extracellular Flux Analyzer was used to measure mitochondrial respiration using the Mito Stress Test Kit (Seahorse Bioscience, # 103015-100). Mitochondrial respiration assay consisted of an equilibration, 1 min mixing, 3 min pause, 1 min mixing, 3 min pause, 0.5 min mixing, 3 min measurement, 1 min mixing, 3 min measurement, 0.5 min mixing, injection of 4 mM ADP, 1 min mixing, 3 min measurement, 1 min mixing, injection of 3.125 µM Oligomycin, 0.5 min mixing, 3 min measurement, 1 min mixing, injection of 4 µM FCCP, 0.5 min mixing, 3 min measurement, 1 min mixing, injection of 4 µM Antimycin, 0.5 min mixing, 3 min measurement. All of the experimental steps prior to plate loading were performed at 4°C.

Blood cholesterol

Blood was collected from the tail vein or post-mortem from heart puncture. Blood samples were centrifuged for 10 min at 2000 g in tri-potassium-EDTA tubes before plasma was recovered. Plasma cholesterol levels were measured with a COBAS c111 analyzer (Roche Diagnostics).

Statistical analysis

Data were analyzed with two-way ANOVA (GraphPad Prism software). Sidak post-tests were used to do multiple comparison analysis following two-way ANOVA. All data are plotted as mean ± SEM.

D. Results

Exercise-associated mitochondrial improvement in old muscle is dependent on muscle PGC-1α

Mitochondrial number and function are boosted by endurance training, while inversely reduced mitochondrial activity has been associated with muscle aging. We therefore investigated the impact of exercise and modulation of muscle PGC-1α expression in old skeletal muscle in vivo. As expected, endurance training induced PGC-1α expression in the gastrocnemius and tibialis anterior (TA) muscle of WT animals (Fig. 1a). Surprisingly however, the genotype effect of overexpression and knockout of PGC-1α on mitochondrial gene expression was not replicated by exercise (Fig. 1b). Nevertheless, oxidative phosphorylation (OXPHOS) protein levels were elevated by PGC-1α and exercise (Fig. 1c). Importantly, the exercise-controlled increase in OXPHOS proteins was blunted in the mKO animals. Even though mitochondrial gene expression and protein levels were already substantially boosted in mTg mice beyond the levels that were achieved by endurance exercise in WT animals, an additional training-effect was still observed in the mTg model in regard to mitochondrial DNA copy number (Fig. 1d) and oxygen consumption rates of isolated mitochondria (Fig. 1e).

Muscle PGC-1α affects endurance capacity of untrained and trained skeletal muscle in old mice

Mitochondrial function is closely linked to endurance performance in skeletal muscle. Based on the observed modulation of mitochondrial properties by exercise and muscle PGC-1α, we next studied the impact the corresponding interventions on muscle endurance during aging. In all three genotypes, exercise training improved endurance performance of old mice, even though at lower and higher levels in mKO and mTg animals, respectively (Fig. 2a). Similarly, circulating lactate was affected in a genotype-dependent manner (Fig. 2b). Surprisingly, an additional lactate-lowering effect of exercise was only observed in mKO and mTg, but not WT mice. At least for the muscle-specific overexpression of PGC-1α, this observation could be based

on the exercise-linked potentiation of the proportion of type I muscle fibers, which was not recorded in WT or mKO animals (Fig. 2c).

Aging, exercise and muscle PGC-1α modulate muscle mass and metabolic parameters

Besides reduced muscle function, a loss of muscle mass and force are the key hallmarks of sarcopenia. An assessment of grip strength accordingly revealed an age-dependent diminution in maximal and isometric force in all genotypes (Fig. 3a and b). Correlating with reduced muscle force, loss of individual muscles masses with age further suggested the onset of sarcopenia in the WT group, while no changes could be observed in lean mass or fiber size in those animals (Fig. 3 c, d and e). Strikingly, old mice deficient for muscle PGC-1α presented an accelerated age-related loss of isometric strength, in addition to diminished maximal force, lean mass, muscle weights and fibers size compared to WT animals, highlighting the development of a pre-mature sarcopenia in those mice. Although mTg animals exhibited a slight age-related loss of lean mass, it was not significantly different from WT animals and the impact of PGC-1α overexpression on individual muscle weights reduction was muscle specific. Even though endurance training is not the most efficient intervention to improve muscle mass, exercise partially rescued the loss maximal grip strength, lean mass and muscle weight in old mKO mice, although maxiimal force and lean mass remained lower than in the trained and untrained WT groups (Fig. 4a, b and d). In WT animals, only gastrocnemius was similarly affected by exercise. Interestingly, exercise however improved isometric strength in old WT mice, but not in mice lacking muscle PGC-1α (Fig. 4b), possibly because of their reduced maximal force and muscle mass. Surprisingly, this absence of exercise effect was also found in mice overexpressing PGC-1α and likely originates from distinct alteration in mTg and mKO animals. In an opposite manner PGC-1α overexpression potentiated the effect of exercise on maximal force in old animals (Fig. 4a).

Muscle mass and function are important predictors of metabolic homeostasis. Thus, the age-associated decrease in muscle mass and function was linked to a deterioration of glucose tolerance in WT and mTg animals, whereas young mKO mice already exhibited a glucose

tolerance similar to the one of 2 years old animals (Fig. S1a). While blood cholesterol remained largely unchanged (Fig. S1b), down- and upregulation of PGC-1α both lowered diastolic blood pressure. However, more importantly, blood pressure increased in old WT and mKO, but not in mTg mice (Fig. S1c). Exercise dramatically reversed age-associated glucose intolerance in the WT group and failed to significantly improve glucose clearance in mTg animals, whereas it did not lead to any changes in mKO mice (Fig. S2a). Blood cholesterol levels were not affected to a large extent by exercise, even though a slight elevation and a significant reduction were observed in the mKO and mTg mice, respectively (Fig. S2b). Diastolic blood pressure was not affected by exercise in any of the genotypes (Fig. S2c).

Exercise and PGC-1α improve age-related impairments in motor coordination and balance

The detrimental effects of sarcopenia in elderly individuals are compounded by a loss of motor coordination and balance. We thus recorded how motor coordination and balance in old mice can be modulated by exercise and muscle PGC-1α using balance beam- and Rotarod-based tests. The number of foot slips and the time needed to cross the balance beam rise dramatically with increasing age in all three genotypes (Fig. 5a). Strikingly however, the mKO mice exhibited the phenotype of 24-months old WT mice already at the age of 12 months. Inversely, the age-dependent increase in both parameters was blunted in mTg animals. Curiously, young mKO and mTg mice performed better in Rotarod tests at young age (Fig. 5b). Exercise training improved the balance performances in old WT and mKO mice to the level observed in sedentary mTg mice (Fig. 6a). Similarly, Rotarod performance in WT mice after exercise reached the level of sedentary mTg animals (Fig. 6b). Strikingly, mKO mice did not respond to exercise in the Rotarod-based assessment of motor coordination.

E. Discussion

Exercise is widely accepted as one of the most efficient treatment against whole-body and muscle aging **(Gremeaux, Gayda et al. 2012, Garatachea, Pareja-Galeano et al. 2015)**. While evidences has been presented that demonstrate a role for PGC-1α modulation in the muscle of old animals **(Wenz, Rossi et al. 2009, Sczelecki, Besse-Patin et al. 2014)**, we now have studied the role of this coactivator in muscle aging in the context of exercise. Most surprisingly, we observed a strong involvement of muscle PGC-1α in the control of motor coordination and balance. An improvement of these parameters has been shown in exercise, which exerts pleiotropic effects on different organs and cell types. Our findings now demonstrate that muscle-intrinsic changes in PGC-1α expression are sufficient to affect motor coordination and balance in old mice. Prior work revealed that central nervous system-specific expression of PGC-1α is essential for motor function **(Zhao, Varghese et al. 2011, Lucas, Dougherty et al. 2012)**. Our present data indicate that skeletal muscle can initiate a retrograde signaling to the nervous system with a similar outcome. Analogous findings have been reported in the remodeling of the neuromuscular junction **(Arnold, Gill et al. 2014)**. Several muscle intrinsic and extrinsic factors contribute to maintenance of motor coordination and balance besides neuronal changes, including motor planning, strength and endurance, all of which are decreased with age **(Garatachea, Pareja-Galeano et al. 2015)**. Thus, reduced muscle mass in aged mKO-PGC-1α mice compared to age-matched WT animals and the ensuing reduction in maximal force could be important for balance and motor coordination **(Schultz, Ashton-Miller et al. 1997)**. Additionally, muscle fiber contraction speed is essential to counteract disequilibrium or to initiate adaptations to changes in motor planning **(Schultz, Ashton-Miller et al. 1997)**. This parameter is highly dependent on calcium homeostasis **(Berchtold, Brinkmeier et al. 2000)**. Interestingly, our group demonstrated that muscle PGC-1α remodel sarcoplasmic reticulum and mitochondrial calcium handling **(Summermatter, Thurnheer et al. 2012)** and thereby possibly affects muscle contraction and ultimately locomotor function. On top of that, muscle PGC-1α overexpression ameliorates neuromuscular junction morphology and function, which might also contribute to the amelioration of age-related motor dysfunction **(Arnold, Gill et al. 2014)**.

Finally the proprioceptive and the vestibular systems are key players for motor coordination and balance performance **(Baloh 1996)** and decline with age **(Johnsson 1971)**. Vestibulo-spinal neuronal projections preferentially connect to slow over fast motor neurons that innervate slow type muscles **(Basaldella, Takeoka et al. 2015)**. Inversely, proprioceptive projections are more predominantly linked to fast motor neurons and hence fast fibers. By promoting a shift towards slow fiber types **(Lin, Wu et al. 2002)**, PGC-1α might also consequently improve vestibular-muscle connection and ultimately balance performance. Of note, ablation of muscle PGC-1α abolished the effect of exercise on motor coordination and bunted exercise-associated increase in endurance. Conversely, overexpression of muscle PGC-1α boosted the effect of exercise on both muscle endurance and maximal strength. Those data indicate that exercise effects on motor coordination, endurance and maximal force rely on muscle PGC-1α levels. Interestingly, similarly to PGC-1α, exercise influences age-related neuromuscular junction alterations **(Wenz, Rossi et al. 2009, Valdez, Tapia et al. 2010)** and drives a shift toward a more oxidative muscle metabolism **(Holloszy and Coyle 1984)** which could thereby also improve vestibulo-muscular communication **(Basaldella, Takeoka et al. 2015)**. In the light of those elements, it would be interesting to investigate if exercise affects motor function through those types of remodeling in a PGC-1α dependent manner.

In our experimental paradigm, exercise did not affect whole body muscle mass and only quadriceps muscle mass in our WT animals. In line with these results, maximal strength was likewise unaffected. Resistance exercise would probably be more effective in improving these parameters. Nevertheless, endurance exercise training boosted isometric muscle force and running capacity in WT mice. Endurance exercise might thus be sufficient to improve sarcopenia at a later age and upon higher muscle strength loss. This is supported by the partial restoration of both muscle mass and muscle force in mKO mice, which displayed an advanced stage in sarcopenia.

Interestingly, even though exercise improved PGC-1α muscle gene expression in some muscles, no changes in mitochondrial mRNA levels were observed. In contrast, an elevation in OXPHOS protein levels correlated with the increased endurance performance of these mice. Strikingly, PGC-1α muscle deletion blunted exercise induction of OXPHOS proteins and PGC-1α overexpression potentiated the effects of exercise on mitochondrial mass, mitochondria respiration and on the proportion of type I fibers. This demonstrates that exercise improves mitochondrial fitness and oxidative metabolism in a PGC-1α-dependent manner, also in old muscles. Correlating with our results, previous work showed that global and muscle-specific PGC-1α deletion prevents exercise-associated amelioration of mitochondria **(Leick, Lyngby et al. 2010)**.

Skeletal muscle is the largest tissue responding to insulin and therefore is a primary site for whole body glucose uptake, utilization and storage. Glucose intolerance, increased blood pressure and altered cholesterol levels are all hallmarks of the age-related metabolic syndrome **(Hildrum, Mykletun et al. 2007)**. Conversely to the aggravated glucose intolerance in old mKO animals described previously **(Sczelecki, Besse-Patin et al. 2014)**, we observed that absence of muscle PGC-1α accelerated glucose intolerance during aging. However, the fact that only genotype and not age association was evaluated and presented in the previous work makes the comparison with the present study difficult. For example, in the data presented in the earlier work, glucose tolerance seems unexpectedly lower in 12 months than in 24 months old WT mice. In mTg mice, even though no overt amelioration in glucose tolerance was observed, overexpression of muscle PGC-1α improved cholesterol levels and abolished the age-associated blood pressure increase. Therefore overall, muscle PGC-1α mildly reduces the development of an age-related metabolic syndrome. Since the metabolic syndrome is highly predictive for heart disorders and failure **(Perrone-Filardi, Paolillo et al. 2015)**, it would be interesting to study the effect of exercise combined with modulation of muscle PGC-1α on the aging heart.

In conclusion, our findings demonstrate that many of the beneficial effects of exercise involve the action of muscle PGC-1α. Of note, some parameters show an additive effect of endurance exercise with overexpression of PGC-1α, suggesting an obvious therapeutic relevance. While an improvement of motor coordination and balance could have been expected from the exercise intervention, the striking effect of overexpression and ablation of muscle PGC-1α reveal a hitherto unsuspected contribution of muscle fibers to these neuronally controlled processes. More work is required to identify the molecular mechanisms that underlie this observation. Nevertheless, our data highlight the importance of proper muscle function on aging-related deteriorations that transcend the immediate effects on muscle fibers.

F. Figures

Figure 1

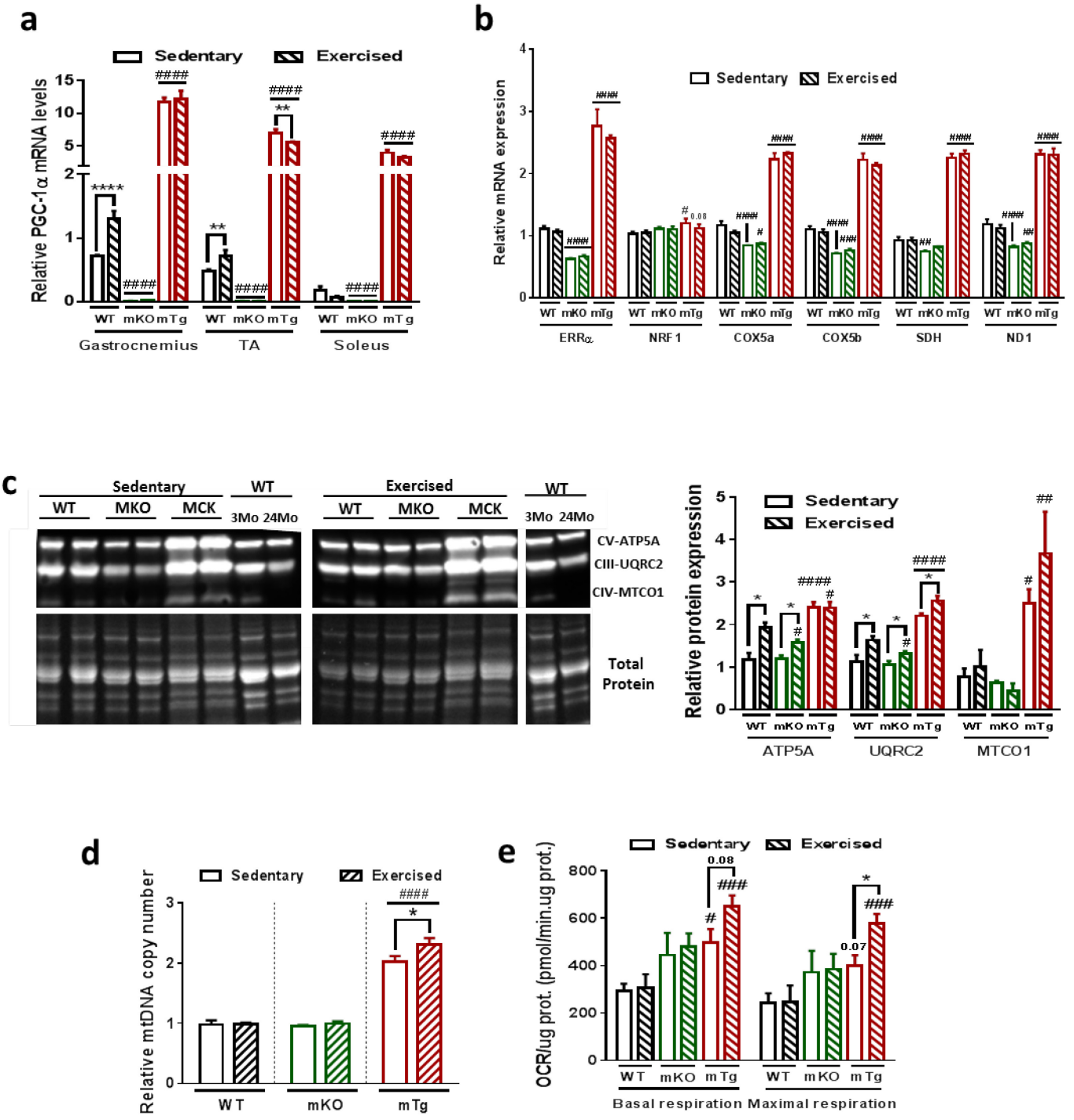

Figure 1: mPGC-1α levels determine exercise-related mitochondrial improvements (a) Relative mPGC-1α gene expression (n=3-6). **(b)** Relative mRNA levels of mitochondrial genes (n=6) **(c)** Mitochondrial OXPHOS protein levels (n=6). **(d)** Mitochondrial mass (n=6) and **(e)** mitochondrial basal and maximal respiration capacities (n=5-6). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between sedentary and exercised animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes for sedentary and exercised animals.

Figure 2

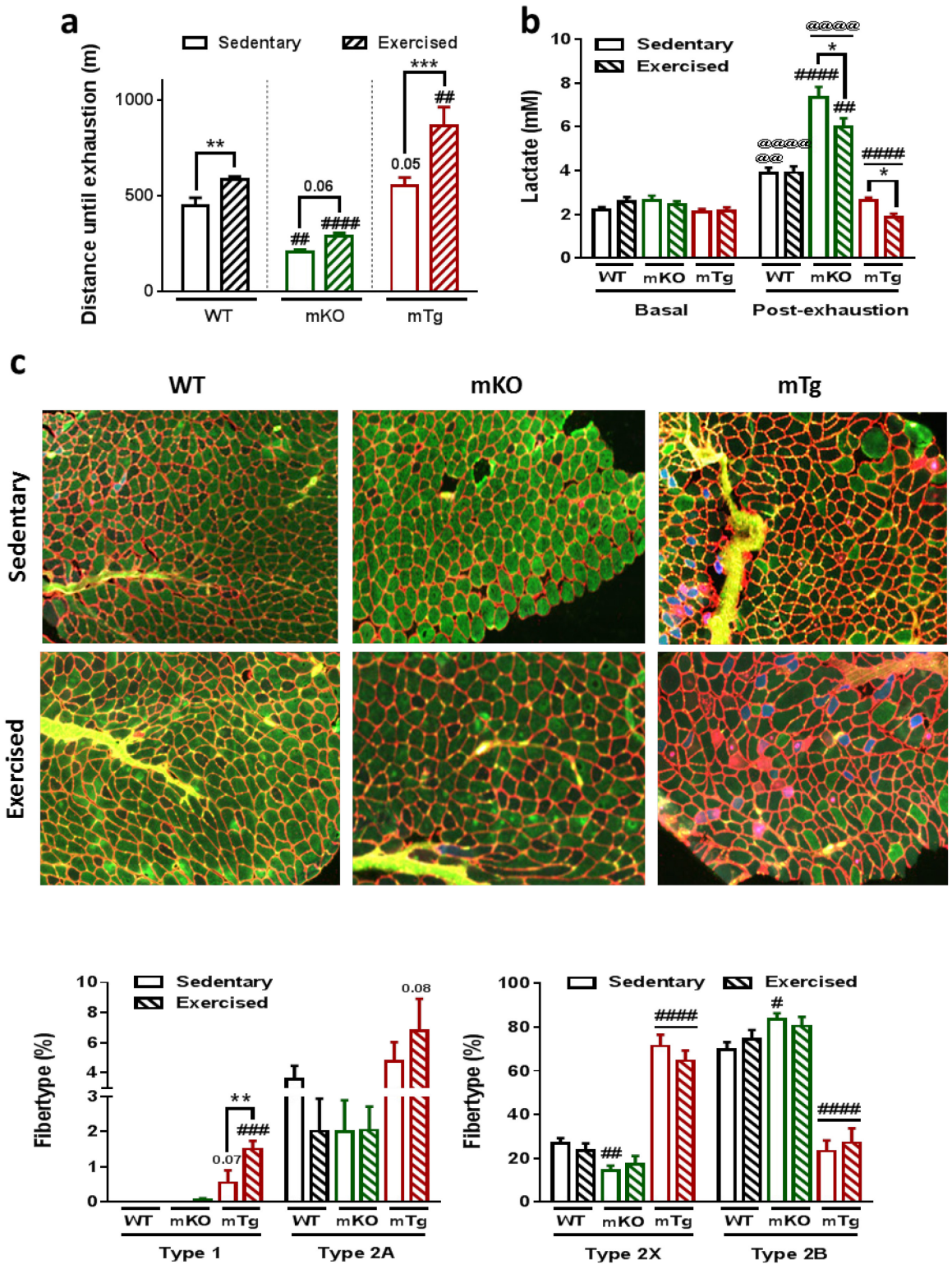
a
Sedentary
Exercised
Distance until exhaustion (m)
1000
500
0
**
0.06
##
####
***
0.05
##
WT
mKO
mTg
b
Sedentary
Exercised
Lactate (mM)
10
8
6
4
2
0
@@@@
*
####
##
@@@@
@@
####
*
WT
mKO
mTg
WT
mKO
mTg
Basal
Post-exhaustion
c
WT
mKO
mTg
Sedentary
Exercised
Sedentary
Exercised
Fibertype (%)
10
8
6
4
3
2
1
0
**
###
0.07
0.08
WT
mKO
mTg
WT
mKO
mTg
Type 1
Type 2A
Sedentary
Exercised
Fibertype (%)
100
80
60
40
20
0
##
####
#
####
WT
mKO
mTg
WT
mKO
mTg
Type 2X
Type 2B

Figure 2: mPGC-1α potentiates exercise-dependent endurance increase (a) Endurance level of mice challenged on a treadmill (n=8-10). **(b)** Pre- and post-exercise blood lactate levels (n=8-10) **(c)** Representative pictures and quantification of fiber type staining (red = type 1 fibers; blue = type 2A fibers ; green = type 2B fibers ; black = type 2X fibers) (n=4-6). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between sedentary and exercised animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes for sedentary and exercised animals.

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Figure 3

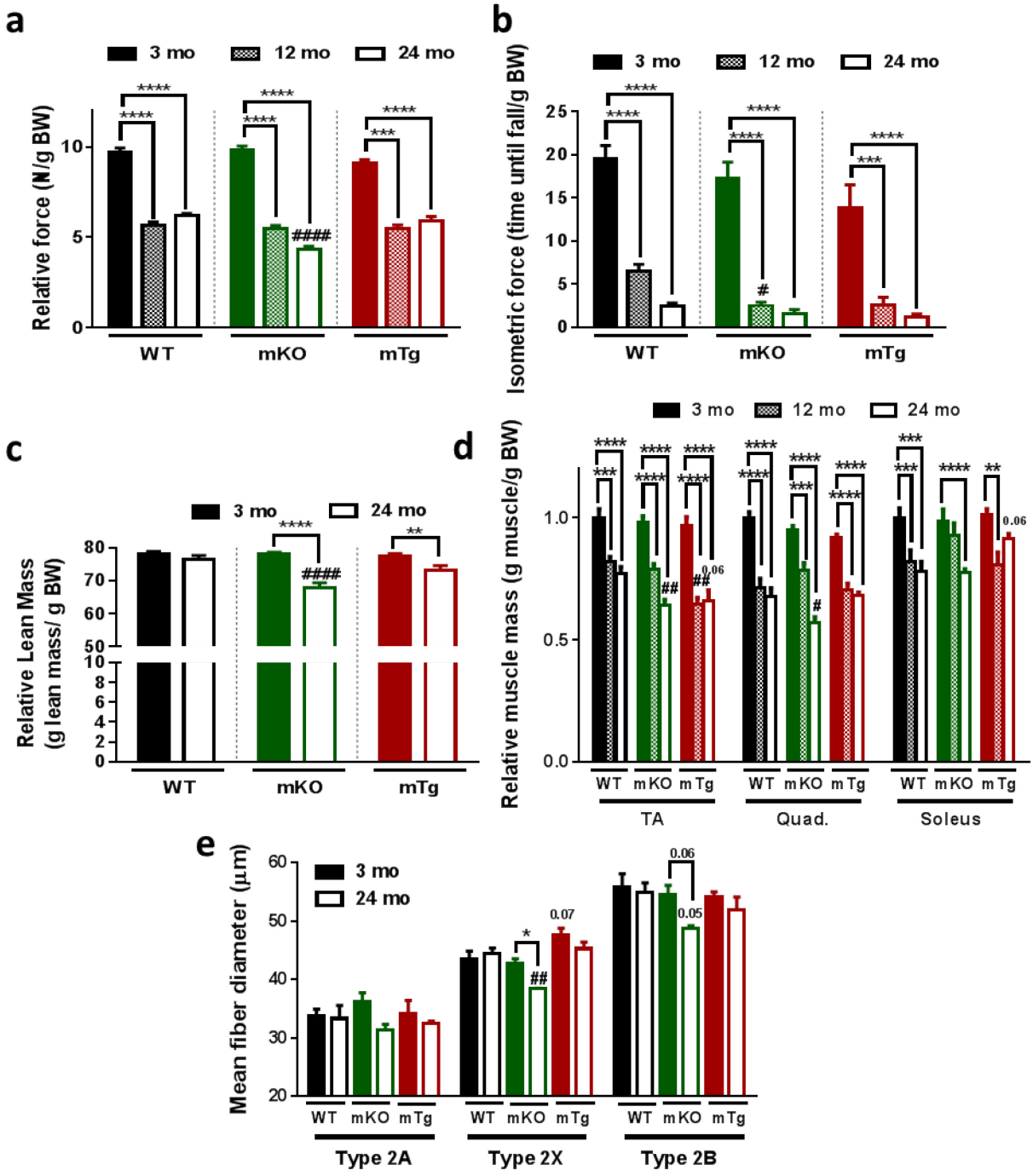

a
3 mo
12 mo
24 mo
Relative force (N/g BW)
WT
mKO
mTg
b
Isometric force (time until fall/g BW)
c
Relative Lean Mass (g lean mass/ g BW)
d
Relative muscle mass (g muscle/g BW)
TA
Quad.
Soleus
e
Mean fiber diameter (µm)
Type 2A
Type 2X
Type 2B

Figure 3: Absence of mPGC-1α accelerates sarcopenia (a) Maximal force (n=10-12), **(b)** isometric force (n=10-12), **(c)** lean relative to body mass (n=10) and **(d)** muscle mass relative to body weight (n=5-6) and **(e)** Fiber type specific minimum ferret diameter (n=3-4) Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals.

Figure 4

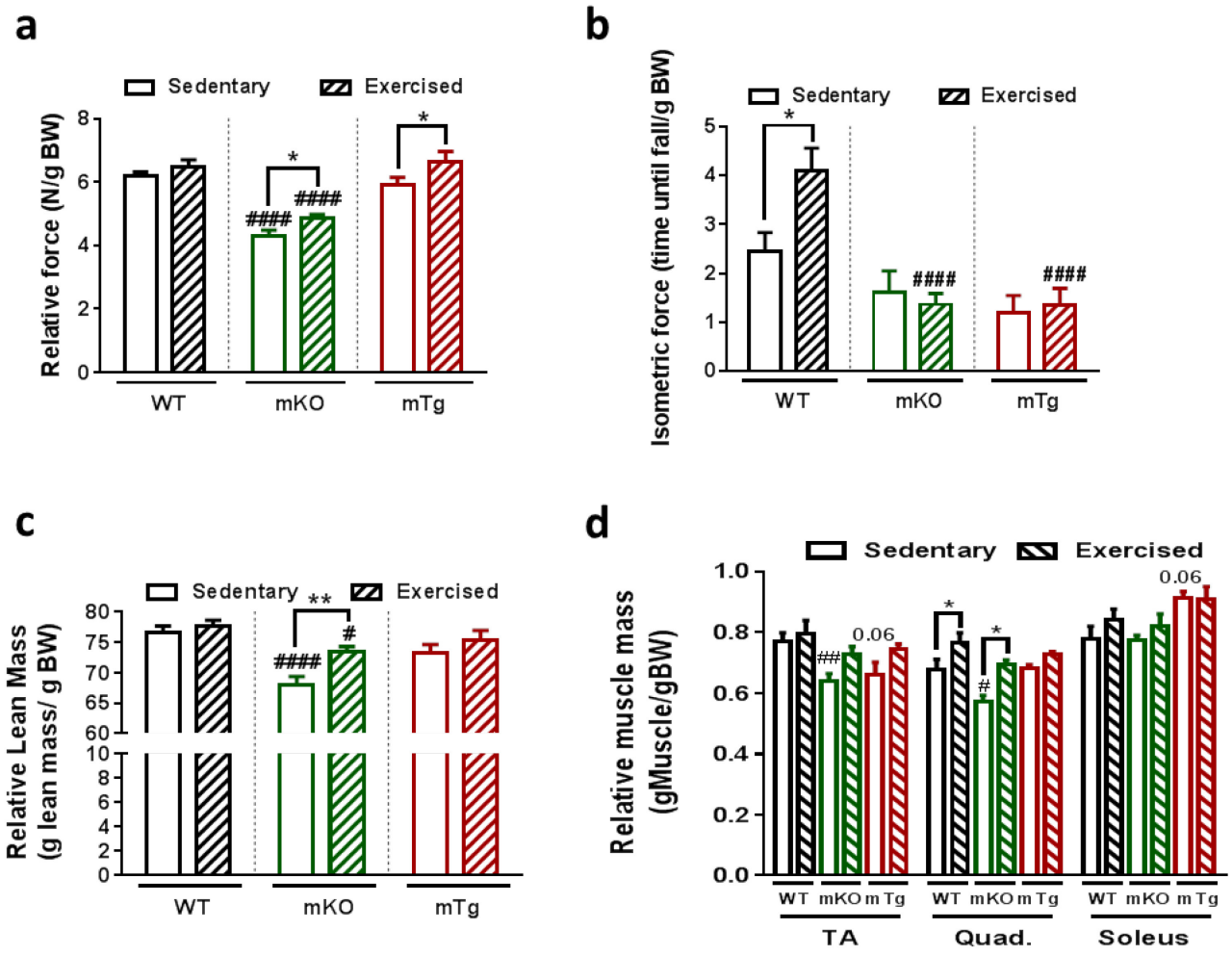

a
Sedentary
Exercised
Relative force (N/g BW)
WT
mKO
mTg
b
Isometric force (time until fall/g BW)
c
Relative Lean Mass (g lean mass/ g BW)
d
Relative muscle mass (gMuscle/gBW)
TA
Quad.
Soleus
0.06

Figure 4: Exercise reduces pre-mature sarcopenia in the absence of mPGC-1α (a) Maximal force (n=8-10), **(b)** isometric force (n=8-10), **(c)** lean relative to body mass (n=8-10) and **(d)** muscle mass relative to body weight (n=5-6). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between sedentary and exercised animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes for sedentary and exercised animals.

Figure 5

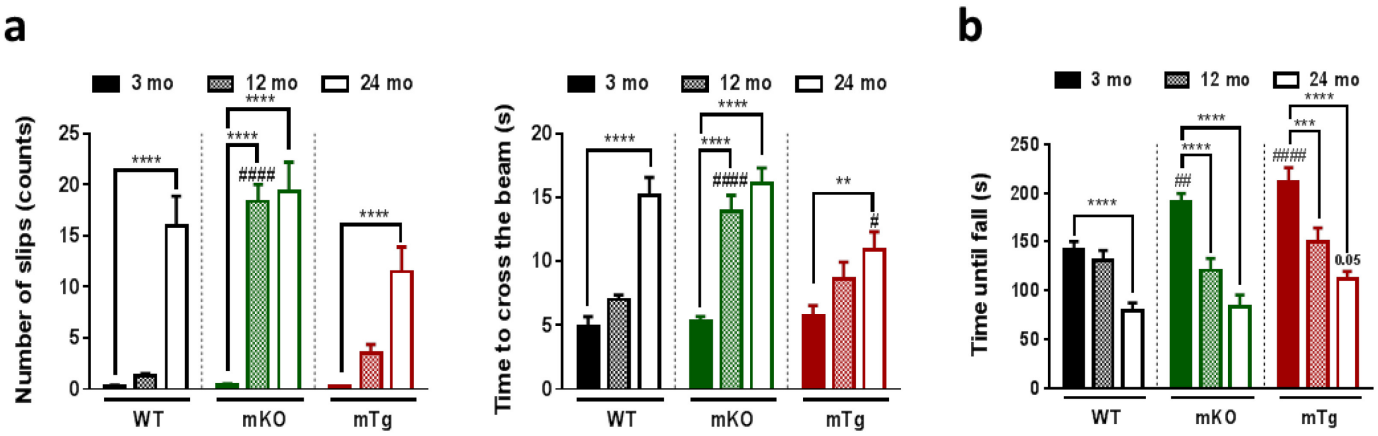

a
b
3 mo
12 mo
24 mo
Number of slips (counts)
Time to cross the beam (s)
Time until fall (s)
WT
mKO
mTg
****
####
**
#
##
###
0.05

Figure 5: mPGC-1α delays locomotor dysfunction during aging (a) Balance performances measured during balance beam crossing (n=10-12). **(b)** Motor coordination of mice challenged with a rotarod (n=10-12). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals.

Figure 6

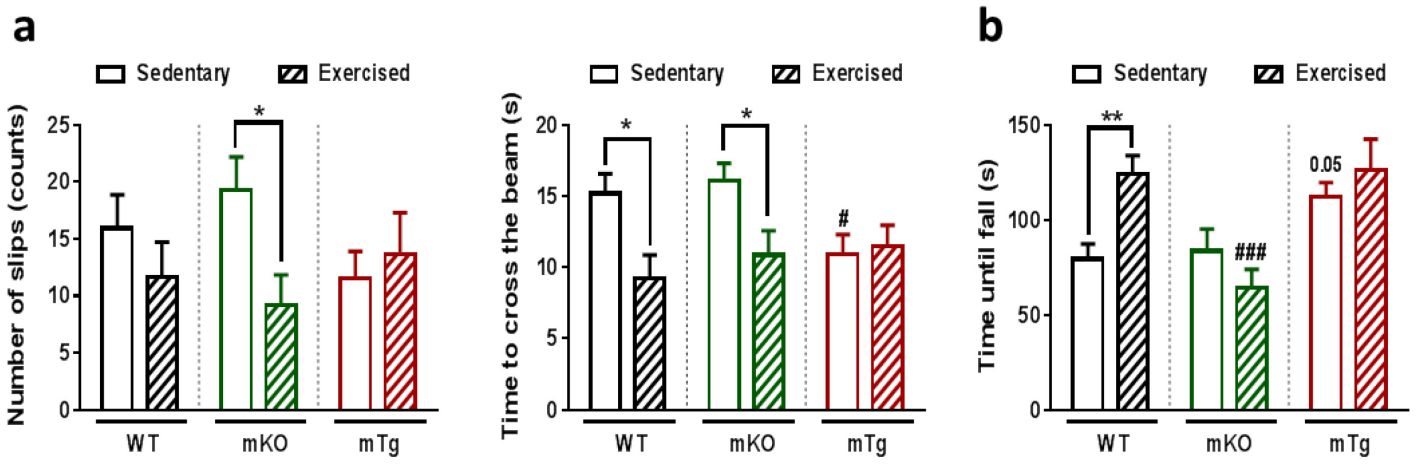

a
Sedentary
Exercised
Number of slips (counts)
25
20
15
10
5
0
*
WT
mKO
mTg
Time to cross the beam (s)
20
15
10
5
0
*
*
#
b
Time until fall (s)
150
100
50
0
**
###
0.05

Figure 6: mPGC-1α elevation mimics exercise to improve motor function in aged mice (a) Balance performances measured during balance beam crossing (n=8-10). **(b)**Mmotor coordination of mice challenged with a rotarod (n=10). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between sedentary and exercised animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes for sedentary and exercised animals.

Figure S1

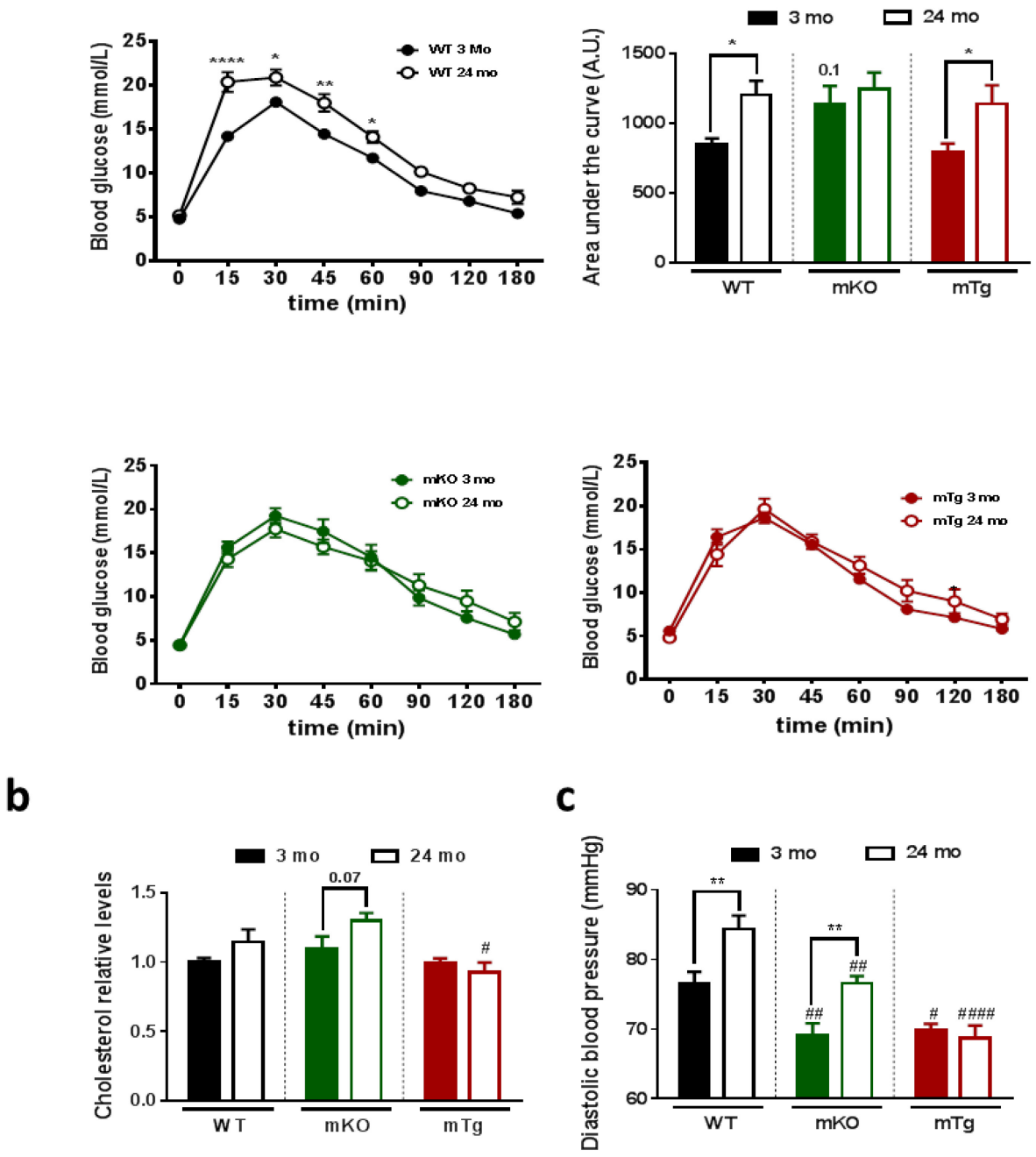

a
Blood glucose (mmol/L)
time (min)
WT 3 Mo
WT 24 mo
Area under the curve (A.U.)
3 mo
24 mo
WT
mKO
mTg
mKO 3 mo
mKO 24 mo
mTg 3 mo
mTg 24 mo
b
Cholesterol relative levels
0.07
c
Diastolic blood pressure (mmHg)

Figure S1: mPGC-1α slightly improves systemic aging (a) Glucose tolerance test curves and area under the curves (n=10), **(b)** total blood cholesterol levels (n=9-10) and **(c)** diastolic blood pressure (n=10). Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes of age-matched animals.

Figure S2

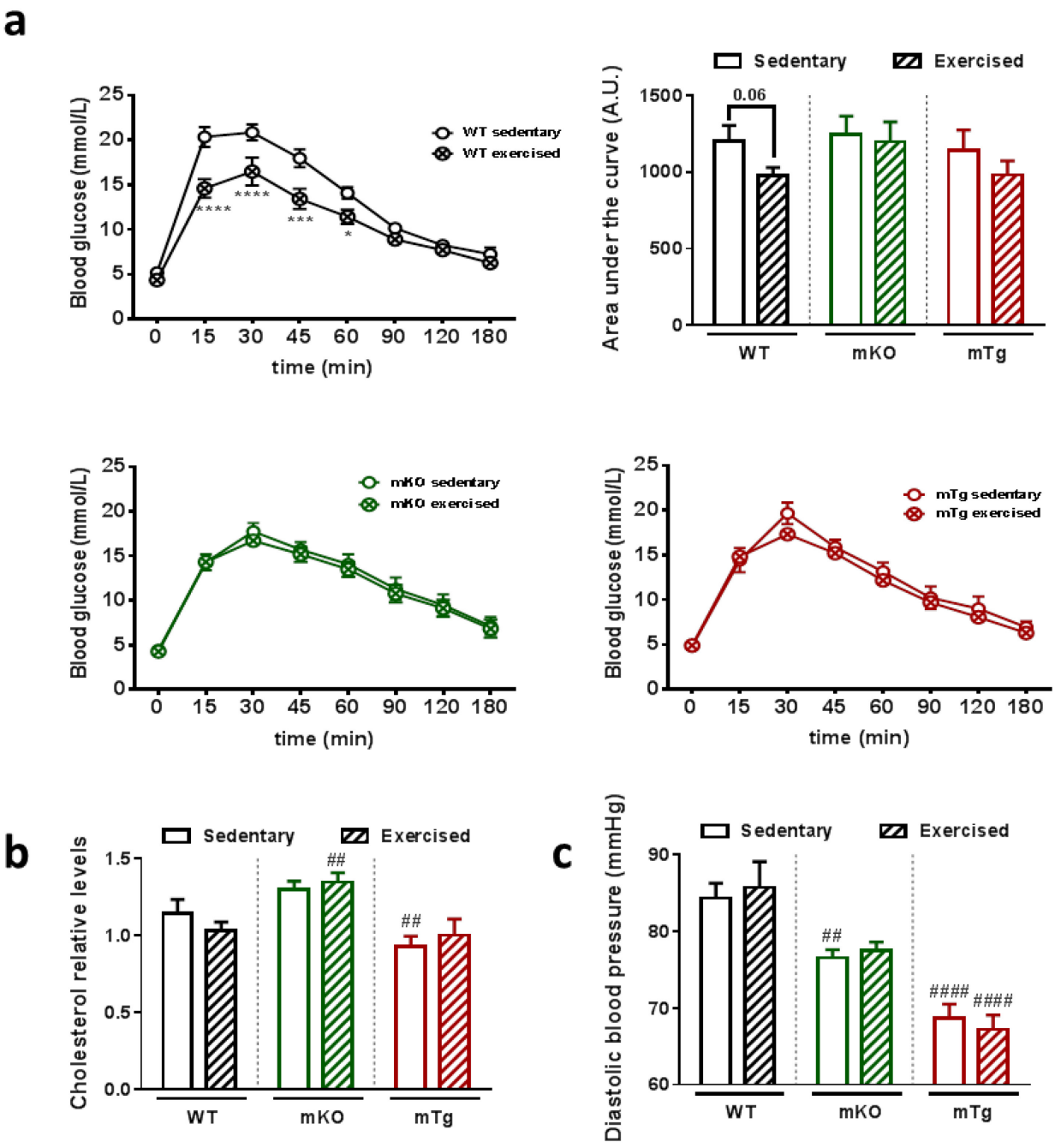

a
Blood glucose (mmol/L)
time (min)
WT sedentary
WT exercised
mKO sedentary
mKO exercised
mTg sedentary
mTg exercised
Area under the curve (A.U.)
Sedentary
Exercised
0.06
WT
mKO
mTg
b
Cholesterol relative levels
c
Diastolic blood pressure (mmHg)

Figure S2: Exercise and mPGC-1α modulation impacts on systemic aging (a) Glucose tolerance test curves and area under the curves (n=8-10), **(b)** total blood cholesterol levels (n=8-10) and **(c)** diastolic blood pressure (n=10-12) Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between sedentary and exercised animals of the same genotype, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between genotypes for sedentary and exercised animals.

G. References

Arnold, A. S., J. Gill, M. Christe, R. Ruiz, S. McGuirk, J. St-Pierre, L. Tabares and C. Handschin (2014). "Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1alpha." Nature communications **5**: 3569.

Baloh, R. W. (1996). "Vestibular and auditory disorders." Curr Opin Neurol **9**(1): 32-36.

Basaldella, E., A. Takeoka, M. Sigrist and S. Arber (2015). "Multisensory Signaling Shapes Vestibulo-Motor Circuit Specificity." Cell **163**(2): 301-312.

Berchtold, M. W., H. Brinkmeier and M. Muntener (2000). "Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease." Physiol Rev **80**(3): 1215-1265.

Booth, F. W., C. K. Roberts and M. J. Laye (2012). "Lack of exercise is a major cause of chronic diseases." Comprehensive Physiology **2**(2): 1143-1211.

Cannavino, J., L. Brocca, M. Sandri, B. Grassi, R. Bottinelli and M. A. Pellegrino (2015). "The role of alterations in mitochondrial dynamics and PGC-1alpha over-expression in fast muscle atrophy following hindlimb unloading." The Journal of physiology **593**(8): 1981-1995.

Cartee, G. D., R. T. Hepple, M. M. Bamman and J. R. Zierath (2016). "Exercise Promotes Healthy Aging of Skeletal Muscle." Cell metabolism **23**(6): 1034-1047.

Egan, B. and J. R. Zierath (2013). "Exercise metabolism and the molecular regulation of skeletal muscle adaptation." Cell metabolism **17**(2): 162-184.

Garatachea, N., H. Pareja-Galeano, F. Sanchis-Gomar, A. Santos-Lozano, C. Fiuza-Luces, M. Moran, E. Emanuele, M. J. Joyner and A. Lucia (2015). "Exercise attenuates the major hallmarks of aging." Rejuvenation research **18**(1): 57-89.

Ghosh, S., R. Lertwattanarak, N. Lefort, M. Molina-Carrion, J. Joya-Galeana, B. P. Bowen, J. Garduno-Garcia Jde, M. Abdul-Ghani, A. Richardson, R. A. DeFronzo, L. Mandarino, H. Van Remmen and N. Musi (2011). "Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance." Diabetes **60**(8): 2051-2060.

Gouspillou, G., N. Sgarioto, S. Kapchinsky, F. Purves-Smith, B. Norris, C. H. Pion, S. Barbat-Artigas, F. Lemieux, T. Taivassalo, J. A. Morais, M. Aubertin-Leheudre and R. T. Hepple (2014). "Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **28**(4): 1621-1633.

Gremeaux, V., M. Gayda, R. Lepers, P. Sosner, M. Juneau and A. Nigam (2012). "Exercise and longevity." Maturitas **73**(4): 312-317.

Handschin, C., C. S. Choi, S. Chin, S. Kim, D. Kawamori, A. J. Kurpad, N. Neubauer, J. Hu, V. K. Mootha, Y. B. Kim, R. N. Kulkarni, G. I. Shulman and B. M. Spiegelman (2007). "Abnormal glucose homeostasis in

skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk." The Journal of clinical investigation **117**(11): 3463-3474.

Handschin, C., Y. M. Kobayashi, S. Chin, P. Seale, K. P. Campbell and B. M. Spiegelman (2007). "PGC-1alpha regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy." Genes & development **21**(7): 770-783.

Hildrum, B., A. Mykletun, T. Hole, K. Midthjell and A. A. Dahl (2007). "Age-specific prevalence of the metabolic syndrome defined by the International Diabetes Federation and the National Cholesterol Education Program: the Norwegian HUNT 2 study." BMC Public Health **7**: 220.

Hoeks, J., Z. Arany, E. Phielix, E. Moonen-Kornips, M. K. Hesselink and P. Schrauwen (2012). "Enhanced lipid-but not carbohydrate-supported mitochondrial respiration in skeletal muscle of PGC-1alpha overexpressing mice." Journal of cellular physiology **227**(3): 1026-1033.

Holloszy, J. O. and E. F. Coyle (1984). "Adaptations of skeletal muscle to endurance exercise and their metabolic consequences." J Appl Physiol Respir Environ Exerc Physiol **56**(4): 831-838.

Johnsson, L. G. (1971). "Degenerative changes and anomalies of the vestibular system in man." Laryngoscope **81**(10): 1682-1694.

Kang, C. H., E. Chung, G. Diffee and L. L. Ji (2013). "Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: Role of PGC-1 alpha." Experimental Gerontology **48**(11): 1343-1350.

Leick, L., S. S. Lyngby, J. F. Wojtaszewski and H. Pilegaard (2010). "PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle." Experimental gerontology **45**(5): 336-342.

Lin, J., H. Wu, P. T. Tarr, C. Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby and B. M. Spiegelman (2002). "Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres." Nature **418**(6899): 797-801.

Lord, S. R., R. D. Clark and I. W. Webster (1991). "Physiological factors associated with falls in an elderly population." Journal of the American Geriatrics Society **39**(12): 1194-1200.

Lucas, E. K., S. E. Dougherty, L. J. McMeekin, A. T. Trinh, C. S. Reid and R. M. Cowell (2012). "Developmental alterations in motor coordination and medium spiny neuron markers in mice lacking pgc-1alpha." PloS one **7**(8): e42878.

Marcell, T. J. (2003). "Sarcopenia: causes, consequences, and preventions." The journals of gerontology. Series A, Biological sciences and medical sciences **58**(10): M911-916.

Nair, K. S. (2005). "Aging muscle." The American journal of clinical nutrition **81**(5): 953-963.

Perez-Schindler, J., S. Summermatter, G. Santos, F. Zorzato and C. Handschin (2013). "The transcriptional coactivator PGC-1alpha is dispensable for chronic overload-induced skeletal muscle hypertrophy and metabolic remodeling." Proceedings of the National Academy of Sciences of the United States of America **110**(50): 20314-20319.

Perrone-Filardi, P., S. Paolillo, P. Costanzo, G. Savarese, B. Trimarco and R. O. Bonow (2015). "The role of metabolic syndrome in heart failure." Eur Heart J **36**(39): 2630-2634.

Rolland, Y., S. Czerwinski, G. Abellan Van Kan, J. E. Morley, M. Cesari, G. Onder, J. Woo, R. Baumgartner, F. Pillard, Y. Boirie, W. M. Chumlea and B. Vellas (2008). "Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives." The journal of nutrition, health & aging **12**(7): 433-450.

Safdar, A., J. P. Little, A. J. Stokl, B. P. Hettinga, M. Akhtar and M. A. Tarnopolsky (2011). "Exercise increases mitochondrial PGC-1alpha content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis." The Journal of biological chemistry **286**(12): 10605-10617.

Sahin, E., S. Colla, M. Liesa, J. Moslehi, F. L. Muller, M. Guo, M. Cooper, D. Kotton, A. J. Fabian, C. Walkey, R. S. Maser, G. Tonon, F. Foerster, R. Xiong, Y. A. Wang, S. A. Shukla, M. Jaskelioff, E. S. Martin, T. P. Heffernan, A. Protopopov, E. Ivanova, J. E. Mahoney, M. Kost-Alimova, S. R. Perry, R. Bronson, R. Liao, R. Mulligan, O. S. Shirihai, L. Chin and R. A. DePinho (2011). "Telomere dysfunction induces metabolic and mitochondrial compromise." Nature **470**(7334): 359-365.

Sandri, M., J. Lin, C. Handschin, W. Yang, Z. P. Arany, S. H. Lecker, A. L. Goldberg and B. M. Spiegelman (2006). "PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription." Proceedings of the National Academy of Sciences of the United States of America **103**(44): 16260-16265.

Schreiber, S. N., R. Emter, M. B. Hock, D. Knutti, J. Cardenas, M. Podvinec, E. J. Oakeley and A. Kralli (2004). "The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis." Proceedings of the National Academy of Sciences of the United States of America **101**(17): 6472-6477.

Schultz, A. B., J. A. Ashton-Miller and N. B. Alexander (1997). "What leads to age and gender differences in balance maintenance and recovery?" Muscle Nerve Suppl **5**: S60-64.

Sczelecki, S., A. Besse-Patin, A. Abboud, S. Kleiner, D. Laznik-Bogoslavski, C. D. Wrann, J. L. Ruas, B. Haibe-Kains and J. L. Estall (2014). "Loss of Pgc-1alpha expression in aging mouse muscle potentiates glucose intolerance and systemic inflammation." American journal of physiology. Endocrinology and metabolism **306**(2): E157-167.

Short, K. R., M. L. Bigelow, J. Kahl, R. Singh, J. Coenen-Schimke, S. Raghavakaimal and K. S. Nair (2005). "Decline in skeletal muscle mitochondrial function with aging in humans." Proceedings of the National Academy of Sciences of the United States of America **102**(15): 5618-5623.

St-Pierre, J., S. Drori, M. Uldry, J. M. Silvaggi, J. Rhee, S. Jager, C. Handschin, K. Zheng, J. Lin, W. Yang, D. K. Simon, R. Bachoo and B. M. Spiegelman (2006). "Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators." Cell **127**(2): 397-408.

Summermatter, S., R. Thurnheer, G. Santos, B. Mosca, O. Baum, S. Treves, H. Hoppeler, F. Zorzato and C. Handschin (2012). "Remodeling of calcium handling in skeletal muscle through PGC-1alpha: impact on force, fatigability, and fiber type." American journal of physiology. Cell physiology **302**(1): C88-99.

Valdez, G., J. C. Tapia, H. Kang, G. D. Clemenson, Jr., F. H. Gage, J. W. Lichtman and J. R. Sanes (2010). "Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise." Proceedings of the National Academy of Sciences of the United States of America **107**(33): 14863-14868.

Ventura-Clapier, R., B. Mettauer and X. Bigard (2007). "Beneficial effects of endurance training on cardiac and skeletal muscle energy metabolism in heart failure." Cardiovascular research **73**(1): 10-18.

Vina, J., M. C. Gomez-Cabrera, C. Borras, T. Froio, F. Sanchis-Gomar, V. E. Martinez-Bello and F. V. Pallardo (2009). "Mitochondrial biogenesis in exercise and in ageing." Advanced Drug Delivery Reviews **61**(14): 1369-1374.

Wenz, T. (2011). "Mitochondria and PGC-1alpha in Aging and Age-Associated Diseases." Journal of aging research **2011**: 810619.

Wenz, T., F. Diaz, B. M. Spiegelman and C. T. Moraes (2008). "Activation of the PPAR/PGC-1alpha pathway prevents a bioenergetic deficit and effectively improves a mitochondrial myopathy phenotype." Cell metabolism **8**(3): 249-256.

Wenz, T., S. G. Rossi, R. L. Rotundo, B. M. Spiegelman and C. T. Moraes (2009). "Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging." Proceedings of the National Academy of Sciences of the United States of America **106**(48): 20405-20410.

Wu, Z., P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell and R. Scarpulla (1999). "Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1." Cell **98**(1): 115-124.

Zhao, W., M. Varghese, S. Yemul, Y. Pan, A. Cheng, P. Marano, S. Hassan, P. Vempati, F. Chen, X. Qian and G. M. Pasinetti (2011). "Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis." Molecular neurodegeneration **6**(1): 51.

V. Discussion and outlook

A. The role of PGC-1α in the arcuate nucleus

Since its identification and characterization as an important regulator of BAT thermogenesis in 1998 **(Puigserver, Wu et al. 1998)**, PGC-1α has been defined as a key player for cellular metabolic transcriptional adaptation in various tissues in response to different environmental conditions such as cold exposure, fasting and exercise. For example, for more than a decade, it has been extensively described in the muscle as an inducer of mitochondrial biogenesis and oxidative metabolism **(Lin, Wu et al. 2002, Pagel-Langenickel, Bao et al. 2008)**. Interestingly, identification of a broad panel of novel functions in multiple tissues has been made regarding this coactivator of transcription and the list keeps increasing even today. Notably, while it was largely described as a major regulator of muscle metabolism, its recent association with the regulation and secretion of emerging myokines indicates that PGC-1α also regulates whole body metabolism and homeostasis via crosstalks between muscle and other tissues. Importantly, the secretion of several muscle factors induced by PGC-1α triggers whole body homeostasis adaptations upon exercise **(Schnyder and Handschin 2015)**. Muscle PGC-1α is therefore not only used to promote a response to exercise in myofibers but also acts as a physical activity sensor for the whole body. For example, the release of irisin, BAIBA and Metrnl from the muscle, controlled by PGC-1α and induced upon exercise, all increase WAT browning and energy expenditure and improve whole body glucose homeostasis **(Bostrom, Wu et al. 2012, Lee, Linderman et al. 2014, Rao, Long et al. 2014, Roberts, Bostrom et al. 2014)**. In addition, PGC-1α further influences glucose homeostasis and insulin sensitivity by regulating insulin-stimulated glucose uptake and disposal in the muscle **(Pagel-Langenickel, Bao et al. 2008, Bonen 2009)** and hepatic gluconeogenesis upon fasting **(Lin, Handschin et al. 2005)**. The role of muscle and liver PGC-1α as a metabolic sensor and regulator of whole body glucose homeostasis raised the question as to whether it could have a similar function in other tissues that require sensing of metabolic signals to control whole body energy homeostasis.

1. PGC-1α is important for energy balance regulation in AgRP neurons

Since the arcuate nucleus is a main regulator of systemic energy balance, we generated mouse models with PGC-1α ablation in AgRP and POMC neurons and unraveled its importance in the control of feeding and energy expenditure by this brain region. We and others found that PGC-1α is induced in the hypothalamus upon fasting **(Coppari, Ramadori et al. 2009)** and in an immortalized AgRP cell line upon serum starvation. This is reminiscent of its regulation in the liver. Interestingly, FOXO1 is necessary for PGC-1α mediated insulin stimulation of gluconeogenesis in the liver **(Puigserver, Rhee et al. 2003)** and also required for insulin and leptin induction of food intake in AgRP neurons **(Kitamura, Feng et al. 2006)**. Moreover, PGC-1α deletion in AgRP neurons prevents leptin-mediated inhibition of food intake. It is therefore tempting to hypothesize that PGC-1α works together with FOXO1 to regulate food intake through the integration of hormonal signaling in the arcuate nucleus. In addition to inhibiting leptin action on AgRP neurons, fasting induction of both food intake and AgRP expression are blunted in mice lacking PGC-1α in AgRP neurons. Interestingly, as PGC-1α, Sirt1 is induced in AgRP neurons upon fasting and its inhibition diminishes fasting-stimulated AgRP expression **(Cakir, Perello et al. 2009)**. Since SIRT1 is a very well-known activator of PGC-1α, the similarities between the phenotypes triggered by SITR1 and PGC-1α inhibition suggest that food intake stimulation upon fasting is mediated by a SIRT1-PGC-1α axis that promotes AgRP upregulation. On the other hand, AMPK, another regulator of PGC-1α, increases fasting-induced AgRP expression **(Minokoshi, Alquier et al. 2004)**. Its activation upon food deprivation in AgRP neurons also drives fasting-induced synaptic remodeling that is required to promote feeding **(Kong, Dagon et al. 2016)**. PGC-1α, in addition to allowing fasting induction of AgRP expression, also regulates synaptic plasticity in muscle **(Arnold, Gill et al. 2014)** and in neurons **(Cheng, Wan et al. 2012)** and might thus be involved in AMPK control of AgRP synaptic remodeling upon fasting.

Intriguingly, despite observing hypophagia in both ad-libitum and fasting conditions, we found that mice with deletion of PGC-1α in AgRP neurons exhibit an increase in body weight and fat mass. This paradoxical phenotype was reminiscent of alterations observed in AgRP neurons ablated mice, which display higher feeding efficiency and higher body weight in spite of lower feeding response upon food intake stimulation **(Joly-Amado, Denis et al. 2012)**. Thus, similarly to AgRP neurons ablated mice, increased body mass observed in our mice could be due to altered nutrient partitioning and utilization in peripheral tissues, such as muscle or WAT. Of note, whole brain deletion of PGC-1α leads to hepatic steatosis, which can be caused by alterations in nutrient and especially lipid partitioning in the liver. It might therefore be of interest to evaluate any potential liver damage in our AgRP-PGC1α KO mice to determine if the liver steatosis observed in mice with whole brain deletion of PGC-1α arises from arcuate nucleus alterations. Aside from nutrient partitioning, reduction of locomotor activity and body temperature in mice lacking PGC-1α in AgRP neurons indicate that they waste less energy. This may also contribute to their increase in fat mass. It is worth noting that AgRP neurons have been shown to regulate BAT thermogenesis and WAT browning **(Ruan, Dietrich et al. 2014)**. It would thus be interesting to further determine whether alterations of AgRP neurons upon PGC-1α deletion lead to reduced BAT thermogenesis and WAT browning, which might result in the observed diminished body temperature.

At the time of this thesis, other studies were unraveling novel processes involved in the arcuate nucleus' control of energy homeostasis, which could be impaired by PGC-1α deletion. For example, mitofusin 1 and 2 have been implicated in the regulation of energy homeostasis and obesity by AgRP neurons **(Dietrich, Liu et al. 2013)**. Interestingly, while studying the role of PGC-1α in muscle aging we observed that PGC-1α strongly regulates mitofusins in myofibers, as it was previously described **(Cartoni, Leger et al. 2005)**. It has been reported that hepatic PGC-1α elevation upon leptin treatment stimulates Mfn1 activity to improve hyperlipidemia **(Hsu, Lee et al. 2015)**. Thus, the control of mitochondrial fusion and fission regulating energy homeostasis within AgRP neurons could also be impaired by PGC-1α deletion in those neurons and contribute the disruption of energy balance that we observed in our mice. Also,

mitochondrial content is increased in AgRP neurons, which is activated by a negative energy balance and is decreased in diet induced obese animals **(Dietrich, Liu et al. 2013)**. Given the master role of PGC-1α in mitochondrial biogenesis, one could postulate that it is also involved in the regulation of mitochondrial mass in AgRP neurons which is important for energy balance regulation. Likewise, PPARγ, an important regulator of FAO that interacts with PGC-1α **(Puigserver, Wu et al. 1998)**, has been recently shown to control energy balance in the arcuate nucleus. Its activation is sufficient to promote AgRP/NPY gene expression and increase food intake through AgRP neurons **(Garretson, Teubner et al. 2015)**. Whether it is also interacting with PGC-1α in AgRP neurons to modulate food intake remains unclear and would be worth studying.

2. Limitation of the study and potential complementary experiment

Although we reported alterations in energy balance upon PGC-1α deletion in AgRP neurons, we observed only a mild phenotype in this mouse model and no phenotype upon PGC-1α ablation in POMC neurons. This could be due to residual expression of PGC-1α in AgRP/POMC neurons. Indeed, even though we detected a recombination indicating the deletion of PGC-1α in arcuate nucleus punches and the restriction of this deletion to the arcuate nucleus, this does not preclude that some AgRP/POMC neurons still express normal PGC-1α levels and does not indicate how much PGC-1α expression is reduced in the targeted neurons. Multiple methods have been used to determine the efficiency of PGC-1α deletion with a lack of success for different reasons. In a first attempt, we measured PGC-1α mRNA levels in arcuate nucleus punches without observing a significant reduction. This is likely due to non-AgRP/POMC neurons present in the cell extract that still express the coactivator. Reporter mouse lines expressing EGFP in AgRP or POMC neurons have been generated to visualize PGC-1α content in those neurons after immunostaining. Unfortunately, several PGC-1α antibodies have been tested and none of them was specific as indicated by the positive signal detected in the arcuate nucleus of PGC-1α global knock-out mice. Therefore, residual PGC-1α expression might still enable most of

its activity and mask a more serious phenotype. On the other hand, we experienced difficulties in generating large groups of animals for the AgRP-PGC-1α KO line because half of the littermates were genotyped as global knock out animals. This forced us to conduct experiments with several cohorts of animals when high numbers of mice were required, which increased variability and reduced the number of animals that could be used for some experiments. To avoid this issue, we could have tried to generate a second knock out mouse line with the tamoxifen-inducible AgRP-cre mouse models recently developed by Elmquist and colleagues **(Berglund, Liu et al. 2013, Wang, Liu et al. 2014)**.

In addition to the results obtained in this study, complementary experiments supporting the effect of PGC-1α in the arcuate nucleus could have been performed. For example, stereotaxic injections of resveratrol could have increased the AMPK/SIRT1-mediated activation of our transcriptional coactivator. This would have allowed us to measure the consequences of PGC-1α activation in AgRP neurons. In addition, the abrogation of the resveratrol effect upon PGC-1α deletion would have confirmed the role of the coactivator in mediating resveratrol action in the arcuate nucleus. However, while some works indeed presented an activation of the SIRT1/PGC1α pathway in muscle **(Lagouge, Argmann et al. 2006)**, our laboratory recently showed that PGC-1α was dispensable for the systemic effect resveratrol and observed only marginal effects of this compound in muscle **(Svensson, Schnyder et al. 2015)**. Moreover, our research group lacked the expertise in that kind of neuronal treatment. We therefore decided that those experiments were not valuable enough regarding the mild phenotype we observed in our mice and the possible failure of PGC-1α activation upon resveratrol treatment.

Similarly, since the deletion of PGC-1α resulted in a mild or a complete absence of phenotype our mice, another interesting long term experiment would have been to establish mouse models with constant overexpression of PGC-1α in AgRP and POMC neurons. Indeed, in muscle, PGC-1α overexpression leads to much more drastic effects and remodeling than its ablation. For example, as we showed in this thesis, muscle loss of PGC-1α only impaired OXPHOS protein and mRNA levels while its overexpression led to a greater increase in

mitochondrial densities and function. It would therefore be interesting to evaluate whether increased PGC-1α content in POMC and AgRP neurons would result in larger alterations of whole body homeostasis than its deletion.

3. PGC-1α and PGC-1β share a redundant function in the arcuate nucleus

Alternatively, compensatory mechanisms may have blunted more severe dysfunctions caused by the absence of PGC-1α in AgRP and POMC neurons. PGC-1β shares many common functions and expression levels with its close homolog PGC-1α in several organs **(Wu, Puigserver et al. 1999, Lin, Puigserver et al. 2002, Uldry, Yang et al. 2006, Lai, Leone et al. 2008, Wareski, Vaarmann et al. 2009, Wenz, Rossi et al. 2009, Shao, Liu et al. 2010, Mitra, Nogee et al. 2012)**. For example, both PGC-1α and PGC-1β regulate mitochondrial mass in neurons **(Wareski, Vaarmann et al. 2009)**. Moreover, PGC-1β is up and downregulated upon PGC-1α ablation and overexpression, respectively, in muscle (data not shown), strongly indicating a compensatory role for PGC-1β in the absence of PGC-1α. Finally, PGC-1β has been shown to control basal mitochondrial dynamics, whereas PGC-1α controls mitochondrial function upon enhanced energetic demand, such as exercise challenge **(Baar, Wende et al. 2002, Zorzano 2009)**. Thus, AgRP and POMC neuronal function might rely on basal mitochondrial metabolism and dynamics that can be sustained by PGC-1β in the absence of PGC-1α. For instance, PGC-1α does not influence AgRP expression in basal conditions but is important for AgRP induction upon fasting, thus supporting a role for PGC-1α in AgRP neurons' adaptability to alterations in the energetic status. In this context, further studies including both single knock-outs of PGC-1β and double knock-outs of PGC-1α/PGC-1β in AgRP and POMC neurons are required to address the role of PGC-1β in the arcuate nucleus and its redundancy with PGC-1α. In light of those elements, we recently decided to generate mice with PGC-1β ablation in AgRP and POMC neurons with the same methods that were used for the generation of the AgRP-PGC1α KO and POMC-PGC1α KO mice.

Similarly to what we did to study the role of PGC-1α in the arcuate nucleus, we characterized the phenotype of AgRP-PGC1β KO and POMC-PGC1β KO animals by measuring body composition, whole body metabolism and energy expenditure. In line with what we observed in AgRP-PGC1α KO mice, deletion of PGC-1β in AgRP neurons tended to increase relative fat mass in the mice (Fig. 1a). Although RER was not changed, mice lacking PGC-1β in AgRP cells presented significantly reduced oxygen consumption during the light phase in basal conditions and a tendency towards lower O2 consumption during the dark period (Fig. 1b and c). Correlating to lower VO2 uptake, AgRP-PGC1β KO animals exhibited diminished energy expenditure during light time (Fig. 1d), reminiscent of the lower body temperature and locomotor activity we observed in AgRP-PGC1α KO mice. To evaluate how PGC-1β deletion would affect the mice's adaptability to energetic challenges, AgRP-PGC1β KO and controls animals were fasted for 24h. In agreement with data observed in basal conditions, AgRP-PGC1β KO mice presented a reduction in both oxygen consumption and a tendency towards lower energy expenditure during the re-feeding period compared to control animals (Fig. 1e and f). Similarly to AgRP-PGC1α KO animals, this preliminary data suggests that lower energy expenditure of AgRP-PGC1β KO mice could result in the tendency towards higher fat mass observed in those animals. Interestingly, the redundant results between the phenotypes triggered by PGC-1α and PGC-1β deletion in AgRP neurons strongly suggest that both coactivators share similar functions in those cells and that they can each compensate for the deletion of the other. Regarding the consequences of the deletion of PGC-1β in POMC neurons, no differences were observed in body composition, oxygen consumption, fat utilization or energy expenditure in both basal and fasted conditions (Fig. 2a-f). This was again in agreement with what we observed when PGC-1α was ablated in those neurons. To complete the phenotypic characterization of POMC-PGC1β KO mice we implanted transmitters in their belly as we did with our AgRP-PGC1α KO mice in our previous study. In addition to the absence of changes in basal metabolism, no alteration of locomotor activity was detected in mice with PGC-1β deletion in POMC neurons (Fig .2g). However, POMC-PGC1β KO presented an increase in their core body temperature, which neared significance in the light period and was significant in the dark period (Fig. 2h). Interestingly, in a very short pilot experiment, we evaluated the body

temperature of POMC-PGC1α KO animals and found that they also had a reduced body temperature compared to control animals, especially during fasting (data not shown), further attesting to the redundancy of the phenotypes triggered by the single deletion of each homolog. This strengthens the hypothesis of a compensatory mechanism upon single deletion of each coactivator in those neuronal cells. Taken together, the data seems to indicate distinct roles for the coactivators in AgRP and POMC neuronal cells. To follow up on those interesting results, a complete characterization of AgRP-PGC1β KO animals should be done, similarly to what has been done in AgRP-PGC1α KO mice, to define how similar the functions of the different PGC-1 factors are in AgRP cells. Also, according to our new preliminary data, PGC-1β and possibly PGC-1α regulates core body temperature in POMC neurons. Consequently, further experiments modulating core body temperature, such as leptin or noradrenaline injection and cold exposure, should be done with POMC-PGC1α/β KO animals to evaluate the role of the coactivators in adaptation to such energetic challenges. Additionally, it would be of interest to determine BAT activation, WAT browning and heat dissipation in those animals. Finally, since PGC-1β and PGC-1α might share a similar role in the arcuate nucleus, it would be particularly informative to compare single and double knock-out animals in order to establish how redundant the two coactivators are and possibly unravel a much stronger phenotype by preventing their compensatory functions.

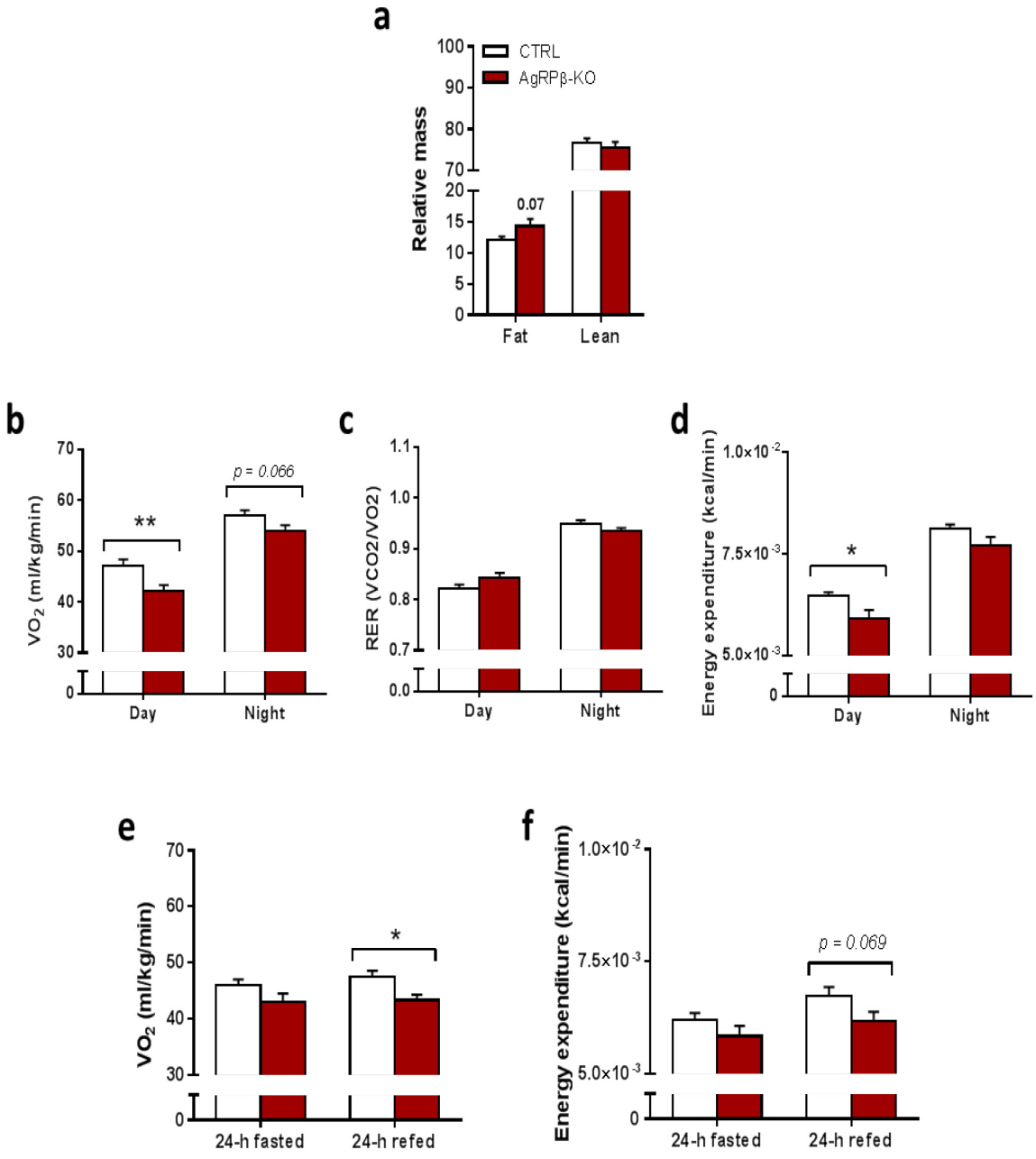

a
CTRL
AgRPβ-KO
Relative mass
0.07
Fat
Lean
b
VO2 (ml/kg/min)
**
p = 0.066
Day
Night
c
RER (VCO2/VO2)
Day
Night
d
Energy expenditure (kcal/min)
*
Day
Night
e
VO2 (ml/kg/min)
*
24-h fasted
24-h refed
f
Energy expenditure (kcal/min)
p = 0.069
24-h fasted
24-h refed

Figure 1: Consequences of PGC-1β deletion in AgRP neurons on whole body metabolism (a) Relative body composition measured by echoMRI (n=12-16). (**b-f)** Oxygen consumption, respiratory exchange ratio and energy expenditure measured in the CLAMS system in fed and fasted conditions in AgRP-PGC1β KO and WT mice (n=8). Values and error bars represent the mean ± SEM. *p<0.05 ; **p<0.01 indicate statistically significant differences between genotypes.

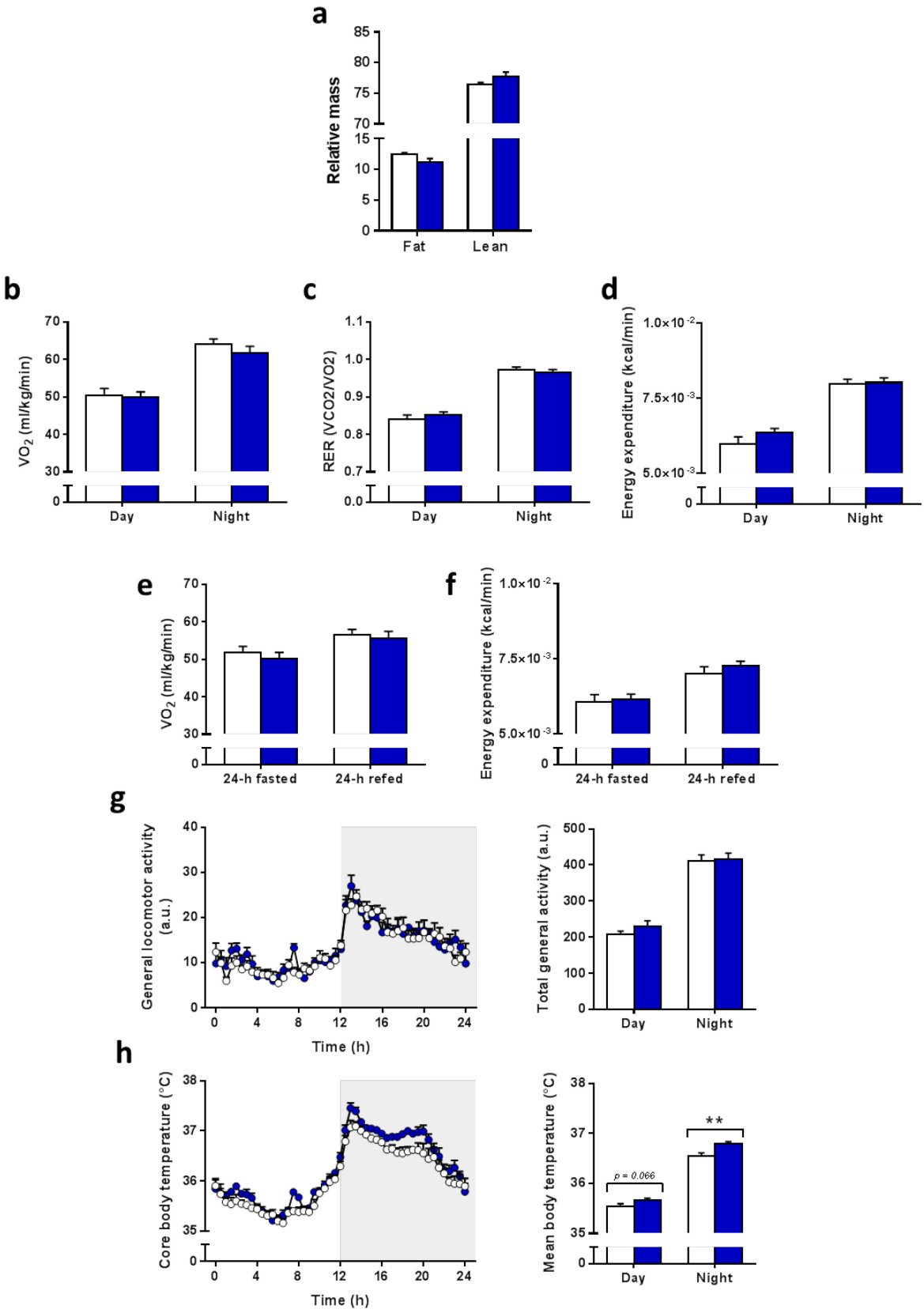

a
Relative mass
85
80
75
70
15
10
5
0
Fat
Lean
b
VO2 (ml/kg/min)
70
60
50
40
30
0
Day
Night
c
RER (VCO2/VO2)
1.1
1.0
0.9
0.8
0.7
0.0
Day
Night
d
Energy expenditure (kcal/min)
1.0×10-2
7.5×10-3
5.0×10-3
0
Day
Night
e
VO2 (ml/kg/min)
70
60
50
40
30
0
24-h fasted
24-h refed
f
Energy expenditure (kcal/min)
1.0×10-2
7.5×10-3
5.0×10-3
0
24-h fasted
24-h refed
g
General locomotor activity (a.u.)
40
30
20
10
0
0
4
8
12
16
20
24
Time (h)
Total general activity (a.u.)
500
400
300
200
100
0
Day
Night
h
Core body temperature (°C)
38
37
36
35
0
0
4
8
12
16
20
24
Time (h)
Mean body temperature (°C)
38
37
36
35
0
p = 0.066
**
Day
Night

Figure 2: Consequences of PGC-1β deletion in AgRP neurons on whole body metabolism (a) Relative body composition measured by echoMRI (n=8). (**b-f)** Oxygen consumption, respiratory exchange ratio and energy expenditure measured in the CLAMS system in fed and fasted conditions in AgRP-PGC1β KO and WT mice (n=7-8). **(g and h)** Locomotor activity and core body temperature measured by intraperitoneally implanted transmitters (n=7-8). Values and error bars represent the mean ± SEM. **p<0.01 indicate statistically significant differences between genotypes.

4. Summary and outlook of the general role of PGC-1α in the brain as a metabolic sensor

Given the effect of PGC-1α modulation on mouse models of neurodegenerative diseases, its low expression in patients with neurological disorders and the neurodegeneration triggered by its ablation in the brain, studies determining the role of PGC-1α in the central nervous system have been highly focused on neuronal survival to date. Very few studies suggested a role for PGC-1α in the control of energy balance in the brain. One of them demonstrated that PGC-1α controls oxytocin expression, a hypothalamic neuropeptide responsible of appetite regulation, in zebrafish **(Blechman, Amir-Zilberstein et al. 2011)**. Yet, no data showing actual dysregulation of food intake or energy balance was presented in this work. Another study reported increased food intake, locomotor activity and body temperature and reduced fasting induction of feeding peptides expression in PGC-1α brain knock-out mice, suggesting a possible role for the transcriptional coactivator in energy balance regulation. Nevertheless, the aforementioned alterations could also arise from indirect consequences of PGC-1α deletion in different brain areas or secondary peripheral tissue dysregulations. The dramatically opposite phenotype triggered by specific ablation of PGC-1α in AgRP neurons compared to the one observed upon PGC-1α whole brain or whole body deletion strongly support this hypothesis. Therefore, our study revealed for the first time the true function of PGC-1α in the arcuate nucleus and its role as a metabolic sensor in the brain. Notably, its increase upon fasting in the AgRP neuronal cell line and in the hypothalamus, as well as the abrogation of leptin response upon its deletion, demonstrates that PGC-1α is able to sense metabolic signals in the arcuate nucleus. Moreover, similarly to the exercise induced production of myokines in muscle, PGC-1α regulates AgRP expression in the arcuate nucleus, which in turn affects various tissues via the regulation of other brain areas. This reveals a central function for PGC-1α in the integration of metabolic signals by AgRP neurons. Finally, the dysregulation of food intake and energy expenditure in AgRP-PGC1α KO animals establishes the importance of neuronal PGC-1α expression for downstream regulation of whole body energy balance. PGC-1α is therefore necessary for both signal integration and the according appropriate control of whole body metabolism by AgRP neurons.

The role of PGC-1α in the regulation of whole body homeostasis is further supported by the fact that global deletion of PGC-1α impairs circadian rhythms in mice **(Liu, Li et al. 2007)** and that its expression follows a circadian pattern in the arcuate nucleus. However, the circadian oscillation of PGC-1α has only been established in POMC neurons **(Agapito, Zhang et al. 2014)** and it would be interesting to investigate whether it also follows a similar pattern of expression in AgRP neurons. Given the highlighted role of PGC-1α in arcuate nucleus nutrient sensing, the transcriptional coactivator now becomes a potential player in other brain processes requiring integration of metabolic signals. Nutrient sensing is not only important for food intake and energy expenditure regulation, but also for the control of multiple whole body homeostatic parameters that are regulated by the brain. For example, glucose sensing in the brain is very important because neurons requires a constant supply of glucose as fuel and for the regulation of their activity. Glucose transporter 2-dependent glucose sensing is an important mechanism for glucose level evaluation **(Steinbusch, Labouebe et al. 2015)**. Interestingly, PGC-1α has been shown to regulate this glucose transporter **(Yoon, Xu et al. 2003, Valtat, Riveline et al. 2013)** and could therefore be important for glucose sensing in neurons. Furthermore, PGC-1α also influences expression of glucokinases **(Yoon, Xu et al. 2003, Zhu, Liu et al. 2010)**, which play an important role in glucose sensing in the ventromedial nucleus of the hypothalamus **(Kang, Dunn-Meynell et al. 2006)**.

Fatty acids sensing in the brain is another mechanism for controlling energy balance and glucose homeostasis. Central administration of long-chain FA oleate reduces feeding and glucose production in rats **(Obici, Feng et al. 2002, Morgan, Obici et al. 2004)**. Moreover, animals with specific ventromedial hypothalamus deletion CD36 present higher plasma leptin levels and exhibit subcutaneous fat depots with an abnormal glucose tolerance. Of note, PGC-1α promotes lipogenesis in muscle **(Summermatter, Baum et al. 2010)**, fatty acid metabolism in different tissue **(Gerhart-Hines, Rodgers et al. 2007, Morris, Meers et al. 2012)** and regulates CD36 levels in muscle **(Summermatter, Baum et al. 2010)**. Thus, PGC-1α might also be implicated in fatty acids signaling in the brain. Furthermore, PPARγ activation induces AgRP expression increase in AgRP neurons and food intake **(Garretson, Teubner et al. 2015)**. Given

PPARs function in fatty acids metabolism **(Georgiadi and Kersten 2012)** and the known interaction between PPARγ and PGC-1α, their collaboration could also help in fatty acids sensing and downstream regulation of energy homeostasis in AgRP neurons. Finally, high fat diet and fatty acids treatments downregulate hypothalamic PGC-1α expression, thereby dysregulating ERα levels and inducing hypothalamic inflammation **(Morselli, Fuente-Martin et al. 2014)**. This data further attests for a critical function of PGC-1α in cellular responses to fatty acids signaling in the hypothalamus.

To conclude, this work has now unraveled a new function for PGC-1 coactivators in the brain and identifies them as metabolic sensors in this tissue. It also shows that the recent emerging function of PGC-1α as a regulator of systemic homeostasis, discovered in the muscle through the regulation of myokines secretions, extend to the brain and therefore probably to other tissues. On the other side, this global coordinated response of PGC-1α in many metabolic tissues upon specific stimuli, followed by the coordinated output given by the coactivator further establishes him as a powerful controller of systemic homeostasis.

B. The role of PGC-1α in skeletal muscle aging

Skeletal muscle undergoes broad and varied changes while it ages, ultimately leading to muscle atrophy and dysfunction (see Introduction Fig. 6). During the past decades, an increasing number of studies reported that muscle aging was associated with mitochondrial dysfunctions, which occur at many different levels in the old mitochondria. Notably, muscle mitochondrial aging includes reduced mitochondrial mass **(Barazzoni, Short et al. 2000, Conley, Jubrias et al. 2000, Short, Bigelow et al. 2005)**, diminished respiratory capacities **(Short, Vittone et al. 2004, Short, Bigelow et al. 2005)**, increased oxidative stress **(Mansouri, Muller et al. 2006, Chabi, Ljubicic et al. 2008)** leading to mtDNA mutations **(Chabi, Mousson de Camaret et al. 2005)**, impaired mitochondrial dynamics and turnover **(Crane, Devries et al. 2010, Ibebunjo, Chick et al. 2013)**, and compromised calcium homeostasis regulation **(Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**.

This large panel of mitochondrial alterations prompted the scientific community to look for treatments, or signaling pathway modifications, to improve mitochondrial wellness as a potential therapy against skeletal muscle aging. For example, a study took advantage of a mouse model expressing the human catalase targeted to the mitochondria to study the effect of reduced oxidative stress on muscle aging. As expected, these animals exhibited lower oxidative stress, but also lower mtDNA deletion and an increased lifespan, thus demonstrating the importance of mitochondrial ROS in muscle and whole body aging **(Schriner, Linford et al. 2005)**. A more recent study revealed that this mouse model also presented with increased muscle specific force, with higher tetanic calcium transients, decreased intracellular calcium leakage and higher SR calcium load **(Umanskaya, Santulli et al. 2014)**, further supporting the link between mitochondrial dysfunction and impaired calcium homeostasis during aging. Treatment with SRT1720, a known activator of SIRT1 and mitochondrial function, improved mice lifespan and health span and reduced age-related risk factors of metabolic diseases **(Mitchell, Martin-Montalvo et al. 2014)**. In addition, caloric restriction and exercise, which both increase mitochondrial function **(Lee, Aspnes et al. 1998, Konopka and Sreekumaran Nair 2013, Barbieri, Agostini et al. 2015)**, are considered as some of the best treatments against age-associated muscle dysfunctions and substantially ameliorate mitochondrial disorders in skeletal muscle **(Marzetti, Lawler et al. 2008, Anderson, Shanmuganayagam et al. 2009, Konopka and Sreekumaran Nair 2013, Cartee, Hepple et al. 2016)**.

PGC-1α expression levels decrease with aging **(Chabi, Ljubicic et al. 2008, Wenz, Rossi et al. 2009, Ghosh, Lertwattanarak et al. 2011, Kang, Chung et al. 2013)**. Since PGC-1α is a master mitochondrial regulator, several studies made use of its regulatory function to improve mitochondrial capacities in the context of muscle aging. PGC-1α overexpression alleviated age-associated muscle disorders in models of pre-mature aging **(Sahin, Colla et al. 2011, Dillon, Williams et al. 2012)** as well as during natural aging in the mouse **(Wenz, Rossi et al. 2009)**.

In this thesis, we confirmed that muscle PGC-1α overexpression ameliorates the overall phenotype of aged mitochondria, notably by regulating mitochondrial respiration, but also by

modulating mitochondrial dynamics and calcium handling in aging muscle. This contributes to preserve cellular calcium content and homeostasis, thereby preventing tubular aggregate formation and protecting against ER stress, which ultimately alleviates age-associated muscle apoptosis. For example, PGC-1α prevented the downregulation of several proteins involved in mitochondrial OXPHOS, dynamics and calcium import in aged muscles and blunted the age-dependent increase of important ER stress and cell death markers. During this thesis, we additionally reported that the above-mentioned PGC-1α-controlled mitochondrial regulations were ERRα dependent. Moreover, this thesis showed that muscle PGC-1α overexpression rescued the majority of the dysfunctions observed in old muscles, including endurance, balance and motor coordination, which ultimately led to preserved locomotor activity. Finally, this thesis also used a mouse model that is deficient in muscular PGC-1α expression to show that loss of PGC-1α accelerates the age-related loss of balance and muscle force and results in a premature sarcopenia.

1. PGC-1α counters aging via regulation of mitochondrial function and dynamics

In a previous study investigating the impact of PGC-1α overexpression in muscle aging, authors demonstrated that OXPHOS decline, increased oxidative stress and impairments of other non-mitochondrial muscle processes could be improved by PGC-1α elevation in the old muscle **(Wenz, Rossi et al. 2009)**. While we confirmed that PGC-1α muscle overexpression preserved mitochondrial respiratory capacities, our results from the PGC-1α muscle deficient mice now further support the idea that PGC-1α downregulation in aging muscle might be one of the triggers for age-associated muscle disorders. For example, young PGC-1α deficient mice presented with reduced OXPHOS protein expression and premature sarcopenia, suggesting that mitochondrial defects contribute to premature muscle dysfunctions. However, our data from the young PGC-1α deficient mice did not reveal the expected changes in mitochondrial densities and mitochondrial respiration, implying that aggravated muscle disorders are not linked to an impaired mitochondrial oxidative capacity. Yet, other age-associated mitochondrial or cellular

dysregulations not studied in this work, such as increased oxidative stress **(Bejma and Ji 1999, Capel, Rimbert et al. 2005)** or excitation-contraction uncoupling **(Renganathan, Messi et al. 1997, Delbono 2000)**, may be mimicked by genetic deletion of PGC-1α and lead to the observed phenotype. Alternatively, our data on post exercise blood lactate amount and mRNA levels of β-oxidation genes indicated a lower fatty acids oxidation rate in young and old muscle lacking PGC-1α. The impaired fatty acid oxidation could therefore lead to lower muscle ATP production and function in spite of normal mitochondrial oxidative function. Finally, in addition to preserving mitochondrial respiration in the old muscle, we now also showed that PGC-1α alleviated muscle and mitochondrial aging by improving other components of mitochondrial metabolism, namely mitochondrial dynamics and mitochondria-SR association via MAM protein regulation.

Mitochondrial dynamics are an important element of mitochondrial homeostasis. Mouse models with specific muscle deletions of Mfn 1 or 2, genes regulating mitochondrial fusion, exhibit increased mtDNA deletion, higher depletion rates in mitochondrial DNA copy numbers and elevated muscle loss **(Chen, Vermulst et al. 2010)**. On the other hand, fission proteins, such as Fis1 or Drp1, are also essential for mitochondrial quality control as they allow damaged mitochondria to be severed from the network and to enter mitophagy. Yet, they also represent an amplifying circle in muscle atrophy, where the muscle is already often subjected to mitochondrial depletion **(Romanello, Guadagnin et al. 2010)**. Therefore, age-associated imbalance in mitochondrial dynamics through lower and higher expression of fusion and fission proteins, respectively, shown in this work and by others **(Crane, Devries et al. 2010)**, certainly impacts mtDNA quality, oxidative capacities, ROS production and cell death. Recently reported in a model of disuse muscle atrophy, PGC-1α prevented reduction of mitochondrial fusion proteins and muscle mass loss **(Cannavino, Brocca et al. 2015)**, further supporting the positive effect mediated by increased mitochondrial dynamics. The increase of Mfn2 and Drp1 gene expression in a mouse model of Duchenne muscular dystrophy was proposed to be a compensatory mechanism to meet the increased energetic demand of dystrophic muscles **(Pant, Sopariwala et al. 2015)**. This constitutes another possible mechanism by which PGC-1α

overexpression alleviates the Duchenne muscular dystrophy phenotype in this mouse model **(Handschin, Kobayashi et al. 2007)**. In the context of muscle aging, the increase of both fusion and fission protein expression in our PGC-1α muscle overexpressing animals likely restores the balance in mitochondrial dynamics during muscle aging and improves muscle function.

Mitochondrial interaction with the SR is another important feature of mitochondrial metabolism that is impaired with age. Mitochondrial ATP production relies on calcium stimulations that mitochondria receive from calcium release subunits contained in the junctional SR and T tubules **(Brookes, Yoon et al. 2004)**, which requires proper associations between mitochondria and SR **(Franzini-Armstrong and Boncompagni 2011)**. Recent investigations in old mouse muscle showed age-associated mitochondria-SR disruption, misplaced mitochondria with regard to their normal position along calcium release subunits, and increased distances between mitochondria and SR **(Fernandez-Sanz, Ruiz-Meana et al. 2014, Pietrangelo, D'Incecco et al. 2015)**. In addition, disruption of mitochondria-SR association in young cardiomyocytes mimicked age-induced changes in calcium handling and impaired calcium transfer to the mitochondria **(Fernandez-Sanz, Ruiz-Meana et al. 2014)**. Our work indicated that PGC-1α could control and improve mitochondrial association with SR through regulation of MAM protein expression, physically connecting mitochondria and SR. We found lower gene and protein expression levels of several MAM elements in the old muscle, including mitofusins, GRP75 and VDAC. We also reported increased levels of those MAM proteins in young and old animals with elevated PGC-1α expression, and conversely a premature decline in their levels in young muscle lacking PGC-1α. These results demonstrate that these genes are tightly regulated by the transcriptional coactivator and strongly suggest that PGC-1α could also control and restore functional mitochondrial-SR association in the old muscle. A key experiment firmly confirming this hypothesis would be the actual quantification of mitochondria-SR contacts and assessment of proper mitochondrial positioning. While preliminary measurements indicated an unchanged number of overall muscle mitochondrial-SR contacts with age and upon PGC-1α modulation (data not shown), it would be more informative to evaluate the proportion of SR that is in contact with mitochondria and not just the total number of contacts. In addition,

increased levels of MAM proteins by PGC-1α might not lead to increased number of contacts but might rather strengthen and improve each connection between mitochondria and SR, or increase the capacity of mitochondria to create new associations with SR when required. This is also in line with the fact that PGC-1α ameliorates mitochondrial responses to environmental changes and improves mitochondria dynamics.

2. PGC-1α regulates calcium content and homeostasis during aging

Our laboratory previously showed that PGC-1α could remodel cellular calcium homeostasis by altering SR protein expression and activity, thereby impacting muscle function **(Summermatter, Thurnheer et al. 2012)**. The alterations of MAM protein expression mentioned above indicates that PGC-1α might also affect cellular calcium balance by improving the connection between SR and the mitochondria. Finally, in this thesis we demonstrated that PGC-1α modulated cellular calcium handling through elevation of mitochondrial calcium uptake in muscle overexpressing PGC-1α. Increased calcium uptake was correlated with higher expression of genes and proteins crucial for mitochondrial calcium import such as MCU and VDAC. Importantly, increased calcium uptake volume for each mitochondrion combined with the larger number of mitochondria found in muscles with elevated PGC-1α levels confers those muscles with an extreme potential to modulate calcium import and excessive intracellular calcium levels. For example, upon high energetic demand—when mitochondria require more calcium to increase oxidative metabolism **(McMillin and Madden 1989)**—or upon toxic myoplasmic calcium concentrations **(Kass and Orrenius 1999)**, increased mitochondrial calcium import can be beneficial and allow mitochondria to better adapt to environmental changes. Interestingly, Pietrangelo and collaborators reported that mitochondrial calcium uptake is reduced in skeletal muscle of aged mice, in addition to the disruption of mitochondrial-SR communication **(Kavanagh, Ainscow et al. 2000, Pietrangelo, D'Incecco et al. 2015)**, thereby possibly contributing to the reduction in activity-dependent ATP production in old muscles **(Drew, Phaneuf et al. 2003, Nair 2005)**. Correlating with those data, mitochondrial calcium uptake

upon SR stimulated calcium release was found to be reduced in the hearts of old mice. This was concomitant with impaired VDAC-RYR communication, which reflects dysfunctional mitochondrial-SR interactions, and decreased NADPH regeneration **(Fernandez-Sanz, Ruiz-Meana et al. 2014)**. In addition, muscle specific deletion of the mitochondria uniporter MCU leads to reduced pyruvate dehydrogenase activity and O2 consumption upon calcium stimulation **(Pan, Liu et al. 2013)**. Improved mitochondrial calcium uptake and mitochondrial-SR communication thus likely contribute to preserve ATP production and mitochondrial respiration in old muscle containing increased PGC-1α content.

In addition to reduced oxidative functions, age-associated decrease in mitochondrial calcium uptake also correlates with increased resting cytosolic calcium content in both heart **(Nitahara, Cheng et al. 1998)** and muscle **(Squier and Bigelow 2000, Fraysse, Desaphy et al. 2006)** of aged animals. Although age-related increase in SR calcium leak due to RYR oxidation **(Andersson, Betzenhauser et al. 2011)** and impaired calcium SR re-uptake **(Hunter, Thompson et al. 1999)** definitely account for the increased calcium levels in old muscle, mitochondria have also been shown to modulate intracellular calcium homeostasis and signaling, as well as cellular metabolism by buffering cytosolic calcium **(Rizzuto, De Stefani et al. 2012)**. Notably, an amyotrophic lateral sclerosis mouse model (that displays impaired mitochondrial inner membrane potential and dysfunctional mitochondrial calcium uptake at the NMJ) allowed researchers to study the effect of mitochondrial calcium uptake defect **(Yi, Ma et al. 2011)**. Using the mitochondria-targeted calcium biosensor, mt11-YC3.6, the authors found a diminished calcium uptake only in the fiber segment with depolarized mitochondria after voltage-stimulated SR calcium release, which mirrored an increased cytosolic calcium level in the same region. This clearly demonstrated the key role of mitochondrial calcium uptake in maintaining cytosolic calcium concentrations during excitation-contraction coupling and suggests that dysfunctional mitochondrial calcium import could participate to muscle degeneration and force reduction in amyotrophic lateral sclerosis and therefore possibly in the aged muscle. To follow up on this hypothesis, it would be interesting to investigate the consequences of impaired mitochondrial calcium uptake on cellular calcium homeostasis during

muscle aging. Of note, muscle specific deletion of MCU mimics age-associated reduction in mitochondrial calcium import in young animals, as well as loss of endurance and maximal and isometric strength **(Pan, Liu et al. 2013)**. Other recent studies showed that MCU-dependent calcium uptake prevents muscle atrophy through the control of hypertrophic pathways in the muscle **(Chemello, Mammucari et al. 2015, Mammucari, Gherardi et al. 2015)**. One could thus evaluate whether MCU deletion in muscle would promote age-induced intracellular calcium homeostasis disruption and the resulting consequences on excitation-contraction coupling, muscle mass and muscle dysfunction during aging. Intriguingly, one of the hypertrophy axis controlled by MCU implicates the PGC-1α4 isoform, indicating that while PGC-1α regulates MCU expression, the calcium uniporter feeds a positive regulatory feedback loop resulting in increased PGC-1α4 expression **(Mammucari, Gherardi et al. 2015)**. This increases the interest in assessing the outcomes of combined modulation of PGC-1α and MCU levels in muscle to investigated how the alteration of one factor would abrogate or potentiate the effect of the other, in particular in the context of muscle aging.

3. PGC-1α protects against tubular aggregate formation

Since calcium controls many key cellular functions, disruption of calcium homeostasis in muscle can lead to cell damage **(Kass and Orrenius 1999)** and cellular stress responses, such as ER stress, which is designed to restore cellular homeostasis **(Kania, Pajak et al. 2015, Krebs, Agellon et al. 2015)**. During muscle aging, we and others found increased levels of ER stress markers **(Dirks and Leeuwenburgh 2004, Chung and Ng 2006, Ogata, Machida et al. 2009)**, correlating with impaired mitochondrial calcium uptake and mitochondria-SR interaction and with the rise in cytoplasmic calcium levels **(Fraysse, Desaphy et al. 2006, Pietrangelo, D'Incecco et al. 2015)**. Age-induced tubular aggregates constitute another indicator of the strong remodeling through which SR goes during muscle aging **(Agbulut, Destombes et al. 2000)**. Nevertheless, it is unknown whether those structures represent a consequence of unresolved ER stress or a compensatory mechanism of the SR to buffer excessive intracellular calcium upon diminished mitochondrial calcium import. In line with the latter hypothesis, we reported that

tubular aggregates cannot be found in aged muscles overexpressing PGC-1α, which present an enhanced capacity to buffer intracellular calcium. A recent publication showed that mice with muscle specific deletion of the MCU regulator, MICU1, exhibited both impaired mitochondrial calcium intake and tubular aggregate formation at a young age **(Liu, Liu et al. 2016)**. This further strengthens the connection between tubular aggregate development, calcium homeostasis disruption and mitochondrial calcium import decline. Other experiments linking calcium dysregulation and SR masses showed that Csq1 and SERCA1, two SR regulators important for calcium homeostasis, are required to accumulate during SR clustering for the formation of tubular aggregate **(Boncompagni, Protasi et al. 2012)**. Csq1 muscle-specific deletion led to incomplete development of tubular aggregate. Taken together, those results suggest that reduced accumulation of Csq1 in old muscle with elevated PGC-1α levels might contribute to prevent tubular aggregate formation. This is supported by our findings demonstrating that PGC-1α controls Csq1 levels in a cell autonomous manner, which strongly indicates that Csq1 downregulation in old transgenic muscle is not a secondary consequence of improved calcium homeostasis or absent tubular aggregates. Finally, we reported that old muscle lacking PGC-1α presented a large increase in number of fibers containing tubular aggregates. This is in agreement with another study showing that muscle specific PGC-1α deletion triggers the formation of SR aggregates in myofibers of a muscle disuse mouse model **(Vainshtein, Desjardins et al. 2015)**. These data further support the key role of PGC-1α in the protection against tubular aggregate development in the context of muscle disorders and atrophy.

To investigate the exact processes through which PGC-1α protects muscle from tubular aggregate formation, one should take advantage of mouse models exhibiting tubular aggregates and evaluate how PGC-1α modulation affects tubular aggregate development in the muscles of those mice and through which mechanisms. For example, in muscle with PGC-1α ablation, it would be of interest to define which elements or pathways modulated by PGC-1α trigger tubular aggregate formation upon denervation induced atrophy **(Vainshtein, Desjardins et al. 2015)**. Mice with muscle specific ablation of Caveolin protein expression develop tubular aggregates **(Schubert, Sotgia et al. 2007)** and represent another great model to evaluate how

PGC-1α protects muscle from such abnormalities. Notably, mice with caveolin 2 muscle specific deletion present abnormal SR organization, and therefore possible dysregulation of mitochondrial-SR communication, as well as impaired mitochondrial calcium regulation, possibly resulting in the observed tubular aggregates. These mice constitute an excellent model to determine whether PGC-1α prevents the development of tubular aggregates by improving mitochondrial control of calcium homeostasis. Alternatively, a combination of muscle-specific PGC-1α overexpression and MCU deletion would allow us to define the role of mitochondrial calcium uptake in the PGC-1α-mediated protection against tubular aggregates during muscle aging. Lastly, mutations in both STIM1 and ORA1 genes have recently been shown to trigger tubular aggregate myopathies in humans, accompanied by altered calcium homeostasis **(Bohm, Chevessier et al. 2013, Endo, Noguchi et al. 2015, Okuma, Saito et al. 2016)**. Expression of the STIM1 mutant in cells similarly alters calcium homeostasis **(Okuma, Saito et al. 2016)**. Based on these results and ours, one could overexpress PGC-1α concomitantly with the STIM1 mutant to see if PGC-1α can restore calcium homeostasis in those cells. Even more interesting would be to express those different mutated proteins in mouse muscles to determine whether alterations of calcium homeostasis indeed generate tubular aggregates and ultimately study the mechanism of their development and the impact of PGC-1α modulation on their formation.

It is interesting to note that tubular aggregates are completely absent in aged oxidative soleus muscle and are only present in glycolytic fibers weakly stained for SDH in the TA of old WT mice in our study. Additionally, patients presenting tubular aggregates exhibit lower muscle fiber respiration rates and reduced OXPHOS complex activities than healthy patients **(Vielhaber, Schroder et al. 2001)**. Intriguingly, muscle of patients that displayed tubular aggregates that did not stain for SR proteins – implying that the aggregates did not originate from the SR – showed a concomitant lack of mitochondrial defects. This strongly suggests a functional link between SR generated tubular aggregates and mitochondrial dysfunctions. On top of that, one study showed in a human patient that 96% of fibers presenting tubular aggregates were cytochrome C oxidase deficient and that tubular aggregates and cytochrome C oxidase labeling were mutually exclusive, further strengthening the association between mitochondrial dysfunction and tubular

aggregate formation. Taken together, these results strongly suggest that improved oxidative metabolism and enhanced mitochondrial mass and function play a central role in the protection against tubular aggregate formation in old muscles with elevated PGC-1α levels. Whether this protection is associated with improved mitochondrial calcium buffering capacities enabled by the enriched mitochondrial mass and function of those muscles is still unclear.

4. PGC-1α reduces age-associated ER stress and apoptosis

PGC-1α was previously shown to induce ER stress response after exercise, which is required for muscle adaptation to physical activity **(Wu, Ruas et al. 2011)**. In the same study, authors showed that increased PGC-1α levels were sufficient to promote ER stress responses. While the ER stress response represents a mechanism to preserve cellular homeostasis upon an acute exercise stimulus, the increased level of ER stress markers in old muscle likely originated from prolonged and unresolved ER stress. Thus, it is tempting to think that PGC-1α promotion of ER stress response in the muscles of young animals helps the muscle to respond faster and better to any future stimulus causing ER stress, thereby preventing muscles from developing unresolved ER stress and preventing stable age-related increase of ER stress markers.

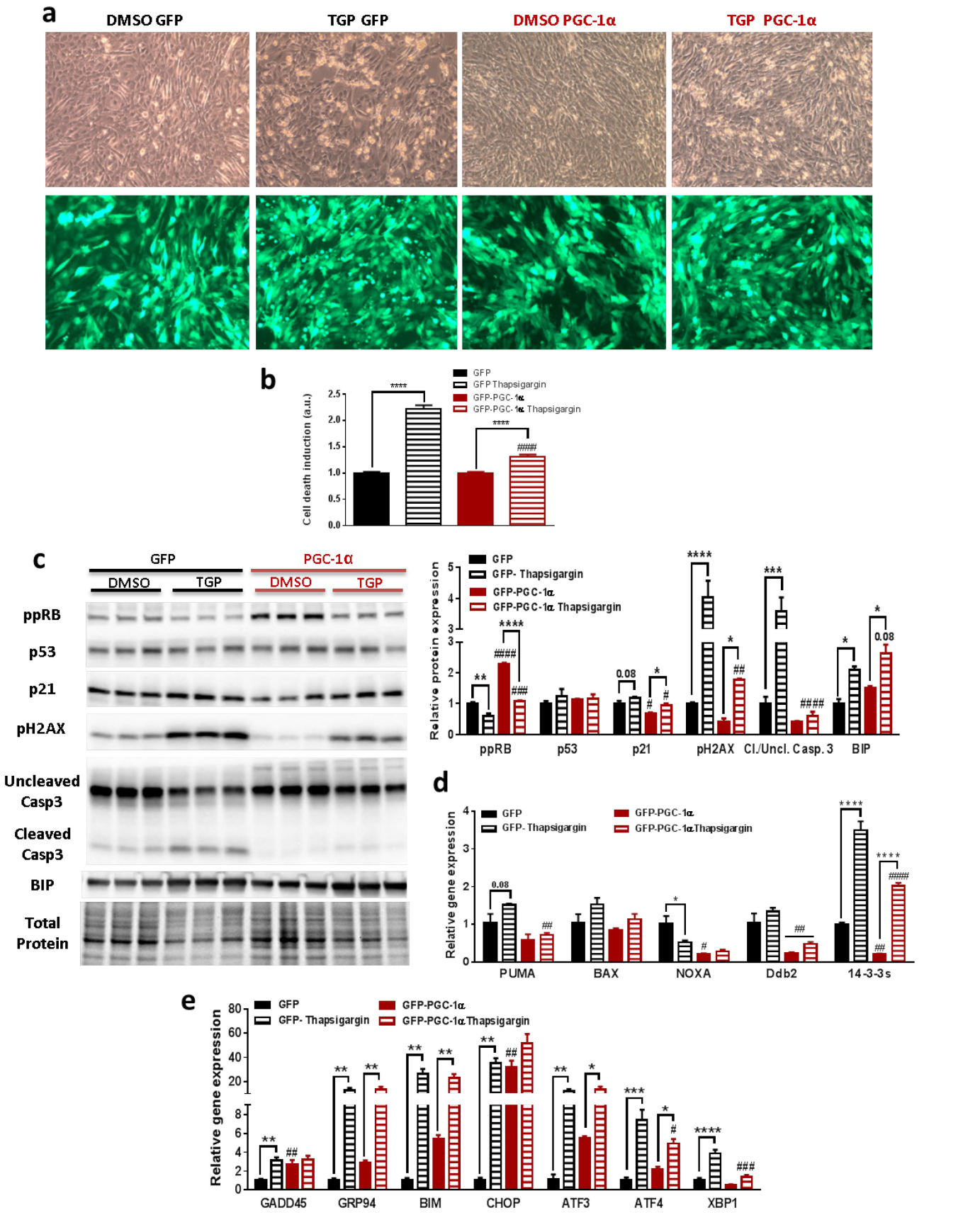
Prolonged ER stress—as well as other cellular dysfunctions, such as mitochondrial defects and altered calcium homeostasis—are strong promoters of muscle apoptosis **(Kass and Orrenius 1999, Singh 2004, Dirks, Hofer et al. 2006, Szegezdi, Logue et al. 2006, Braga, Sinha Hikim et al. 2008, Meng and Yu 2010, Sano and Reed 2013)**. Interestingly, reduction in age-related apoptosis induction in old muscles with enhanced PGC-1α content strongly correlated with alleviations of SR-burden, mitochondrial decline and dysregulation of calcium metabolism. This suggests that the lower cell death observed in muscle of old transgenic PGC-1α mice is a secondary consequence of the substantial reduction in myofiber damages and improved cellular homeostasis. However, a direct anti-apoptotic role for PGC-1α has previously been postulated in various tissues **(Valle, Alvarez-Barrientos et al. 2005, Luo, Zhu et al. 2009, Wenz, Rossi et al. 2009, Egger, Samardzija et al. 2012)**. We therefore induced apoptosis in C2C12 cells using

ceramide and showed that PGC-1α was able to protect cells even after acute cellular damages were initiated. This is done through the positive and negative regulation of anti- and pro-apoptotic genes, respectively. Ceramide induces cell death by the dysregulation of multiple pathways and through damage of several cellular components, including mitochondria, ER stress response and calcium metabolism **(Siskind 2005, Thon, Mohlig et al. 2005, Liu, Xia et al. 2014)**. Ceramide thus represented a chemical of choice to mimic age-induced apoptosis in a cell culture model. However, this did not allow us to determine whether PGC-1α could actually protect myofibers from cell death induced specifically by calcium homeostasis dysregulation.

Thapsigargin (TGP) is a non-competitive inhibitor of SERCA that blocks SR calcium uptake **(Lytton, Westlin et al. 1991)** and promotes cell apoptosis through a dramatic increase of cytosolic calcium and ER stress **(Chen, Chiang et al. 2000, Rao, Hermel et al. 2001)**. To establish whether PGC-1α helps muscle cells to deal with altered calcium homeostasis and protects myocytes from cell death induced by cytosolic calcium increase, we treated control cells and myocytes overexpressing PGC-1α with TGP in a short pilot study. Similarly to what we observed after ceramide treatment, PGC-1α protected muscle cells from TGP-induced apoptosis as indicated by microscopic pictures of C2C12 cells and a cell death assay. (Fig.3a and b). In line with reduced cell death, PGC-1α overexpression blunted the activation of caspase 3 and pH2AX protein induction, as well as the decrease in ppRB protein levels (Fig. 3c) upon TGP treatment. PGC-1α upregulation diminished p21 protein expression after both DMSO and TGP exposure but did not prevent its induction by TGP. Moreover, PGC-1α abolished or alleviated the upregulation of several cell death related genes after TGP treatment, further supporting a protective function against TGP apoptosis induction in muscle cells (Fig. 3d). Surprisingly however, when PGC-1α overexpression led to the downregulation of Noxa, similarly to what we observed during the ceramide experiment, TGP exposure reduced Noxa expression too. Also, contrary to ceramide, TGP did not increase p53 protein levels, indicating that ceramide and TGP induces apoptosis through different pathways. Conversely to cell death markers, PGC-1α did not prevent the induction of BIP protein expression and the upregulation of many ER stress genes upon the acute stimulus of TGP, which is in agreement with previously reported data **(Wu, Ruas et al.**

2011). PGC-1α overexpression even promoted the expression of several unfolded protein response genes in the absence of TGP exposure, further supporting the hypothesis that PGC-1α prepares muscle cells to better respond to ER stress stimuli. However, PGC-1α prevented the induction of ATF4 expression and of one of its target genes, xbp1. This suggests that PGC-1α might coordinately promote and blunt the induction of different ER stress pathways to ultimately restore SR homeostasis and prevent apoptosis. Further investigations are required to dissect the exact mechanism by which PGC-1α regulates ER stress. Nevertheless, PGC-1α-mediated protection against TGP-induced cell death confirms that the coactivator protects muscle from cellular damages induced by calcium homeostasis dysregulation and helps muscle to deal with increased cytosolic calcium. This further supports the protective role for PGC-1α against age-related calcium homeostasis disruption during muscle aging. Whether this protective effect is mediated by improved ER stress, enhanced mitochondrial calcium buffering, higher SR calcium handling or through general apoptotic pathway inhibition remains to be determined.

The combined cell death experiments using ceramide and TGP also highlight and firmly confirm the powerful pro-survival action of PGC-1α upon different apoptotic stimulus. Based on those results, one could extend the study on the anti-apoptotic effect of PGC-1α in different contexts, whereby cell death is induced by denervation, muscular dystrophies or metabolic myopathies **(Tews 2002)**. In addition, knock-down or forced expression of different elements involved in the different apoptotic pathways, such as mPTP opening, or caspase-dependent and independent pathways, in combination with PGC-1α overexpression and TGP or ceramide treatment would provide valuable information on the apoptotic or survival mechanism that PGC-1α alters to prevent cell death.


a
DMSO GFP
TGP GFP
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TGP PGC-1α
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Cell death induction (a.u.)
GFP
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GFP-PGC-1α Thapsigargin
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p53
p21
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BAX
NOXA
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GFP- Thapsigargin
GFP-PGC-1α
GFP-PGC-1α Thapsigargin
GADD45
GRP94
BIM
CHOP
ATF3
ATF4
XBP1

Fig. 3: PGC-1α protects from thapsigargin-induced cell death (a) Representative picture of C2C12 myoblasts expressing endogenous or increased PGC-1α levels after TGP or DMSO treatment **(b)** relative propidium ioide incorporation in C2C12 myoblasts after TGP or DMSO treatment **(c-d)** relative protein and mRNA levels of cell death and cell survival markers in C2C12 myoblasts after TGP or DMSO treatment **(e)** relative mRNA levels of ER stress markers in C2C12 myoblasts after TGP or DMSO treatment. Values represent 3-4 technical replicates in one experiment. Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals of the same genotype or between cells treated with DMSO and TGP, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between cells with endogenous and overexpressed PGC-1α levels.

5. PGC-1α improves muscle motor skills and preserves locomotor activity during aging

Results regarding balance performance, motor coordination and locomotor activity in old mice with abrogated and elevated PGC-1α expression not only demonstrated the ability of the coactivator to alleviate age-related muscle disorders but also reveals novel PGC-1α-controlled motor skills. These ameliorations could arise from many different changes known to be triggered by PGC-1α in the muscle, such as enhanced muscle intrinsic function and metabolism **(Lin, Handschin et al. 2005)**, improved neuromuscular junction communication **(Handschin, Kobayashi et al. 2007, Arnold, Gill et al. 2014)** and indirect central neuronal remodeling via muscle factor secretions **(Kuipers and Bramham 2006, Wrann, White et al. 2013, Agudelo, Femenia et al. 2014)**. In any case, the motor function data described above suggest that elevating muscle PGC-1α might be helpful to improve balance and motor coordination in the elderly, thereby improving life quality and preventing falls, which results in fractures and hospitalizations. In addition, PGC-1α modulation might be relevant for the treatment of other diseases that present with motor skills dysfunction, thus opening a whole new field of investigation. For example, amyotrophic lateral sclerosis is a motor neuron degenerative disorder that results in muscle weakness with loss of balance and motor coordination **(Rowland and Shneider 2001, Lui and Byl 2009)**. Interestingly, it was reported that deletion of PGC-1α caused an earlier onset of the motor diseases in the amyotrophic lateral sclerosis SOD1(G93A) mouse model **(Eschbach, Schwalenstocker et al. 2013)**. Another study demonstrated that overexpression of the transcriptional coactivator in muscle ameliorated endurance performance and locomotor activity in those mice **(Da Cruz, Parone et al. 2012)**; however, potential ameliorations in balance and motor coordination were not assessed in this study. Moreover, kynurenine metabolism has been identified as a pathomechanism of amyotrophic lateral sclerosis and multiple sclerosis, and kynurenic acid synthesis is considered to be a possible therapeutic candidate for the fight those diseases **(Fuvesi, Rajda et al. 2012)**. The established role of muscle PGC-1α in the kynurenine pathway **(Agudelo, Femenia et al. 2014)** further implicates the coactivator as a potential therapeutic target in the treatment of

those diseases and possibly represents a mechanism by which PGC-1α muscle transgenic expression improved the phenotype of SOD1(G93A) mice in the Da Cruz et al study. Likewise, PGC-1α overexpression improved endurance performance in a Duchenne muscular dystrophy mouse model, but its capacity to ameliorate other motor skill disabilities in those mice had not been tested yet **(Handschin, Kobayashi et al. 2007)**.

6. Limitations of the study

While the work performed during this thesis allowed us to better understand the role of PGC-1α in muscle aging and to discover new functions of PGC-1α in muscle, different aspects of the study could have been optimized. Notably, we decided to use animals of 24 months of age as our aged groups, whereas older animals might have shown a broader and stronger phenotype and might have led to the discovery of a greater protective effect of PGC-1α. For example, while we did observe an age-associated loss of muscle strength and decline in isolated muscle masses when normalized to body weight in WT animals, we did not find a reduction in lean mass or muscle fiber cross-sectional area in those animals, suggesting that they were just at the onset of sarcopenia. This possibly masked a protective effect of PGC-1α in the old muscle against age-induced loss of muscle mass and precluded us from actually studying the mechanism of sarcopenia. Yet, the ideal age to study sarcopenia in mice is still a matter of debate. Notably, while sarcopenia occurs similarly in rats and humans, with a progressive loss of muscle mass and muscle function, mice tend to sustain their muscle mass for a longer time before a steep drop around 28 months of age. This severe drop might indeed represent a sarcopenia associated muscle mass reduction but could also arise from a drastic systemic failure and cancer-related cachexia, which would then make impossible to investigate the exclusive effect of sarcopenia. Further studies should be attempted to assess the best time point to study sarcopenia in mice, or in rats which would be an even better sarcopenic model according to the similar muscle aging pattern that they share with humans. Nevertheless, the decision to use 24 month old animals was mainly based on the fact that the lifespan of PGC-1α muscle knock-out animals was unknown at this time. In addition, the previous work investigating the consequences of PGC-1α overexpression on muscle aging showed an increased lifespan of those

animals compared to WT mice **(Wenz, Rossi et al. 2009)**. It was therefore very possible that mice lacking muscle PGC-1α would exhibit an opposite phenotype with a reduced lifespan. Since an aging study takes a considerable amount of time, we could not afford the risk of choosing an older time point and lose the majority of our knock-out animals. However, we found that mice with muscle deletion of PGC-1α had a similar lifespan compared to WT animals (data not shown). To our surprise, we also observed that mice with PGC-1α overexpression had a lifespan comparable to the one of WT animals (data not shown). The fact that 80% of WT animals were dead at the age of 24 months in the previous publication **(Wenz, Rossi et al. 2009)** whereas almost 90% of our WT mice were still alive at this age, similarly to what Jackson laboratories shows (https://www.jax.org/jax-mice-and-services/find-and-order-jax-mice/most-popular-jax-mice-strains/aged-b6), strongly suggests that different housekeeping conditions between their study and our work drove the discrepancy in the observed lifespan and muscle mass loss. In addition, while we used 26 WT and 42 mTg animals for our survival curve, data presented in the previous study only included 12 WT and 17 PGC-1α overexpressing mice which might not be representative enough. Furthermore, the lifespan prolonging effect of PGC-1α could not be reproduced in later studies from the same group (interpersonal communication) making their assumptions about the effect of muscle PGC-1α on animal lifespan even less conclusive.

In addition to the possibly suboptimal chosen animal age, the design of the study with the many different groups of mice forced us to test the animals in a staggered manner. Although the experiments with the different groups were all performed following the exact same protocol at the exact same period of the day, some parameters could not be controlled and might have led to increased variability between the groups. For example, construction works triggering vibrations were occurring sporadically for several days or weeks. Mice were always tested in the absence of such perturbations but the noise and the vibrations occurring outside of the test period might still have raised the general level of stress of some tested mice. Likewise, diverse animal caretakers handling the animals differently might also have generated various levels of anxiety in those animals.

Another problem that we encountered during the thesis was that although we tried to collect all possible organs in order to maximize the number of experiments that we could perform, we needed to decide the method for harvesting and treating all of the samples before having collected preliminary and informative molecular data. This limited us to perform only experiments that were compatible with the way we collected our samples. Especially, information about mitochondrial calcium uptake in aging muscles, as well as basal cytosolic calcium levels, would have provided valuable additional information about the protective role of PGC-1α against calcium dyshomeostasis in the aging myofiber. However, as we learned out about this particular function of PGC-1α only at the late stage of the thesis we did not have sufficient living animals at the appropriate age in all the groups of interest to perform those calcium assays that require freshly harvested muscle. Additionally, since PGC-1α has been linked to telomere protection in aged aortas **(Xiong, Patrushev et al. 2015)**, it would have been interesting to correlate a protective role of PGC-1α in telomerase activity and telomere length in aged PGC-1α transgenic muscles, with concomitant reductions in cell death marker levels we observed. Unfortunately, those assays likewise necessitated fresh muscles. Furthermore, samples that were harvested at the beginning of the project were stored for a very long time. Following our results pointing towards a preventative role for PGC-1α against age-related apoptosis, we were eager to measure apoptosis with a Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling immunostaining assay; however, it either failed to show a positive apoptotic signal in aging muscles or led to a false positive signal in young muscles, according to the protocol used. Immunolabeling of freshly isolated muscles from one young and one old animal showed the expected age-related increase in apoptotic signaling, demonstrating that long time storage made our samples unusable at least for some immunostainings.

7. Summary and outlook of the role of PGC-1α in skeletal muscle aging

During this thesis we unraveled a novel aspect of metabolism in which PGC-1α contributes to slowing down skeletal muscle aging. While previously the main protective function of PGC-1α in the prevention of age-related muscle disorders was the preservation of mitochondrial oxidative functions **(Wenz, Rossi et al. 2009)**, we now show that PGC-1α also controls and improves calcium homeostasis and ER stress through the regulation of mitochondrial calcium uptake and connection with SR during aging. At the same time, this thesis highlights a connection between mitochondrial dysfunction and calcium dyshomeostasis in aging muscle and shows that these are not necessarily distinct dysfunctional processes. Collectively, this improves our general understanding of transcriptional regulation of calcium metabolism in muscle and how PGC-1α can affect muscle metabolism. In addition, we demonstrated that positive effects of PGC-1α on age-associated muscle dysfunctions were not restricted to improving muscle endurance as previously described **(Wenz, Rossi et al. 2009)**, but also impacted motor skills and muscle force. Taken together, these results suggest a therapeutic role for PGC-1α in muscle aging, but also in motor neuron disorders, such as amyotrophic lateral sclerosis and spinal muscle atrophy on one side and in calcium dysregulation associated diseases, such as central core disease or tubular aggregate myopathies on the other side.

The newly highlighted functions of PGC-1α in calcium regulation and cell death prevention also open new domains of investigation regarding potential neuronal salvaging effects of the transcriptional co-activator in motor and neuromuscular diseases or other conditions involving calcium dysregulation in the central nervous system. Of note would be neuronal disorders, for many of which calcium dysregulations has been implicated in the pathogenesis. Upon ischemic injury, cellular calcium overload is thought to be a key player in neuronal death; addition of intracellular calcium chelators have been shown to protect neurons after stokes **(Tymianski, Wallace et al. 1993)**. In addition, ER stress induced by calcium dyshomeostasis has been shown to contribute to neuronal cell death after a stroke **(Paschen 2003)**. Evaluations of brain tissue from patients with Parkinson, Huntington and Alzheimer

diseases all indicate a role for calcium overload in neuronal vulnerability to death. In Alzheimer disease patients, free and protein-bound calcium content, as well as activity of calcium-dependent proteases, are increased in neurons containing neurofibrillary tangles as compared to tangle-free neurons **(Murray, Landsberg et al. 1992, Nixon 2003)**. Amyloid β-peptide causes an elevation of basal intracellular calcium levels through a mechanism involving the production of 4-hydroxy-2,3-nonenal, which sensitizes neurons to excitotoxicity and apoptosis **(Mattson, Cheng et al. 1992, Mark, Hensley et al. 1995, Keller, Mark et al. 1997)**. Calcium metabolism is dysregulated in lymphoblast mitochondria and in the brain of Huntington disease patients, as well as in the central nervous system of Huntington disease mice **(Bezprozvanny and Hayden 2004)**. Lastly, spinal cord sections from amyotrophic lateral sclerosis patients showed a significant increase in the calcium content of motoneurons compared to control subjects **(Kasarskis, Tandon et al. 1995)**. Of note, motor neurons exhibiting high levels of calcium-binding proteins presented higher resistance to amyotrophic lateral sclerosis **(Alexianu, Ho et al. 1994)** and overexpression of the calcium-binding protein parvalbumin promotes motor neurons survival and delays disease onset in a mouse model of amyotrophic lateral sclerosis **(Beers, Ho et al. 2001)**.

Interestingly, PGC-1α is repressed in patients carrying these degenerative diseases **(McGill and Beal 2006, Qin, Haroutunian et al. 2009, Zheng, Liao et al. 2010)** and has been shown to exert a protective function against each of those neurological disorders **(Cui, Jeong et al. 2006, Kim, Nguyen et al. 2007, Katsouri, Parr et al. 2011, Zhao, Varghese et al. 2011, Mudo, Makela et al. 2012)**. While until now the salvaging action mediated by PGC-1α and the dysfunction triggered by its repression were thought to be associated with mitochondrial function and oxidative stress alterations in neurodegenerative patients **(Raymond 2016)**, our muscle data suggest that PGC-1α might also prevent neurological disorders by restoring disrupted calcium homeostasis and ameliorating ER stress response in neurons of those patients. In a similar manner, while mitochondrial defects and increased oxidative stress have been postulated to be at the origin of the neurodegenerative phenotype of PGC-1α brain and whole body knock out mice, because of the well-defined master role of PGC-1α in the regulation

of mitochondrial metabolism **(Lin, Wu et al. 2004, Ma, Li et al. 2010)**, no evidence supporting this conclusion was actually provided and calcium homeostasis dysregulation might play a central role in the neurodegeneration of those animals. This leaves open a whole new field of study about the function and the benefits of PGC-1α in the preservation of calcium homeostasis in neurons, and further supports its therapeutic role in the treatment of neurodegenerative disorders.

C. PGC-1α and exercise during muscle aging

In the course of this thesis, the publication of a new paper about the investigations of PGC-1α muscle deletion on aging prompted us to push our investigations further and bring another dimension to the project to be able to differentiate ourselves from what had been done before. PGC-1α and exercise have many similar effects on muscle, such as increased mitochondrial function, NMJ remodeling and production of myokines **(Gremeaux, Gayda et al. 2012, Egan and Zierath 2013, Chan and Arany 2014, Schnyder and Handschin 2015)**. Moreover, exercise improves muscle aging disorders by ameliorating many of the above mentioned functions altered in aged muscles **(Garatachea, Pareja-Galeano et al. 2015, Cartee, Hepple et al. 2016)**. Importantly, at least regarding some of those functions, PGC-1α is thought to mediate and to be required for the beneficial effect of exercise in young mice **(Chan and Arany 2014)** and in mouse models of premature aging **(Leick, Lyngby et al. 2010, Safdar, Annis et al. 2016)**. However, the connection between exercise and PGC-1α during natural muscle aging is unknown. We therefore wanted to study the link between the positive effect of exercise and PGC-1α modulation during muscle aging. For that, we divided our groups of control, PGC-1α muscle deleted and PGC-1α muscle overexpressing mice into sub-groups that either received treadmill exercise for 3 months late in their lifespan or that stayed sedentary. This allowed us to evaluate the effects of exercise on muscle aging in a first time and to determine whether those effects were dependent on muscle PGC-1α expression and if they could be mimicked or even boosted by PGC-1α overexpression in muscle.

1. The effects of PGC-1α on exercise-controlled muscle enhancement during aging

The results of this study revealed that PGC-1α expression at old age is required for some exercise-dependent muscle functions improvements but not for others. For example, increased maximal force and balance in old mice lacking muscle PGC-1α show that it is not required for theses specific beneficial effects of exercise. Conversely, absence of muscle PGC-1α completely abrogated exercise-mediated improvements in motor coordination and isometric strength. To our surprise, higher PGC-1α levels also abolished exercise-induced effects on isometric strength, indicating that there is an expression limit for PGC-1α's beneficial outcomes on muscle strength resistance when coupled with exercise. In addition to precluding exercise-dependent ameliorations on motor coordination and isometric strength, PGC-1α muscle deletion blunted exercise-mediated increase in muscle endurance. In accordance with this result, elevated muscle PGC-1α expression potentiated exercise effects on mice running capacities and lactate usage, further indicating that muscle PGC-1α does impacts specific exercise outcomes affecting muscle aging. In addition, PGC-1α muscle upregulation mimicked or even overridden exercise-mediated improvements in almost all regards in the old muscle. This additionally suggests that exercise is acting through PGC-1α but that other cellular components might compensate for its absence and improve muscle function in PGC-1α deficient muscles upon exercise.

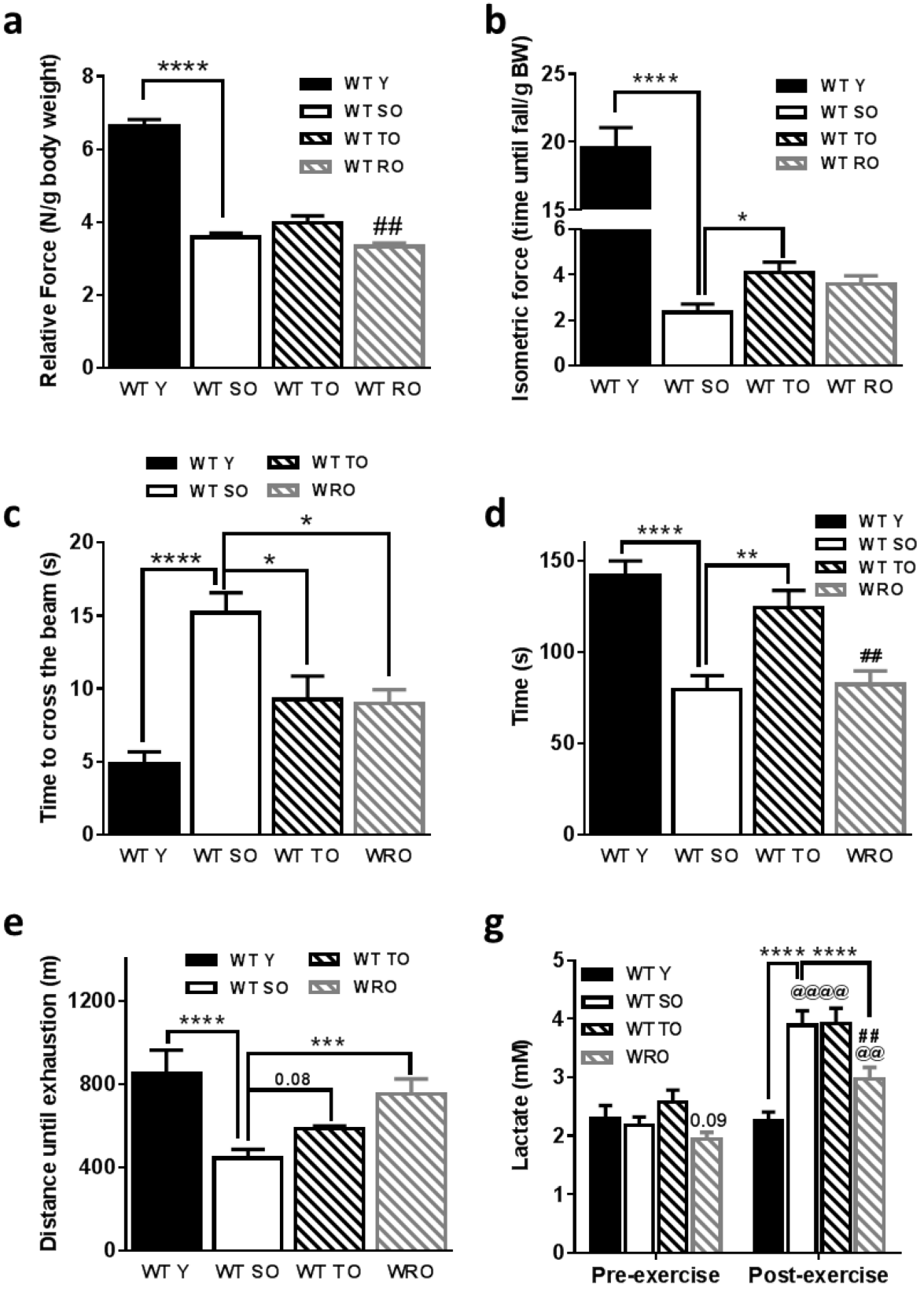
Interestingly, with regard to muscle endurance and mitochondrial function and densities, the synergistic effect of exercise and PGC-1α muscle overexpression was superior to that of either exercise or PGC-1α upregulation alone. In humans, such an improved endurance would not only improve the quality of life of older adults but would also allow them to increase their exercise training intensity and duration, thereby potentiating any amelioration in age-related muscle disorders.

2. PGC-1α, exercise and caloric restriction

While only a few treatments increase muscle PGC-1α levels, caloric restriction, which is one of the best prescription against muscle aging **(Marzetti, Lees et al. 2009, Anderson and Weindruch 2010)**, has been shown to increase PGC-1α content in many tissues, including skeletal muscle **(Ranhotra 2010, Handschin 2016)**. Thus, PGC-1α seems to be linked to both exercise and caloric restriction effects in muscle aging and might represent a common mechanism in those treatments. Similarly to exercise, caloric restriction-mediated induction and activation of PGC-1α is driven by the activation of SIRT1 and AMPK **(Anderson, Barger et al. 2008, Lettieri Barbato, Baldelli et al. 2012)**, which results in elevated mitochondrial biogenesis and function, possibly ultimately leading to improved muscle function and reduced muscle aging **(Anderson, Shanmuganayagam et al. 2009)**. These pathways commonly induced by exercise and caloric restriction could be amplified by the combination or alternation of those treatments and therefore considerably increase muscle performances during aging. However, very strict patients monitoring should be observed during those treatments and appropriate and moderate caloric restriction should be given in order to avoid energy depletion. It would be additionally interesting to test if alterations in muscle PGC-1α expression would modulate those changes. Alternatively, one could conduct an aging study combining caloric restriction and PGC-1α muscle modulation, in a set up similar to our work on PGC-1α and exercise, to evaluate the potential of the coregulator to promote a caloric restriction effect on muscle aging. Although a recent study failed to show an elevation of metabolic improvements with the combination of caloric restriction and PGC-1α muscle upregulation upon high-fat diet **(Wong, Mikus et al. 2015)**, the data mainly focused on systemic metabolism and did not investigate muscle function. In addition, another work showed that PGC-1α mediated mitochondrial adaptation and not metabolic changes upon caloric restriction, which indicates the importance of the transcriptional co-activator for caloric restriction effects in the context of muscle aging in which mitochondrial dysfunction plays a key role **(Finley, Lee et al. 2012)**.

3. Distinct exercise regimes differentially affect muscle aging

Exercise has long been recognized as one of the top prescription to reduce age-related muscle disorders. However, what still remains unclear is how to maximize the effect of exercise training on our muscles during aging. A relevant question is whether it is better to maintain a low intensity but regular physical activity regime throughout life or to start more intense endurance training later in life. In other words, are short and recent chronic exercise muscle adaptations more beneficial than permanent muscle changes acquired from consistent stimulation during life-long physical activity? To answer this question we gave life-long free and continuous running wheel access to a group of WT mice. We then compared the performance of those animals with the group of mice that received forced treadmill exercise only for the last 3 months of their life and with the group of sedentary control mice. As we described in the study about the effect of PGC-1α and exercise, aging led to the reduction of all evaluated muscle functions (fig. 4a-g). Wheel and treadmill running both belong to the endurance type of exercise and therefore did not significantly affect maximal force. While resistance training is very challenging to set up with mice, this kind of exercise would have likely affected maximal muscle force in the aged mice (Fig. 4a). However, late-life treadmill exercise but not life-long physical activity significantly improved isometric force (Fig. 4b). Similarly, while both types of exercise improved balance performance at old age, only late-life treadmill exercise restored motor coordination (Fig.4c and d). Inversely, only life-long physical activity significantly ameliorated endurance and lactate utilization in old mice (Fig. 4e and g). Those results confirm that exercise increases general muscle function during aging but that different types of training impact distinct muscle dysfunctions.


a
Relative Force (N/g body weight)
****
##
WT Y
WT SO
WT TO
WT RO
b
Isometric force (time until fall/g BW)
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*
c
Time to cross the beam (s)
WRO
d
Time (s)
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e
Distance until exhaustion (m)
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0.08
g
Lactate (mM)
0.09
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Pre-exercise
Post-exercise

Fig. 4: Life-long running wheel and late-life forced treadmill exercise effects on age-related muscle dysfunction (a) Maximal grip strength **(b)** isometric strength **(c)** balance **(d)** motor coordination **(e)** running endurance and **(g)** blood lactate levels pre- and post-exercise measured in 3 months old (WT Y), 24 months old sedentary (WT SO), 24 months old late-life treadmill trained (WT TO) and 24 months old life-long voluntary active (WRO) mice (n=8-12). Voluntary active mice were given free access to running wheel during their whole life. Values are mean ± SEM. *P < 0.05; **P < 0.01; ***; P < 0.001; ****p<0.0001 indicate statistically significant differences between young and old animals or between sedentary and exercised/voluntary active animals, # p<0.01; ## p<0.01; ### p<0.01; #### p<0.001 indicate statistically significant differences between late-life forced treadmill exercised and life-long voluntary active mice.

Several elements can explain the divergent impacts of life-long and late-life training. First, consistent life-long and shorter but recent muscle adaptations of late-life chronic exercise might lead to different muscle function ameliorations. Further molecular analyses are required to unravel the potentially divergent muscle adaptations triggered by life-long and shorter late-life chronic exercise. However, some aspects of the exercise set-up could also be responsible for the observed changes. For example, the movements performed during treadmill exercise, where the mice need to adapt their steps to the belt rotation of the treadmill, and the movements on the rotarod are very similar. Therefore, forced treadmill exercise might actually provide a pre-training for the rotarod task and confer a serious advantage to this group of mice. Surprisingly, however, mice that received forced treadmill exercise training performed worse in the treadmill exhaustion test than mice that had free access to running wheels, while mice exercising with treadmill were actually already practicing this task when they were training. This could be due to higher endurance metabolism in mice that had access to running wheels, as suggested by their improved lactate utilization. Alternatively, these mice might be mentally better prepared for endurance test than mice that were forced to train. Indeed, it has been shown that mental preparation has a significant impact on athletic performance in professional sportsmen **(Bar-Eli and Blumenstein 2004)**. In the case of mice, wheel running is addictive, and mice from which running wheels were removed have been reported to show signs of depression, demonstrating that running wheel activity increases the mouse's motivation to run **(Novak, Burghardt et al. 2012, Nishijima, Llorens-Martin et al. 2013)**. On the other hand, It is very likely that forced treadmill training did not have the same positive mental impact on mice. Actually, the aversive and stressful stimulus used during treadmill training probably even increased anxiety levels in this group of mice along the endurance exhaustion test. The combination of improved endurance metabolism and mental resistance to fatigue therefore possibly both contributed to the increased endurance performance of the running wheel group compared to the treadmill exercise group. It would be interesting to dissect and evaluate brain rewarding signaling pathway alterations after forced and voluntary exercise, in addition to characterizing muscle metabolism and signaling pathways

The drawback of using running wheels as a model of life-long physical activity is that the duration and intensity of exercise cannot be controlled, and dramatically drops after 15 months of age (data not shown). Therefore, the absence of force and motor skills improvements in mice with life-long running wheel access might also arise from the fact that they had lower exercise intensities and amounts at the end of their life than the group of mice that received forced treadmill exercise.

4. Limitations of the study

While our results showed that exercise greatly improved age-related muscle and motor dysregulations in many regards, the mechanisms governing those ameliorations remain unclear. As described in the introduction of this thesis, exercise affects many muscle processes. However, we intriguingly did not find any histological or gene or protein expression alterations triggered by exercise (data not shown) in any signaling pathway we looked at in our aging studies, with the exception of increased OXPHOS protein expression and PGC-1α mRNA levels. Although we evaluated some possible changes at the protein levels, the majority of our data originates from gene expression analyses, for which changes are observable during acute exercise but disappear over chronic exercise **(Egan, Hawley et al. 2016)**. It might therefore be more informative to examine protein post-translational modifications that can provide protein stabilization, prevent their degradation, or stimulate their activity, thereby triggering stable and lasting muscle adaptations. In addition, we did not evaluate changes in all processes altered by age and exercise. In an attempt to identify candidates mediating exercise effects and PGC-1α impacts on muscle aging, we sequenced the whole transcriptome of our different groups. The sequencing data are now ready to be analyzed and might reveal interesting candidates induced by PGC-1α modulation and exercise training. Even more informative would have been to perform deep sequencing of ribosome-protected mRNA fragments which reflects protein translation and predict protein abundance of the whole proteome. Unfortunately, lack of material precluded such an experiment.

5. Summary and outlook of the role and complementarity of PGC-1α and exercise in skeletal muscle aging

In conclusion, this study showed that both exercise and PGC-1α improve age-related muscle disorders and that they work synergistically in many regards to achieve this aim. Our work now reveals that PGC-1α can be used to potentiate the beneficial effects of exercise on muscle dysfunctions and mitochondria protein expression during aging. This highlights a therapeutic role for PGC-1α, not only in the prevention of muscle aging, but also in supporting exercise in ameliorating age-related muscle disorders and possibly other muscle conditions for which exercise is advised. Further studies should however investigate the mechanisms and muscle adaptations driven by PGC-1α during exercise in the context of muscle aging. To take full advantage of the benefits of PGC-1α and exercise on muscle aging, it is important to identify which types of training are the most effective in slowing down sarcopenia and what methods could be used to increase PGC-1α levels in humans. The results of this thesis revealed the importance of PGC-1α in maximizing exercise-mediated prevention of muscle aging, settling up the foundations for forthcoming studies aimed at designing better treatments against age-related muscle disorders.

D. References

Agapito, M. A., C. Q. Zhang, S. Murugan and D. K. Sarkar (2014). "Fetal Alcohol Exposure Disrupts Metabolic Signaling in Hypothalamic Proopiomelanocortin Neurons via a Circadian Mechanism in Male Mice." Endocrinology **155**(7): 2578-2588.

Agbulut, O., J. Destombes, D. Thiesson and G. Butler-Browne (2000). "Age-related appearance of tubular aggregates in the skeletal muscle of almost all male inbred mice." Histochemistry and cell biology **114**(6): 477-481.

Agudelo, L. Z., T. Femenia, F. Orhan, M. Porsmyr-Palmertz, M. Goiny, V. Martinez-Redondo, J. C. Correia, M. Izadi, M. Bhat, I. Schuppe-Koistinen, A. T. Pettersson, D. M. Ferreira, A. Krook, R. Barres, J. R. Zierath, S. Erhardt, M. Lindskog and J. L. Ruas (2014). "Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression." Cell **159**(1): 33-45.

Alexianu, M. E., B. K. Ho, A. H. Mohamed, V. La Bella, R. G. Smith and S. H. Appel (1994). "The role of calcium-binding proteins in selective motoneuron vulnerability in amyotrophic lateral sclerosis." Annals of neurology **36**(6): 846-858.

Anderson, R. M., J. L. Barger, M. G. Edwards, K. H. Braun, C. E. O'Connor, T. A. Prolla and R. Weindruch (2008). "Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response." Aging cell **7**(1): 101-111.

Anderson, R. M., D. Shanmuganayagam and R. Weindruch (2009). "Caloric restriction and aging: studies in mice and monkeys." Toxicol Pathol **37**(1): 47-51.

Anderson, R. M. and R. Weindruch (2010). "Metabolic reprogramming, caloric restriction and aging." Trends Endocrinol Metab **21**(3): 134-141.

Andersson, D. C., M. J. Betzenhauser, S. Reiken, A. C. Meli, A. Umanskaya, W. Xie, T. Shiomi, R. Zalk, A. Lacampagne and A. R. Marks (2011). "Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging." Cell Metab **14**(2): 196-207.

Arnold, A. S., J. Gill, M. Christe, R. Ruiz, S. McGuirk, J. St-Pierre, L. Tabares and C. Handschin (2014). "Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1alpha." Nature communications **5**: 3569.

Baar, K., A. R. Wende, T. E. Jones, M. Marison, L. A. Nolte, M. Chen, D. P. Kelly and J. O. Holloszy (2002). "Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1." FASEB journal : official publication of the Federation of American Societies for Experimental Biology **16**(14): 1879-1886.

Bar-Eli, M. and B. Blumenstein (2004). "Performance enhancement in swimming: the effect of mental training with biofeedback." J Sci Med Sport **7**(4): 454-464.

Barazzoni, R., K. R. Short and K. S. Nair (2000). "Effects of aging on mitochondrial DNA copy number and cytochrome c oxidase gene expression in rat skeletal muscle, liver, and heart." The Journal of biological chemistry **275**(5): 3343-3347.

Barbieri, E., D. Agostini, E. Polidori, L. Potenza, M. Guescini, F. Lucertini, G. Annibalini, L. Stocchi, M. De Santi and V. Stocchi (2015). "The pleiotropic effect of physical exercise on mitochondrial dynamics in aging skeletal muscle." Oxidative medicine and cellular longevity **2015**: 917085.

Beers, D. R., B. K. Ho, L. Siklos, M. E. Alexianu, D. R. Mosier, A. H. Mohamed, Y. Otsuka, M. E. Kozovska, R. E. McAlhany, R. G. Smith and S. H. Appel (2001). "Parvalbumin overexpression alters immune-mediated increases in intracellular calcium, and delays disease onset in a transgenic model of familial amyotrophic lateral sclerosis." Journal of neurochemistry **79**(3): 499-509.

Bejma, J. and L. L. Ji (1999). "Aging and acute exercise enhance free radical generation in rat skeletal muscle." J Appl Physiol (1985) **87**(1): 465-470.

Berglund, E. D., C. Liu, J. W. Sohn, T. Liu, M. H. Kim, C. E. Lee, C. R. Vianna, K. W. Williams, Y. Xu and J. K. Elmquist (2013). "Serotonin 2C receptors in pro-opiomelanocortin neurons regulate energy and glucose homeostasis." The Journal of clinical investigation **123**(12): 5061-5070.

Bezprozvanny, I. and M. R. Hayden (2004). "Deranged neuronal calcium signaling and Huntington disease." Biochemical and biophysical research communications **322**(4): 1310-1317.

Blechman, J., L. Amir-Zilberstein, A. Gutnick, S. Ben-Dor and G. Levkowitz (2011). "The metabolic regulator PGC-1alpha directly controls the expression of the hypothalamic neuropeptide oxytocin." The Journal of neuroscience : the official journal of the Society for Neuroscience **31**(42): 14835-14840.

Bohm, J., F. Chevessier, A. Maues De Paula, C. Koch, S. Attarian, C. Feger, D. Hantai, P. Laforet, K. Ghorab, J. M. Vallat, M. Fardeau, D. Figarella-Branger, J. Pouget, N. B. Romero, M. Koch, C. Ebel, N. Levy, M. Krahn, B. Eymard, M. Bartoli and J. Laporte (2013). "Constitutive activation of the calcium sensor STIM1 causes tubular-aggregate myopathy." American journal of human genetics **92**(2): 271-278.

Boncompagni, S., F. Protasi and C. Franzini-Armstrong (2012). "Sequential stages in the age-dependent gradual formation and accumulation of tubular aggregates in fast twitch muscle fibers: SERCA and calsequestrin involvement." Age (Dordr) **34**(1): 27-41.

Bonen, A. (2009). "PGC-1alpha-induced improvements in skeletal muscle metabolism and insulin sensitivity." Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme **34**(3): 307-314.

Bostrom, P., J. Wu, M. P. Jedrychowski, A. Korde, L. Ye, J. C. Lo, K. A. Rasbach, E. A. Bostrom, J. H. Choi, J. Z. Long, S. Kajimura, M. C. Zingaretti, B. F. Vind, H. Tu, S. Cinti, K. Hojlund, S. P. Gygi and B. M. Spiegelman (2012). "A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis." Nature **481**(7382): 463-468.

Braga, M., A. P. Sinha Hikim, S. Datta, M. G. Ferrini, D. Brown, E. L. Kovacheva, N. F. Gonzalez-Cadavid and I. Sinha-Hikim (2008). "Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice." Apoptosis **13**(6): 822-832.

Brookes, P. S., Y. Yoon, J. L. Robotham, M. W. Anders and S. S. Sheu (2004). "Calcium, ATP, and ROS: a mitochondrial love-hate triangle." Am J Physiol Cell Physiol **287**(4): C817-833.

Cakir, I., M. Perello, O. Lansari, N. J. Messier, C. A. Vaslet and E. A. Nillni (2009). "Hypothalamic Sirt1 regulates food intake in a rodent model system." PLoS One **4**(12): e8322.

Cannavino, J., L. Brocca, M. Sandri, B. Grassi, R. Bottinelli and M. A. Pellegrino (2015). "The role of alterations in mitochondrial dynamics and PGC-1alpha over-expression in fast muscle atrophy following hindlimb unloading." The Journal of physiology **593**(8): 1981-1995.

Capel, F., V. Rimbert, D. Lioger, A. Diot, P. Rousset, P. P. Mirand, Y. Boirie, B. Morio and L. Mosoni (2005). "Due to reverse electron transfer, mitochondrial H2O2 release increases with age in human vastus lateralis muscle although oxidative capacity is preserved." Mech Ageing Dev **126**(4): 505-511.

Cartee, G. D., R. T. Hepple, M. M. Bamman and J. R. Zierath (2016). "Exercise Promotes Healthy Aging of Skeletal Muscle." Cell metabolism **23**(6): 1034-1047.

Cartoni, R., B. Leger, M. B. Hock, M. Praz, A. Crettenand, S. Pich, J. L. Ziltener, F. Luthi, O. Deriaz, A. Zorzano, C. Gobelet, A. Kralli and A. P. Russell (2005). "Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise." J Physiol **567**(Pt 1): 349-358.

Chabi, B., V. Ljubicic, K. J. Menzies, J. H. Huang, A. Saleem and D. A. Hood (2008). "Mitochondrial function and apoptotic susceptibility in aging skeletal muscle." Aging Cell **7**(1): 2-12.

Chabi, B., B. Mousson de Camaret, A. Chevrollier, S. Boisgard and G. Stepien (2005). "Random mtDNA deletions and functional consequence in aged human skeletal muscle." Biochem Biophys Res Commun **332**(2): 542-549.

Chan, M. C. and Z. Arany (2014). "The many roles of PGC-1alpha in muscle--recent developments." Metabolism: clinical and experimental **63**(4): 441-451.

Chemello, F., C. Mammucari, G. Gherardi, R. Rizzuto, G. Lanfranchi and S. Cagnin (2015). "Gene expression changes of single skeletal muscle fibers in response to modulation of the mitochondrial calcium uniporter (MCU)." Genomics data **5**: 64-67.

Chen, H., M. Vermulst, Y. E. Wang, A. Chomyn, T. A. Prolla, J. M. McCaffery and D. C. Chan (2010). "Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations." Cell **141**(2): 280-289.

Chen, L. Y., A. S. Chiang, J. J. Hung, H. I. Hung and Y. K. Lai (2000). "Thapsigargin-induced grp78 expression is mediated by the increase of cytosolic free calcium in 9L rat brain tumor cells." J Cell Biochem **78**(3): 404-416.

Cheng, A., R. Wan, J. L. Yang, N. Kamimura, T. G. Son, X. Ouyang, Y. Luo, E. Okun and M. P. Mattson (2012). "Involvement of PGC-1alpha in the formation and maintenance of neuronal dendritic spines." Nat Commun **3**: 1250.

Chung, L. and Y. C. Ng (2006). "Age-related alterations in expression of apoptosis regulatory proteins and heat shock proteins in rat skeletal muscle." Biochimica et biophysica acta **1762**(1): 103-109.

Conley, K. E., S. A. Jubrias and P. C. Esselman (2000). "Oxidative capacity and ageing in human muscle." J Physiol **526 Pt 1**: 203-210.

Coppari, R., G. Ramadori and J. K. Elmquist (2009). "The role of transcriptional regulators in central control of appetite and body weight." Nature clinical practice. Endocrinology & metabolism **5**(3): 160-166.

Crane, J. D., M. C. Devries, A. Safdar, M. J. Hamadeh and M. A. Tarnopolsky (2010). "The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure." J Gerontol A Biol Sci Med Sci **65**(2): 119-128.

Cui, L., H. Jeong, F. Borovecki, C. N. Parkhurst, N. Tanese and D. Krainc (2006). "Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration." Cell **127**(1): 59-69.

Da Cruz, S., P. A. Parone, V. S. Lopes, C. Lillo, M. McAlonis-Downes, S. K. Lee, A. P. Vetto, S. Petrosyan, M. Marsala, A. N. Murphy, D. S. Williams, B. M. Spiegelman and D. W. Cleveland (2012). "Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS." Cell Metab **15**(5): 778-786.

Delbono, O. (2000). "Regulation of excitation contraction coupling by insulin-like growth factor-1 in aging skeletal muscle." J Nutr Health Aging **4**(3): 162-164.

Dietrich, M. O., Z. W. Liu and T. L. Horvath (2013). "Mitochondrial dynamics controlled by mitofusins regulate Agrp neuronal activity and diet-induced obesity." Cell **155**(1): 188-199.

Dillon, L. M., S. L. Williams, A. Hida, J. D. Peacock, T. A. Prolla, J. Lincoln and C. T. Moraes (2012). "Increased mitochondrial biogenesis in muscle improves aging phenotypes in the mtDNA mutator mouse." Hum Mol Genet **21**(10): 2288-2297.

Dirks, A. J., T. Hofer, E. Marzetti, M. Pahor and C. Leeuwenburgh (2006). "Mitochondrial DNA mutations, energy metabolism and apoptosis in aging muscle." Ageing Res Rev **5**(2): 179-195.

Dirks, A. J. and C. Leeuwenburgh (2004). "Aging and lifelong calorie restriction result in adaptations of skeletal muscle apoptosis repressor, apoptosis-inducing factor, X-linked inhibitor of apoptosis, caspase-3, and caspase-12." Free Radical Biology and Medicine **36**(1): 27-39.

Drew, B., S. Phaneuf, A. Dirks, C. Selman, R. Gredilla, A. Lezza, G. Barja and C. Leeuwenburgh (2003). "Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart." Am J Physiol Regul Integr Comp Physiol **284**(2): R474-480.

Egan, B., J. A. Hawley and J. R. Zierath (2016). "SnapShot: Exercise Metabolism." Cell Metab **24**(2): 342-342 e341.

Egan, B. and J. R. Zierath (2013). "Exercise metabolism and the molecular regulation of skeletal muscle adaptation." Cell metabolism **17**(2): 162-184.

Egger, A., M. Samardzija, V. Sothilingam, N. Tanimoto, C. Lange, S. Salatino, L. Fang, M. Garcia-Garrido, S. Beck, M. J. Okoniewski, A. Neutzner, M. W. Seeliger, C. Grimm and C. Handschin (2012). "PGC-1alpha determines light damage susceptibility of the murine retina." PloS one **7**(2): e31272.

Endo, Y., S. Noguchi, Y. Hara, Y. K. Hayashi, K. Motomura, S. Miyatake, N. Murakami, S. Tanaka, S. Yamashita, R. Kizu, M. Bamba, Y. Goto, N. Matsumoto, I. Nonaka and I. Nishino (2015). "Dominant mutations in ORAI1 cause tubular aggregate myopathy with hypocalcemia via constitutive activation of store-operated Ca(2)(+) channels." Hum Mol Genet **24**(3): 637-648.

Eschbach, J., B. Schwalenstocker, S. M. Soyal, H. Bayer, D. Wiesner, C. Akimoto, A. C. Nilsson, A. Birve, T. Meyer, L. Dupuis, K. M. Danzer, P. M. Andersen, A. Witting, A. C. Ludolph, W. Patsch and P. Weydt (2013). "PGC-1alpha is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis." Hum Mol Genet **22**(17): 3477-3484.

Fernandez-Sanz, C., M. Ruiz-Meana, E. Miro-Casas, E. Nunez, J. Castellano, M. Loureiro, I. Barba, M. Poncelas, A. Rodriguez-Sinovas, J. Vazquez and D. Garcia-Dorado (2014). "Defective sarcoplasmic reticulum-mitochondria calcium exchange in aged mouse myocardium." Cell Death Dis **5**: e1573.

Fernandez-Sanz, C., M. Ruiz-Meana, E. Miro-Casas, E. Nunez, J. Castellano, M. Loureiro, I. Barba, M. Poncelas, A. Rodriguez-Sinovas, J. Vazquez and D. Garcia-Dorado (2014). "Defective sarcoplasmic reticulum-mitochondria calcium exchange in aged mouse myocardium." Cell Death & Disease **5**.

Finley, L. W., J. Lee, A. Souza, V. Desquiret-Dumas, K. Bullock, G. C. Rowe, V. Procaccio, C. B. Clish, Z. Arany and M. C. Haigis (2012). "Skeletal muscle transcriptional coactivator PGC-1alpha mediates mitochondrial, but not metabolic, changes during calorie restriction." Proceedings of the National Academy of Sciences of the United States of America **109**(8): 2931-2936.

Franzini-Armstrong, C. and S. Boncompagni (2011). "The evolution of the mitochondria-to-calcium release units relationship in vertebrate skeletal muscles." J Biomed Biotechnol **2011**: 830573.

Fraysse, B., J. F. Desaphy, J. F. Rolland, S. Pierno, A. Liantonio, V. Giannuzzi, C. Camerino, M. P. Didonna, D. Cocchi, A. De Luca and D. Conte Camerino (2006). "Fiber type-related changes in rat skeletal muscle calcium homeostasis during aging and restoration by growth hormone." Neurobiology of disease **21**(2): 372-380.

Fuvesi, J., C. Rajda, K. Bencsik, J. Toldi and L. Vecsei (2012). "The role of kynurenines in the pathomechanism of amyotrophic lateral sclerosis and multiple sclerosis: therapeutic implications." J Neural Transm (Vienna) **119**(2): 225-234.

Garatachea, N., H. Pareja-Galeano, F. Sanchis-Gomar, A. Santos-Lozano, C. Fiuza-Luces, M. Moran, E. Emanuele, M. J. Joyner and A. Lucia (2015). "Exercise attenuates the major hallmarks of aging." Rejuvenation research **18**(1): 57-89.

Garretson, J. T., B. J. Teubner, K. L. Grove, A. Vazdarjanova, V. Ryu and T. J. Bartness (2015). "Peroxisome proliferator-activated receptor gamma controls ingestive behavior, agouti-related protein, and neuropeptide Y mRNA in the arcuate hypothalamus." J Neurosci **35**(11): 4571-4581.

Georgiadi, A. and S. Kersten (2012). "Mechanisms of gene regulation by fatty acids." Adv Nutr **3**(2): 127-134.

Gerhart-Hines, Z., J. T. Rodgers, O. Bare, C. Lerin, S. H. Kim, R. Mostoslavsky, F. W. Alt, Z. Wu and P. Puigserver (2007). "Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha." The EMBO journal **26**(7): 1913-1923.

Ghosh, S., R. Lertwattanarak, N. Lefort, M. Molina-Carrion, J. Joya-Galeana, B. P. Bowen, J. Garduno-Garcia Jde, M. Abdul-Ghani, A. Richardson, R. A. DeFronzo, L. Mandarino, H. Van Remmen and N. Musi (2011). "Reduction in reactive oxygen species production by mitochondria from elderly subjects with normal and impaired glucose tolerance." Diabetes **60**(8): 2051-2060.

Gremeaux, V., M. Gayda, R. Lepers, P. Sosner, M. Juneau and A. Nigam (2012). "Exercise and longevity." Maturitas **73**(4): 312-317.

Handschin, C. (2016). "Caloric restriction and exercise "mimetics": Ready for prime time?" Pharmacol Res **103**: 158-166.

Handschin, C., Y. M. Kobayashi, S. Chin, P. Seale, K. P. Campbell and B. M. Spiegelman (2007). "PGC-1alpha regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy." Genes & development **21**(7): 770-783.

Hsu, W. H., B. H. Lee and T. M. Pan (2015). "Leptin-induced mitochondrial fusion mediates hepatic lipid accumulation." Int J Obes (Lond) **39**(12): 1750-1756.

Hunter, S. K., M. W. Thompson, P. A. Ruell, A. R. Harmer, J. M. Thom, T. H. Gwinn and R. D. Adams (1999). "Human skeletal sarcoplasmic reticulum Ca2+ uptake and muscle function with aging and strength training." J Appl Physiol (1985) **86**(6): 1858-1865.

Ibebunjo, C., J. M. Chick, T. Kendall, J. K. Eash, C. Li, Y. Zhang, C. Vickers, Z. Wu, B. A. Clarke, J. Shi, J. Cruz, B. Fournier, S. Brachat, S. Gutzwiller, Q. Ma, J. Markovits, M. Broome, M. Steinkrauss, E. Skuba, J. R. Galarneau, S. P. Gygi and D. J. Glass (2013). "Genomic and proteomic profiling reveals reduced mitochondrial function and disruption of the neuromuscular junction driving rat sarcopenia." Mol Cell Biol **33**(2): 194-212.

Joly-Amado, A., R. G. Denis, J. Castel, A. Lacombe, C. Cansell, C. Rouch, N. Kassis, J. Dairou, P. D. Cani, R. Ventura-Clapier, A. Prola, M. Flamment, F. Foufelle, C. Magnan and S. Luquet (2012). "Hypothalamic AgRP-neurons control peripheral substrate utilization and nutrient partitioning." EMBO J **31**(22): 4276-4288.

Kang, C., E. Chung, G. Diffee and L. L. Ji (2013). "Exercise training attenuates aging-associated mitochondrial dysfunction in rat skeletal muscle: role of PGC-1alpha." Experimental gerontology **48**(11): 1343-1350.

Kang, L., A. A. Dunn-Meynell, V. H. Routh, L. D. Gaspers, Y. Nagata, T. Nishimura, J. Eiki, B. B. Zhang and B. E. Levin (2006). "Glucokinase is a critical regulator of ventromedial hypothalamic neuronal glucosensing." Diabetes **55**(2): 412-420.

Kania, E., B. Pajak and A. Orzechowski (2015). "Calcium homeostasis and ER stress in control of autophagy in cancer cells." Biomed Res Int **2015**: 352794.

Kasarskis, E. J., L. Tandon, M. A. Lovell and W. D. Ehmann (1995). "Aluminum, calcium, and iron in the spinal cord of patients with sporadic amyotrophic lateral sclerosis using laser microprobe mass spectroscopy: a preliminary study." Journal of the neurological sciences **130**(2): 203-208.

Kass, G. E. and S. Orrenius (1999). "Calcium signaling and cytotoxicity." Environ Health Perspect **107 Suppl 1**: 25-35.

Katsouri, L., C. Parr, N. Bogdanovic, M. Willem and M. Sastre (2011). "PPARgamma co-activator-1alpha (PGC-1alpha) reduces amyloid-beta generation through a PPARgamma-dependent mechanism." Journal of Alzheimer's disease : JAD **25**(1): 151-162.

Kavanagh, N. I., E. K. Ainscow and M. D. Brand (2000). "Calcium regulation of oxidative phosphorylation in rat skeletal muscle mitochondria." Biochimica et biophysica acta **1457**(1-2): 57-70.

Keller, J. N., R. J. Mark, A. J. Bruce, E. Blanc, J. D. Rothstein, K. Uchida, G. Waeg and M. P. Mattson (1997). "4-Hydroxynonenal, an aldehydic product of membrane lipid peroxidation, impairs glutamate transport and mitochondrial function in synaptosomes." Neuroscience **80**(3): 685-696.

Kim, D., M. D. Nguyen, M. M. Dobbin, A. Fischer, F. Sananbenesi, J. T. Rodgers, I. Delalle, J. A. Baur, G. Sui, S. M. Armour, P. Puigserver, D. A. Sinclair and L. H. Tsai (2007). "SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis." EMBO J **26**(13): 3169-3179.

Kitamura, T., Y. Feng, Y. I. Kitamura, S. C. Chua, Jr., A. W. Xu, G. S. Barsh, L. Rossetti and D. Accili (2006). "Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake." Nature medicine **12**(5): 534-540.

Kong, D., Y. Dagon, J. N. Campbell, Y. Guo, Z. Yang, X. Yi, P. Aryal, K. Wellenstein, B. B. Kahn, B. L. Sabatini and B. B. Lowell (2016). "A Postsynaptic AMPK-->p21-Activated Kinase Pathway Drives Fasting-Induced Synaptic Plasticity in AgRP Neurons." Neuron **91**(1): 25-33.

Konopka, A. R. and K. Sreekumaran Nair (2013). "Mitochondrial and skeletal muscle health with advancing age." Molecular and cellular endocrinology **379**(1-2): 19-29.

Krebs, J., L. B. Agellon and M. Michalak (2015). "Ca(2+) homeostasis and endoplasmic reticulum (ER) stress: An integrated view of calcium signaling." Biochemical and biophysical research communications **460**(1): 114-121.

Kuipers, S. D. and C. R. Bramham (2006). "Brain-derived neurotrophic factor mechanisms and function in adult synaptic plasticity: new insights and implications for therapy." Curr Opin Drug Discov Devel **9**(5): 580-586.

Lagouge, M., C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeq, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver and J. Auwerx (2006). "Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha." Cell **127**(6): 1109-1122.

Lai, L., T. C. Leone, C. Zechner, P. J. Schaeffer, S. M. Kelly, D. P. Flanagan, D. M. Medeiros, A. Kovacs and D. P. Kelly (2008). "Transcriptional coactivators PGC-1alpha and PGC-lbeta control overlapping programs required for perinatal maturation of the heart." Genes & development **22**(14): 1948-1961.

Lee, C. M., L. E. Aspnes, S. S. Chung, R. Weindruch and J. M. Aiken (1998). "Influences of caloric restriction on age-associated skeletal muscle fiber characteristics and mitochondrial changes in rats and mice." Ann N Y Acad Sci **854**: 182-191.

Lee, P., J. D. Linderman, S. Smith, R. J. Brychta, J. Wang, C. Idelson, R. M. Perron, C. D. Werner, G. Q. Phan, U. S. Kammula, E. Kebebew, K. Pacak, K. Y. Chen and F. S. Celi (2014). "Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans." Cell Metab **19**(2): 302-309.

Leick, L., S. S. Lyngby, J. F. Wojtaszewski and H. Pilegaard (2010). "PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle." Experimental gerontology **45**(5): 336-342.

Lettieri Barbato, D., S. Baldelli, B. Pagliei, K. Aquilano and M. R. Ciriolo (2012). "Caloric Restriction and the Nutrient-Sensing PGC-1alpha in Mitochondrial Homeostasis: New Perspectives in Neurodegeneration." Int J Cell Biol **2012**: 759583.

Lin, J., C. Handschin and B. M. Spiegelman (2005). "Metabolic control through the PGC-1 family of transcription coactivators." Cell Metab **1**(6): 361-370.

Lin, J., P. Puigserver, J. Donovan, P. Tarr and B. M. Spiegelman (2002). "Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor." The Journal of biological chemistry **277**(3): 1645-1648.

Lin, J., H. Wu, P. T. Tarr, C. Y. Zhang, Z. Wu, O. Boss, L. F. Michael, P. Puigserver, E. Isotani, E. N. Olson, B. B. Lowell, R. Bassel-Duby and B. M. Spiegelman (2002). "Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres." Nature **418**(6899): 797-801.

Lin, J., P. H. Wu, P. T. Tarr, K. S. Lindenberg, J. St-Pierre, C. Y. Zhang, V. K. Mootha, S. Jager, C. R. Vianna, R. M. Reznick, L. Cui, M. Manieri, M. X. Donovan, Z. Wu, M. P. Cooper, M. C. Fan, L. M. Rohas, A. M. Zavacki, S. Cinti, G. I. Shulman, B. B. Lowell, D. Krainc and B. M. Spiegelman (2004). "Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice." Cell **119**(1): 121-135.

Liu, C., S. Li, T. Liu, J. Borjigin and J. D. Lin (2007). "Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism." Nature **447**(7143): 477-481.

Liu, J. C., J. Liu, K. M. Holmstrom, S. Menazza, R. J. Parks, M. M. Fergusson, Z. X. Yu, D. A. Springer, C. Halsey, C. Liu, E. Murphy and T. Finkel (2016). "MICU1 Serves as a Molecular Gatekeeper to Prevent In Vivo Mitochondrial Calcium Overload." Cell Rep **16**(6): 1561-1573.

Liu, Z., Y. Xia, B. Li, H. Xu, C. Wang, Y. Liu, Y. Li, C. Li, N. Gao and L. Li (2014). "Induction of ER stress-mediated apoptosis by ceramide via disruption of ER Ca(2+) homeostasis in human adenoid cystic carcinoma cells." Cell Biosci **4**: 71.

Lui, A. J. and N. N. Byl (2009). "A systematic review of the effect of moderate intensity exercise on function and disease progression in amyotrophic lateral sclerosis." J Neurol Phys Ther **33**(2): 68-87.

Luo, Y., W. Zhu, J. Jia, C. Zhang and Y. Xu (2009). "NMDA receptor dependent PGC-1alpha up-regulation protects the cortical neuron against oxygen-glucose deprivation/reperfusion injury." Journal of molecular neuroscience : MN **39**(1-2): 262-268.

Lytton, J., M. Westlin and M. R. Hanley (1991). "Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca-ATPase family of calcium pumps." J Biol Chem **266**(26): 17067-17071.

Ma, D., S. Li, E. K. Lucas, R. M. Cowell and J. D. Lin (2010). "Neuronal inactivation of peroxisome proliferator-activated receptor gamma coactivator 1alpha (PGC-1alpha) protects mice from diet-induced obesity and leads to degenerative lesions." The Journal of biological chemistry **285**(50): 39087-39095.

Mammucari, C., G. Gherardi, I. Zamparo, A. Raffaello, S. Boncompagni, F. Chemello, S. Cagnin, A. Braga, S. Zanin, G. Pallafacchina, L. Zentilin, M. Sandri, D. De Stefani, F. Protasi, G. Lanfranchi and R. Rizzuto (2015). "The mitochondrial calcium uniporter controls skeletal muscle trophism in vivo." Cell reports **10**(8): 1269-1279.

Mansouri, A., F. L. Muller, Y. Liu, R. Ng, J. Faulkner, M. Hamilton, A. Richardson, T. T. Huang, C. J. Epstein and H. Van Remmen (2006). "Alterations in mitochondrial function, hydrogen peroxide release and oxidative damage in mouse hind-limb skeletal muscle during aging." Mech Ageing Dev **127**(3): 298-306.

Mark, R. J., K. Hensley, D. A. Butterfield and M. P. Mattson (1995). "Amyloid beta-peptide impairs ion-motive ATPase activities: evidence for a role in loss of neuronal Ca2+ homeostasis and cell death." The Journal of neuroscience : the official journal of the Society for Neuroscience **15**(9): 6239-6249.

Marzetti, E., J. M. Lawler, A. Hiona, T. Manini, A. Y. Seo and C. Leeuwenburgh (2008). "Modulation of age-induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle." Free Radic Biol Med **44**(2): 160-168.

Marzetti, E., H. A. Lees, S. E. Wohlgemuth and C. Leeuwenburgh (2009). "Sarcopenia of aging: underlying cellular mechanisms and protection by calorie restriction." Biofactors **35**(1): 28-35.

Mattson, M. P., B. Cheng, D. Davis, K. Bryant, I. Lieberburg and R. E. Rydel (1992). "beta-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity." The Journal of neuroscience : the official journal of the Society for Neuroscience **12**(2): 376-389.

McGill, J. K. and M. F. Beal (2006). "PGC-1alpha, a new therapeutic target in Huntington's disease?" Cell **127**(3): 465-468.

McMillin, J. B. and M. C. Madden (1989). "The role of calcium in the control of respiration by muscle mitochondria." Med Sci Sports Exerc **21**(4): 406-410.

Meng, S. J. and L. J. Yu (2010). "Oxidative stress, molecular inflammation and sarcopenia." Int J Mol Sci **11**(4): 1509-1526.

Minokoshi, Y., T. Alquier, N. Furukawa, Y. B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferre, M. J. Birnbaum, B. J. Stuck and B. B. Kahn (2004). "AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus." Nature **428**(6982): 569-574.

Mitchell, S. J., A. Martin-Montalvo, E. M. Mercken, H. H. Palacios, T. M. Ward, G. Abulwerdi, R. K. Minor, G. P. Vlasuk, J. L. Ellis, D. A. Sinclair, J. Dawson, D. B. Allison, Y. Zhang, K. G. Becker, M. Bernier and R. de Cabo (2014). "The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet." Cell Rep **6**(5): 836-843.

Mitra, R., D. P. Nogee, J. F. Zechner, K. Yea, C. M. Gierasch, A. Kovacs, D. M. Medeiros, D. P. Kelly and J. G. Duncan (2012). "The transcriptional coactivators, PGC-1alpha and beta, cooperate to maintain cardiac mitochondrial function during the early stages of insulin resistance." Journal of molecular and cellular cardiology **52**(3): 701-710.

Morgan, K., S. Obici and L. Rossetti (2004). "Hypothalamic responses to long-chain fatty acids are nutritionally regulated." J Biol Chem **279**(30): 31139-31148.

Morris, E. M., G. M. Meers, F. W. Booth, K. L. Fritsche, C. D. Hardin, J. P. Thyfault and J. A. Ibdah (2012). "PGC-1alpha overexpression results in increased hepatic fatty acid oxidation with reduced triacylglycerol accumulation and secretion." Am J Physiol Gastrointest Liver Physiol **303**(8): G979-992.

Morselli, E., E. Fuente-Martin, B. Finan, M. Kim, A. Frank, C. Garcia-Caceres, C. R. Navas, R. Gordillo, M. Neinast, S. P. Kalainayakan, D. L. Li, Y. Gao, C. X. Yi, L. Hahner, B. F. Palmer, M. H. Tschop and D. J. Clegg (2014). "Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha." Cell Rep **9**(2): 633-645.

Mudo, G., J. Makela, V. Di Liberto, T. V. Tselykh, M. Olivieri, P. Piepponen, O. Eriksson, A. Malkia, A. Bonomo, M. Kairisalo, J. A. Aguirre, L. Korhonen, N. Belluardo and D. Lindholm (2012). "Transgenic expression and activation of PGC-1alpha protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease." Cellular and molecular life sciences : CMLS **69**(7): 1153-1165.

Murray, F. E., J. P. Landsberg, R. J. Williams, M. M. Esiri and F. Watt (1992). "Elemental analysis of neurofibrillary tangles in Alzheimer's disease using proton-induced X-ray analysis." Ciba Found Symp **169**: 201-210; discussion 210-206.

Nair, K. S. (2005). "Aging muscle." The American journal of clinical nutrition **81**(5): 953-963.

Nishijima, T., M. Llorens-Martin, G. S. Tejeda, K. Inoue, Y. Yamamura, H. Soya, J. L. Trejo and I. Torres-Aleman (2013). "Cessation of voluntary wheel running increases anxiety-like behavior and impairs adult hippocampal neurogenesis in mice." Behav Brain Res **245**: 34-41.

Nitahara, J. A., W. Cheng, Y. Liu, B. Li, A. Leri, P. Li, D. Mogul, S. R. Gambert, J. Kajstura and P. Anversa (1998). "Intracellular calcium, DNase activity and myocyte apoptosis in aging Fischer 344 rats." J Mol Cell Cardiol **30**(3): 519-535.

Nixon, R. A. (2003). "The calpains in aging and aging-related diseases." Ageing research reviews **2**(4): 407-418.

Novak, C. M., P. R. Burghardt and J. A. Levine (2012). "The use of a running wheel to measure activity in rodents: relationship to energy balance, general activity, and reward." Neurosci Biobehav Rev **36**(3): 1001-1014.

Obici, S., Z. Feng, K. Morgan, D. Stein, G. Karkanias and L. Rossetti (2002). "Central administration of oleic acid inhibits glucose production and food intake." Diabetes **51**(2): 271-275.

Ogata, T., S. Machida, Y. Oishi, M. Higuchi and I. Muraoka (2009). "Differential cell death regulation between adult-unloaded and aged rat soleus muscle." Mech Ageing Dev **130**(5): 328-336.

Okuma, H., F. Saito, J. Mitsui, Y. Hara, Y. Hatanaka, M. Ikeda, T. Shimizu, K. Matsumura, J. Shimizu, S. Tsuji and M. Sonoo (2016). "Tubular aggregate myopathy caused by a novel mutation in the cytoplasmic domain of STIM1." Neurol Genet **2**(1): e50.

Pagel-Langenickel, I., J. Bao, J. J. Joseph, D. R. Schwartz, B. S. Mantell, X. Xu, N. Raghavachari and M. N. Sack (2008). "PGC-1alpha integrates insulin signaling, mitochondrial regulation, and bioenergetic function in skeletal muscle." The Journal of biological chemistry **283**(33): 22464-22472.

Pan, X., J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, M. M. Fergusson, Rovira, II, M. Allen, D. A. Springer, A. M. Aponte, M. Gucek, R. S. Balaban, E. Murphy and T. Finkel (2013). "The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter." Nat Cell Biol **15**(12): 1464-1472.

Pant, M., D. H. Sopariwala, N. C. Bal, J. Lowe, D. A. Delfin, J. Rafael-Fortney and M. Periasamy (2015). "Metabolic dysfunction and altered mitochondrial dynamics in the utrophin-dystrophin deficient mouse model of duchenne muscular dystrophy." PLoS One **10**(4): e0123875.

Paschen, W. (2003). "Endoplasmic reticulum: a primary target in various acute disorders and degenerative diseases of the brain." Cell Calcium **34**(4-5): 365-383.

Pietrangelo, L., A. D'Incecco, A. Ainbinder, A. Michelucci, H. Kern, R. T. Dirksen, S. Boncompagni and F. Protasi (2015). "Age-dependent uncoupling of mitochondria from Ca2(+) release units in skeletal muscle." Oncotarget **6**(34): 35358-35371.

Pietrangelo, L., A. D'Incecco, A. Ainbinder, A. Michelucci, H. Kern, R. T. Dirksen, S. Boncompagni and F. Protasi (2015). "Age-dependent uncoupling of mitochondria from Ca2+ release units in skeletal muscle." Oncotarget **6**(34): 35358-35371.

Puigserver, P., J. Rhee, J. Donovan, C. J. Walkey, J. C. Yoon, F. Oriente, Y. Kitamura, J. Altomonte, H. Dong, D. Accili and B. M. Spiegelman (2003). "Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction." Nature **423**(6939): 550-555.

Puigserver, P., Z. Wu, C. W. Park, R. Graves, M. Wright and B. M. Spiegelman (1998). "A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis." Cell **92**(6): 829-839.

Qin, W., V. Haroutunian, P. Katsel, C. P. Cardozo, L. Ho, J. D. Buxbaum and G. M. Pasinetti (2009). "PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia." Archives of neurology **66**(3): 352-361.

Ranhotra, H. S. (2010). "Long-term caloric restriction up-regulates PPAR gamma co-activator 1 alpha (PGC-1alpha) expression in mice." Indian journal of biochemistry & biophysics **47**(5): 272-277.

Rao, R. R., J. Z. Long, J. P. White, K. J. Svensson, J. Lou, I. Lokurkar, M. P. Jedrychowski, J. L. Ruas, C. D. Wrann, J. C. Lo, D. M. Camera, J. Lachey, S. Gygi, J. Seehra, J. A. Hawley and B. M. Spiegelman (2014). "Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis." Cell **157**(6): 1279-1291.

Rao, R. V., E. Hermel, S. Castro-Obregon, G. del Rio, L. M. Ellerby, H. M. Ellerby and D. E. Bredesen (2001). "Coupling endoplasmic reticulum stress to the cell death program. Mechanism of caspase activation." J Biol Chem **276**(36): 33869-33874.

Raymond, L. A. (2016). "Striatal synaptic dysfunction and altered calcium regulation in Huntington disease." Biochemical and biophysical research communications.

Renganathan, M., M. L. Messi and O. Delbono (1997). "Dihydropyridine receptor-ryanodine receptor uncoupling in aged skeletal muscle." J Membr Biol **157**(3): 247-253.

Rizzuto, R., D. De Stefani, A. Raffaello and C. Mammucari (2012). "Mitochondria as sensors and regulators of calcium signalling." Nat Rev Mol Cell Biol **13**(9): 566-578.

Roberts, L. D., P. Bostrom, J. F. O'Sullivan, R. T. Schinzel, G. D. Lewis, A. Dejam, Y. K. Lee, M. J. Palma, S. Calhoun, A. Georgiadi, M. H. Chen, V. S. Ramachandran, M. G. Larson, C. Bouchard, T. Rankinen, A. L. Souza, C. B. Clish, T. J. Wang, J. L. Estall, A. A. Soukas, C. A. Cowan, B. M. Spiegelman and R. E. Gerszten (2014). "beta-Aminoisobutyric acid induces browning of white fat and hepatic beta-oxidation and is inversely correlated with cardiometabolic risk factors." Cell Metab **19**(1): 96-108.

Romanello, V., E. Guadagnin, L. Gomes, I. Roder, C. Sandri, Y. Petersen, G. Milan, E. Masiero, P. Del Piccolo, M. Foretz, L. Scorrano, R. Rudolf and M. Sandri (2010). "Mitochondrial fission and remodelling contributes to muscle atrophy." EMBO J **29**(10): 1774-1785.

Rowland, L. P. and N. A. Shneider (2001). "Amyotrophic lateral sclerosis." N Engl J Med **344**(22): 1688-1700.

Ruan, H. B., M. O. Dietrich, Z. W. Liu, M. R. Zimmer, M. D. Li, J. P. Singh, K. Zhang, R. Yin, J. Wu, T. L. Horvath and X. Yang (2014). "O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat." Cell **159**(2): 306-317.

Safdar, A., S. Annis, Y. Kraytsberg, C. Laverack, A. Saleem, K. Popadin, D. C. Woods, J. L. Tilly and K. Khrapko (2016). "Amelioration of premature aging in mtDNA mutator mouse by exercise: the interplay of oxidative stress, PGC-1alpha, p53, and DNA damage. A hypothesis." Curr Opin Genet Dev **38**: 127-132.

Sahin, E., S. Colla, M. Liesa, J. Moslehi, F. L. Muller, M. Guo, M. Cooper, D. Kotton, A. J. Fabian, C. Walkey, R. S. Maser, G. Tonon, F. Foerster, R. Xiong, Y. A. Wang, S. A. Shukla, M. Jaskelioff, E. S. Martin, T. P. Heffernan, A. Protopopov, E. Ivanova, J. E. Mahoney, M. Kost-Alimova, S. R. Perry, R. Bronson, R. Liao, R. Mulligan, O. S. Shirihai, L. Chin and R. A. DePinho (2011). "Telomere dysfunction induces metabolic and mitochondrial compromise." Nature **470**(7334): 359-365.

Sano, R. and J. C. Reed (2013). "ER stress-induced cell death mechanisms." Biochim Biophys Acta **1833**(12): 3460-3470.

Schnyder, S. and C. Handschin (2015). "Skeletal muscle as an endocrine organ: PGC-1alpha, myokines and exercise." Bone **80**: 115-125.

Schriner, S. E., N. J. Linford, G. M. Martin, P. Treuting, C. E. Ogburn, M. Emond, P. E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D. C. Wallace and P. S. Rabinovitch (2005). "Extension of murine life span by overexpression of catalase targeted to mitochondria." Science **308**(5730): 1909-1911.

Schubert, W., F. Sotgia, A. W. Cohen, F. Capozza, G. Bonuccelli, C. Bruno, C. Minetti, E. Bonilla, S. Dimauro and M. P. Lisanti (2007). "Caveolin-1(-/-)- and caveolin-2(-/-)-deficient mice both display numerous skeletal muscle abnormalities, with tubular aggregate formation." The American journal of pathology **170**(1): 316-333.

Shao, D., Y. Liu, X. Liu, L. Zhu, Y. Cui, A. Cui, A. Qiao, X. Kong, Q. Chen, N. Gupta, F. Fang and Y. Chang (2010). "PGC-1 beta-regulated mitochondrial biogenesis and function in myotubes is mediated by NRF-1 and ERR alpha." Mitochondrion **10**(5): 516-527.

Short, K. R., M. L. Bigelow, J. Kahl, R. Singh, J. Coenen-Schimke, S. Raghavakaimal and K. S. Nair (2005). "Decline in skeletal muscle mitochondrial function with aging in humans." Proceedings of the National Academy of Sciences of the United States of America **102**(15): 5618-5623.

Short, K. R., J. L. Vittone, M. L. Bigelow, D. N. Proctor and K. S. Nair (2004). "Age and aerobic exercise training effects on whole body and muscle protein metabolism." Am J Physiol Endocrinol Metab **286**(1): E92-101.

Singh, K. K. (2004). "Mitochondria damage checkpoint in apoptosis and genome stability." FEMS Yeast Res **5**(2): 127-132.

Siskind, L. J. (2005). "Mitochondrial ceramide and the induction of apoptosis." J Bioenerg Biomembr **37**(3): 143-153.

Squier, T. C. and D. J. Bigelow (2000). "Protein oxidation and age-dependent alterations in calcium homeostasis." Front Biosci **5**: D504-526.

Steinbusch, L., G. Labouebe and B. Thorens (2015). "Brain glucose sensing in homeostatic and hedonic regulation." Trends Endocrinol Metab **26**(9): 455-466.

Summermatter, S., O. Baum, G. Santos, H. Hoppeler and C. Handschin (2010). "Peroxisome proliferator-activated receptor {gamma} coactivator 1{alpha} (PGC-1{alpha}) promotes skeletal muscle lipid refueling in vivo by activating de novo lipogenesis and the pentose phosphate pathway." The Journal of biological chemistry **285**(43): 32793-32800.

Summermatter, S., R. Thurnheer, G. Santos, B. Mosca, O. Baum, S. Treves, H. Hoppeler, F. Zorzato and C. Handschin (2012). "Remodeling of calcium handling in skeletal muscle through PGC-1alpha: impact on force, fatigability, and fiber type." American journal of physiology. Cell physiology **302**(1): C88-99.

Svensson, K., S. Schnyder, V. Albert, B. Cardel, L. Quagliata, L. M. Terracciano and C. Handschin (2015). "Resveratrol and SRT1720 Elicit Differential Effects in Metabolic Organs and Modulate Systemic Parameters Independently of Skeletal Muscle Peroxisome Proliferator-activated Receptor gamma Co-activator 1alpha (PGC-1alpha)." J Biol Chem **290**(26): 16059-16076.

Szegezdi, E., S. E. Logue, A. M. Gorman and A. Samali (2006). "Mediators of endoplasmic reticulum stress-induced apoptosis." EMBO Rep **7**(9): 880-885.

Tews, D. S. (2002). "Apoptosis and muscle fibre loss in neuromuscular disorders." Neuromuscul Disord **12**(7-8): 613-622.

Thon, L., H. Mohlig, S. Mathieu, A. Lange, E. Bulanova, S. Winoto-Morbach, S. Schutze, S. Bulfone-Paus and D. Adam (2005). "Ceramide mediates caspase-independent programmed cell death." FASEB J **19**(14): 1945-1956.

Tymianski, M., M. C. Wallace, I. Spigelman, M. Uno, P. L. Carlen, C. H. Tator and M. P. Charlton (1993). "Cell-permeant Ca2+ chelators reduce early excitotoxic and ischemic neuronal injury in vitro and in vivo." Neuron **11**(2): 221-235.

Uldry, M., W. Yang, J. St-Pierre, J. Lin, P. Seale and B. M. Spiegelman (2006). "Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation." Cell metabolism **3**(5): 333-341.

Umanskaya, A., G. Santulli, W. Xie, D. C. Andersson, S. R. Reiken and A. R. Marks (2014). "Genetically enhancing mitochondrial antioxidant activity improves muscle function in aging." Proc Natl Acad Sci U S A **111**(42): 15250-15255.

Vainshtein, A., E. M. Desjardins, A. Armani, M. Sandri and D. A. Hood (2015). "PGC-1alpha modulates denervation-induced mitophagy in skeletal muscle." Skeletal muscle **5**: 9.

Valle, I., A. Alvarez-Barrientos, E. Arza, S. Lamas and M. Monsalve (2005). "PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells." Cardiovascular research **66**(3): 562-573.

Valtat, B., J. P. Riveline, P. Zhang, A. Singh-Estivalet, M. Armanet, N. Venteclef, A. Besseiche, D. P. Kelly, F. Tronche, P. Ferre, J. F. Gautier, B. Breant and B. Blondeau (2013). "Fetal PGC-1alpha overexpression programs adult pancreatic beta-cell dysfunction." Diabetes **62**(4): 1206-1216.

Vielhaber, S., R. Schroder, K. Winkler, S. Weis, M. Sailer, H. Feistner, H. J. Heinze, J. M. Schroder and W. S. Kunz (2001). "Defective mitochondrial oxidative phosphorylation in myopathies with tubular aggregates originating from sarcoplasmic reticulum." Journal of neuropathology and experimental neurology **60**(11): 1032-1040.

Wang, Q., C. Liu, A. Uchida, J. C. Chuang, A. Walker, T. Liu, S. Osborne-Lawrence, B. L. Mason, C. Mosher, E. D. Berglund, J. K. Elmquist and J. M. Zigman (2014). "Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin." Molecular metabolism **3**(1): 64-72.

Wareski, P., A. Vaarmann, V. Choubey, D. Safiulina, J. Liiv, M. Kuum and A. Kaasik (2009). "PGC-1{alpha} and PGC-1{beta} regulate mitochondrial density in neurons." The Journal of biological chemistry **284**(32): 21379-21385.

Wenz, T., S. G. Rossi, R. L. Rotundo, B. M. Spiegelman and C. T. Moraes (2009). "Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging." Proceedings of the National Academy of Sciences of the United States of America **106**(48): 20405-20410.

Wong, K. E., C. R. Mikus, D. H. Slentz, S. E. Seiler, K. L. DeBalsi, O. R. Ilkayeva, K. I. Crain, M. T. Kinter, C. L. Kien, R. D. Stevens and D. M. Muoio (2015). "Muscle-Specific Overexpression of PGC-1alpha Does Not Augment Metabolic Improvements in Response to Exercise and Caloric Restriction." Diabetes **64**(5): 1532-1543.

Wrann, C. D., J. P. White, J. Salogiannnis, D. Laznik-Bogoslavski, J. Wu, D. Ma, J. D. Lin, M. E. Greenberg and B. M. Spiegelman (2013). "Exercise induces hippocampal BDNF through a PGC-1alpha/FNDC5 pathway." Cell Metab **18**(5): 649-659.

Wu, J., J. L. Ruas, J. L. Estall, K. A. Rasbach, J. H. Choi, L. Ye, P. Bostrom, H. M. Tyra, R. W. Crawford, K. P. Campbell, D. T. Rutkowski, R. J. Kaufman and B. M. Spiegelman (2011). "The unfolded protein response mediates adaptation to exercise in skeletal muscle through a PGC-1alpha/ATF6alpha complex." Cell metabolism **13**(2): 160-169.

Wu, Z., P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell and R. Scarpulla (1999). "Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1." Cell **98**(1): 115-124.

Xiong, S., N. Patrushev, F. Forouzandeh, L. Hilenski and R. W. Alexander (2015). "PGC-1alpha Modulates Telomere Function and DNA Damage in Protecting against Aging-Related Chronic Diseases." Cell reports **12**(9): 1391-1399.

Yi, J., C. Ma, Y. Li, N. Weisleder, E. Rios, J. Ma and J. Zhou (2011). "Mitochondrial calcium uptake regulates rapid calcium transients in skeletal muscle during excitation-contraction (E-C) coupling." The Journal of biological chemistry **286**(37): 32436-32443.

Yoon, J. C., G. Xu, J. T. Deeney, S. N. Yang, J. Rhee, P. Puigserver, A. R. Levens, R. Yang, C. Y. Zhang, B. B. Lowell, P. O. Berggren, C. B. Newgard, S. Bonner-Weir, G. Weir and B. M. Spiegelman (2003). "Suppression of beta cell energy metabolism and insulin release by PGC-1alpha." Developmental cell **5**(1): 73-83.

Zhao, W., M. Varghese, S. Yemul, Y. Pan, A. Cheng, P. Marano, S. Hassan, P. Vempati, F. Chen, X. Qian and G. M. Pasinetti (2011). "Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis." Mol Neurodegener **6**(1): 51.

Zheng, B., Z. Liao, J. J. Locascio, K. A. Lesniak, S. S. Roderick, M. L. Watt, A. C. Eklund, Y. Zhang-James, P. D. Kim, M. A. Hauser, E. Grunblatt, L. B. Moran, S. A. Mandel, P. Riederer, R. M. Miller, H. J. Federoff, U. Wullner, S. Papapetropoulos, M. B. Youdim, I. Cantuti-Castelvetri, A. B. Young, J. M. Vance, R. L. Davis, J. C. Hedreen, C. H. Adler, T. G. Beach, M. B. Graeber, F. A. Middleton, J. C. Rochet and C. R. Scherzer (2010). "PGC-1alpha, a potential therapeutic target for early intervention in Parkinson's disease." Science translational medicine **2**(52): 52ra73.

Zhu, L. L., Y. Liu, A. F. Cui, D. Shao, J. C. Liang, X. J. Liu, Y. Chen, N. Gupta, F. D. Fang and Y. S. Chang (2010). "PGC-1alpha coactivates estrogen-related receptor-alpha to induce the expression of glucokinase." American journal of physiology. Endocrinology and metabolism **298**(6): E1210-1218.

Zorzano, A. (2009). "Regulation of mitofusin-2 expression in skeletal muscle." Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme **34**(3): 433-439.

Appendix: Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1α

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ARTICLE

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Morphological and functional remodelling of the neuromuscular junction by skeletal muscle PGC-1α

Anne-Sophie Arnold[1], Jonathan Gill[1], Martine Christe[1,‡], Rocío Ruiz[2], Shawn McGuirk[3], Julie St-Pierre[3], Lucía Tabares[2] & Christoph Handschin[1]

The neuromuscular junction (NMJ) exhibits high morphological and functional plasticity. In the mature muscle, the relative levels of physical activity are the major determinants of NMJ function. Classically, motor neuron-mediated activation patterns of skeletal muscle have been thought of as the major drivers of NMJ plasticity and the ensuing fibre-type determination in muscle. Here we use muscle-specific transgenic animals for the peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) as a genetic model for trained mice to elucidate the contribution of skeletal muscle to activity-induced adaptation of the NMJ. We find that muscle-specific expression of PGC-1α promotes a remodelling of the NMJ, even in the absence of increased physical activity. Importantly, these plastic changes are not restricted to post-synaptic structures, but extended to modulation of presynaptic cell morphology and function. Therefore, our data indicate that skeletal muscle significantly contributes to the adaptation of the NMJ subsequent to physical activity.

[1] Biozentrum, Division of Pharmacology/Neurobiology, University of Basel, Klingelbergstrasse 50/70, CH-4056 Basel, Switzerland. [2] Department of Medical Physiology and Biophysics, School of Medicine University of Seville, Avda. Sánchez Pizjuan 4, 41009 Sevilla, Spain. [3] Department of Biochemistry, Rosalind and Morris Goodman Cancer Centre, McGill University, 3655 promenade Sir William Osler, Montreal, Quebec, Canada H3G 1Y6. Correspondence and requests for materials should be addressed to C.H. (email: christoph.handschin@unibas.ch).
‡deceased.

The neuromuscular junction (NMJ) is a complex structure that mediates the cross-talk between motor neurons and muscle fibres. The embryonic and post-natal installation of NMJs has been studied extensively and the key factors that regulate the NMJ assembly in this context have been described, including agrin and acetylcholine, membrane protein complexes and extracellular matrix components as well as various neurotrophic factors[1–3]. At least some of these regulators are also responsible for guiding NMJ formation in denervation–reinnervation cycles that are observed in fibre damage, several muscle dystrophies or in the aging process[4,5]. Interestingly, while a dominant role for motor neurons in NMJ formation has been postulated, an increasing amount of evidence highlights the important contribution of skeletal muscle to the proper installation of the NMJ[2,6–8]. For example, prepatterning by primitive, aneural acetylcholine receptor (AChR) clusters on the muscle fibre membrane might help axonal guidance[9]. Likewise, skeletal muscle-specific receptor tyrosine-protein kinase (MuSK) and low-density lipoprotein receptor-related protein 4, two key components involved in post-synaptic agrin action, mediate retrograde signaling from the muscle to the motor neuron that is crucial for presynaptic differentiation of the NMJ during embryonic and postnatal development[10]. At least studies in *Drosophila* suggest that these proteins might also be involved in late-stage NMJ plasticity[11]; however, this hypothesis remains to be substantiated in *Drosophila* as well as in vertebrates.

Curiously, in contrast to the widely accepted important contribution of the skeletal muscle to NMJ formation in development and re-innervation, the role of this organ in mediating plastic changes of the mature NMJ is much less appreciated. For example, morphological and functional adaptations of the NMJ to endurance training improve neuromuscular transmission and thereby contribute to an increased fatigue resistance[12,13]. The neuromuscular exercise plasticity however has almost exclusively attributed to the rate and amplitude of myocellular calcium transients that are triggered by distinct patterns of motor neuron firing[14,15]. Thus, exercise adaptations of the NMJ that ultimately lead to a metabolic and myofibrillar fibre-type switch towards oxidative, high endurance muscle fibres are thought to be dominantly controlled by the neuronal input[16]. Accordingly, the plastic changes of NMJs in the trained neuromuscular context have been extensively characterized and include adaptations in endplate size, sprouting of nerve terminal branches, and electrophysiological kinetics[17–19]. However, the anterograde and retrograde signals involved in this remodelling remain largely enigmatic. Similarly, potential contributions of the skeletal muscle to these plastic changes have not been studied.

Overexpression of the peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) in cultured muscle cells is sufficient to promote postsynaptic NMJ gene transcription and the formation of aneural AChR clusters. Moreover, skeletal muscle-specific gain- and loss-of-function mouse models for PGC-1α exhibit altered expression of postsynaptic NMJ genes in muscle *in vivo*[20]. However, the consequence of altered muscle PGC-1α expression on NMJ morphology and function *in vivo* has not been assessed to date.

Besides controlling postsynaptic NMJ gene expression, PGC-1α is a key regulatory nexus of endurance exercise adaptation in the skeletal muscle. Accordingly, PGC-1α levels are strongly regulated by physical activity[21]. Upon activation, PGC-1α promotes many, if not all of the adaptations of muscle to endurance training, including increased mitochondrial biogenesis and oxidative metabolism, vascularization and a switch towards type I and IIa oxidative, high-endurance muscle fibres[22–24]. Inversely, muscle-specific PGC-1α knockout mice exhibit features of pathological inactivity such as a switch towards glycolytic muscle fibres, reduced exercise capacity, fibre damage and local as well as systemic inflammation[25,26]. On the basis of the ability to promote a trained phenotype even in inactive muscle, overexpression of PGC-1α exerts a beneficial therapeutic effect in different contexts of muscular wasting, including Duchenne muscular dystrophy[20], denervation-induced fibre atrophy, statin-mediated muscle dysmorphology, sarcopenia[27] or amyotrophic lateral sclerosis[28,29].

To assess the contribution of skeletal muscle to the plasticity of adult NMJ morphology and function, we now studied the muscle-specific PGC-1α transgenic mice as a genetic model for endurance exercise. Even though these animals exhibit a trained phenotype, the PGC-1α transgenics are not hyperactive and hence do not exhibit increased motor neuron firing when kept in a sedentary state[30]. This allows a dissociation of the effects from skeletal muscle and motor neurons on the NMJ in a mouse model of endurance exercise. Surprisingly, our findings demonstrate a hitherto underappreciated role for skeletal muscle to modulate NMJ plasticity both post- and pre-synaptically by improving NMJ integrity and function. Thus, in the adult neuromuscular system, shifts in the phenotype of the muscle fibres significantly contribute to the NMJ remodelling by activating a novel retrograde signalling.

Results

Transgenic PGC-1α expression in different tissues. In the transgenic animals, the expression of PGC-1α is driven by the muscle creatine kinase (MCK) promoter. To ensure muscle specificity of expression, we measured PGC-1α expression in the MCK mice using the WT samples as reference. As expected, the relative PGC-1α mRNA expression was highest in the predominantly glycolytic muscles tibialis anterior (TA), extensor digitorum longus (EDL), sternocleidomastoid (SCM) and levator auris longus (LAL), followed by the more mixed muscle beds in gastrocnemius and diaphragm (Fig. 1). The overexpression of PGC-1α was not significantly elevated in MCK mice in the oxidative soleus and transversus abdominis (TVA) muscles. Important for the present study, neuronal tissue, including spinal cord and brain, exhibited indistinguishable levels of PGC-1α transcript in WT and MCK animals, thus confirming the muscle specificity of the transgenic PGC-1α expression in this mouse line.

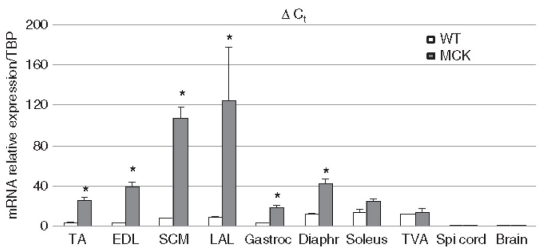

Figure 1 | Level of expression of PGC-1α in different muscles from WT and MCK mice Relative PGC-1α mRNA levels in tissues from WT (white bars, $n=3$) and MCK-PGC-1α mice (grey bars, $n=3$) (6- to 8-week-old). Total RNA was reverse transcribed and the level of expression of PGC-1α was determined by real-time PCR, relative to the TATA-Binding Protein (TBP) expression level, analysed according to the ΔC_t method. Each bar represents mean ± s.e.m. *$P<0.05$ ($n=3$, t-test two-tailed). TA: tibialis anterior, EDL: extensor digitorum longus, SCM: sternocleidomastoidian, LAL: levator auris longus, Gastroc: gastrocnemius, Diaphr: diaphragm, TVA: transversus abdominis.

Elevated muscle PGC-1α modifies NMJ morphology. The effect of muscle PGC-1α on postsynaptic gene expression in muscle cells and on formation of aneural AChR clusters *in vitro* has been demonstrated previously[20]. We now studied the consequences of elevated expression of PGC-1α in skeletal muscle on NMJ morphology *in vivo*. AChR aggregates in the post-synaptic membrane, typically pretzel-shaped, were stained with AF488-α-bungarotoxin (α-Bgtx), a very potent ligand for AChR (visualized in green). The motor neuron was labelled using an anti-neurofilament antibody revealed by a cyanin 5-conjugated secondary antibody (shown in red; Fig. 2a). Quantification of the AChR clusters revealed a significantly smaller area in the glycolytic EDL and SCM of the MCK mice compared with their WT littermates (Fig. 2b). In contrast, the already smaller area of the postsynaptic endplates in the more oxidative diaphragm and soleus muscles was unchanged between MCK and WT animals (Fig. 2b).

NMJs always present a certain percentage of abnormalities at the post- and pre-synaptic levels. For example, the pretzel shape can be irregular, fragmented or the density of the clusters can be faint, a sign of decreased AChR intensity. The percentage of structural NMJ deformities increases as the mouse ages and can be rectified by caloric restriction or exercise[18]. Similar to the improvement mediated by these interventions, the post-synaptic architecture of the NMJs was also significantly improved in the young MCK mice (Fig 2c–f). In particular, SCM and diaphragm

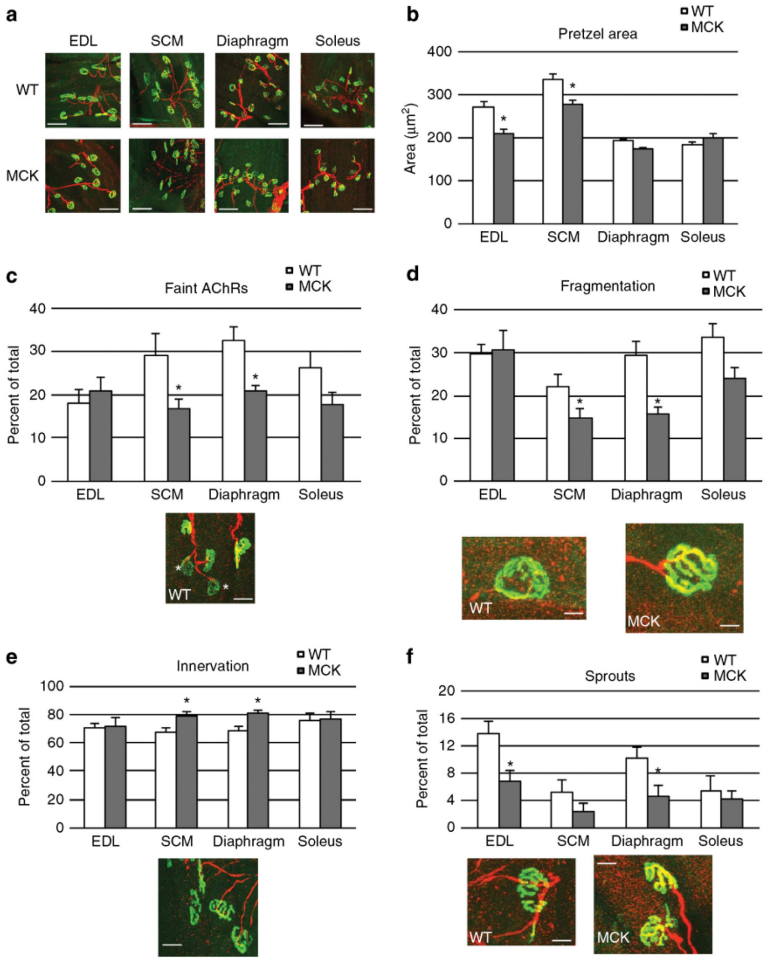

Figure 2 | Structural comparison of the NMJ from WT and transgenic mice. (**a**) Representative confocal stack image of fluorescently labelled NMJ in different muscles. Muscles were stained with AF488-coupled α-bungarotoxin to visualize AChRs (green) and anti-neurofilament antibodies coupled to cyanin 5 to stain the nerve part (red). Calibration bar: 200 μm (**b**) Mean area of the AChRs aggregates was determined using the Image J in arbitrary units. Each bar represents mean ± s.e.m. *$P<0.05$ ($N=3$, *t*-test two-tailed). (**c–f**) The frequency of faint AChRs clusters, highlighted by a white star Calibration bar: 50 μm (**c**), fragmented AChRs clusters Calibration bar: 1.5 μm (**d**), innervated pretzels Calibration bar: 40 μm (**e**) and sprouted AChRs clusters Calibration bar: 25 μm (**f**) was determined on confocal images stack. Each bar represents mean ± s.e.m. from at least 100 NMJ from three mice *$P<0.05$ ($N=3$, $n>100$, *t*-test two-tailed). White bars represent s animals, grey bars represent transgenic mice.

muscles of MCK animals displayed a reduction of faint pretzels (Fig. 2c) and the number of fragmented clusters (Fig. 2d) when compared with the WT counterparts, while no significant differences were detectable in EDL and soleus muscles. Likewise, overexpression of PGC-1α in skeletal muscle increased the number of innervated NMJs with clear co-localization of the AChR and the motor neuron signal in SCM and diaphragm (Fig. 2e) as well a reduction of sprouts, nerve terminals that do not terminate within an AChR cluster in EDL and diaphragm (Fig. 2f) of MCK mice.

PGC-1α-mediated changes in the ultrastructure of the NMJ. Our data indicate that muscle PGC-1α improves NMJ morphology on the histological level. Importantly, structural and functional stabilization of the NMJ is to a large extent governed by the total area of interface between the motor neuron and the muscle and hence the number and size of synaptic folds. Therefore, to investigate how PGC-1α expression in muscle could affect NMJ structure, we studied the synaptic folds on the ultrastructural level using transmission electron microscopy (TEM) of the synaptic regions of SCM and soleus muscle (Fig 3a–f). Interestingly, the NMJs in the SCM of MCK mice not only exhibited more folds per individual synapse (Fig. 3a) but also a significant increase in the average length of these synaptic folds (Fig. 3b), as illustrated by a representative TEM picture shown in Fig. 3c. To confirm the increase in fold density in the NMJ from MCK NMJs, we measured the expression of the $Na_V1.4$ channel concentrated in the depths of the folds using western blot (Fig. 3d, see Supplementary Fig. 1 for representative

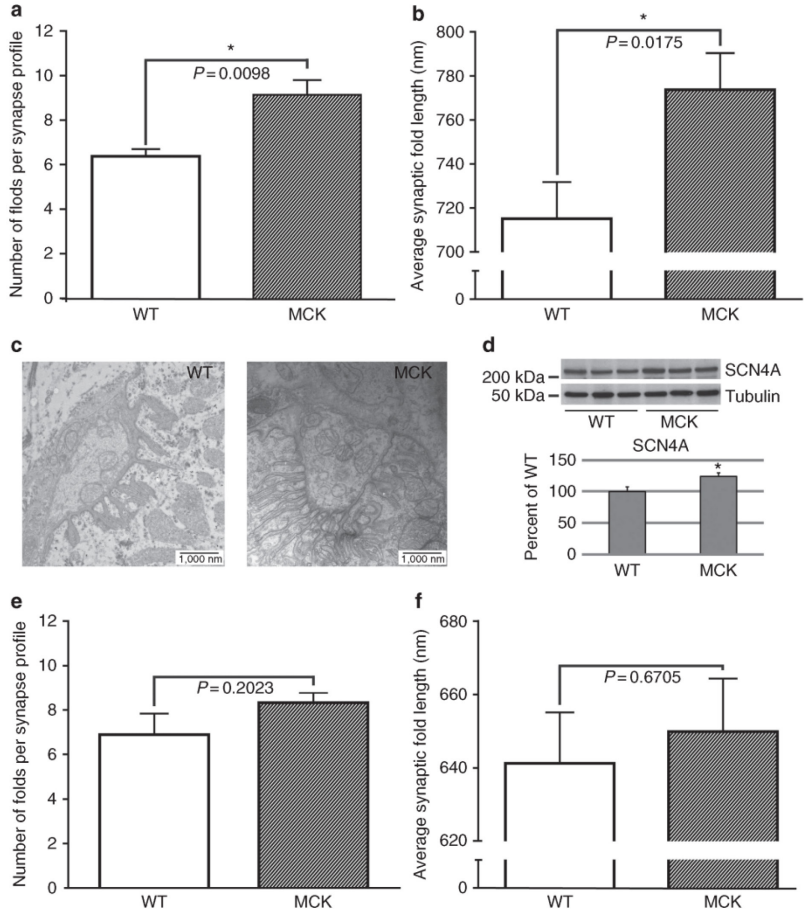

Figure 3 | Synaptic fold characterization on the TEM images from WT and transgenic mice NMJs. (**a**) The average number of folds attached to synapses was determined for WT and MCK mice in SCM muscle. (**b**) Synaptic fold lengths from SCM muscles were scaled and measured with Image J and averaged. Each bar represents mean ± s.e.m. ($n=4$, t-test two-tailed). (**c**) Representative TEM image of the NMJ area of SCM muscle from WT or MCK animals. (**d**) Western blot detection of the $Na_V1.4$ expression in SCM muscles from WT and MCK animals. The relative band intensity was analysed using the Image J. The WT/tubulin ratio was set as 100%. (**e**) The average number of folds attached to synapses was determined for WT and MCK mice in soleus muscle. (**f**) Synaptic fold lengths from soleus muscles were scaled and measured with Image J and averaged. Each bar represents mean ± s.e.m. ($n=4$, t-test two-tailed). SF: Synaptic folds, m: mitochondria.

full-sized western blots). The expression of $Na_V1.4$ was correlated with the fold quantification, being higher in the muscle of transgenic animals. In contrast, the number and average length of the synaptic folds in soleus muscle of the MCK mice were not different from those of the WT controls (Fig. 3e,f, respectively) correlating with the difference in PGC-1α expression in SCM but not the soleus of MCK compared with WT mice.

Muscle PGC-1α promotes slow-type NMJ function. The morphological and ultrastructural analysis of the NMJ in MCK versus WT mice not only suggests stabilization but also a shift towards slow-type NMJs in the MCK mice—for example, indicated by the smaller end plate size in these animals. As a next step, we therefore wanted to test whether the morphological changes induced by overexpressed PGC-1α in the skeletal muscle were sufficient to trigger functional adaptations of motor neuron–muscle fibre interaction. First, compound muscle action potentials in response to repetitive nerve stimulation were assessed by electromyography. Specifically, the train of compound action potentials following direct stimulation of the sciatic nerve with a frequency of 50 Hz was recorded in the gastrocnemius of WT and MCK animals (Fig. 4a,b, respectively). Interestingly, while the amplitude of the first response was similar in the MCK and WT animals, the amplitude and area under the curve of the successive responses showed a significant decrement in the WT animals that was much lower in the MCK mice (Fig. 4c).

To refine the functional studies, we subsequently investigated neurotransmission at the NMJ of the glycolytic LAL, which exhibits similar morphological NMJ plasticity in MCK mice to the leg glycolytic muscles and the oxidative TVA muscle with unchanged morphological properties between the two genotypes (Supplementary Fig. 2). First, we measured muscle resting membrane potential and found no significant differences between MCK and WT mice in either muscle (Supplementary Table 1). Then, when studying the spontaneous miniature end-plate potentials (mEPP) in LAL, a significantly slower mEPP rise time in MCK mice (0.78 ± a s.d. of 0.03 s) in comparison with WT (0.68 ± a s.d. of 0.02 s) was observed ($P = 0.03$, *t*-test), while no significant differences in mEPP amplitude, frequency or signal decay constant were found (Fig. 5a). Similarly, the rising time of evoked endplate potentials (EPPs) in the LAL of MCK mice was significantly slower (0.75 ± a s.d. of 0.02 s) than in WT (0.66 ± a s.d. of 0.02 s; $P = 0.0085$), while the amplitude and decay time constant of the EPPs remained unchanged in MCK mice (Fig. 5b). As EPPs are slower in slow muscles than in fast muscles[31], we next measured the properties of mEPPs and EPPs in the oxidative TVA muscle (Supplementary Fig. 3). As expected, the mean rising times of mEPPs and EPPs in WT TVA were slower than in WT LAL muscles (Tables 1 and 2) but not significantly different to those values measured in MCK LAL muscles. On the other hand, no difference in the rising times of spontaneous and evoked potentials was found between WT and MCK mice in the TVA (Tables 1 and 2).

Short-term synaptic plasticity at the NMJ of the LAL muscle was tested by repetitive trains of stimuli of 1 s at 100 Hz (Fig. 5c). No differences in paired-pulse ratio at interstimulus intervals ranging from 10 to 200 ms were observed between the genotypes (Fig. 5c), suggesting no differences in synaptic release probabilities. In contrast, steady-state depression at the end of the 1 s, 100-Hz train of stimuli were significantly lower in the MCK (39 ± a s.d. of 2%) compared with WT (48 ± a s.d. of 3%) mice ($P = 0.03$, *t*-test; Fig. 5c). The ability of the MCK motor neuron terminals to sustain neurotransmitter release more efficiently than their WT counterparts became evident even after a few stimuli. For example, smaller depression after 10 repetitive stimuli

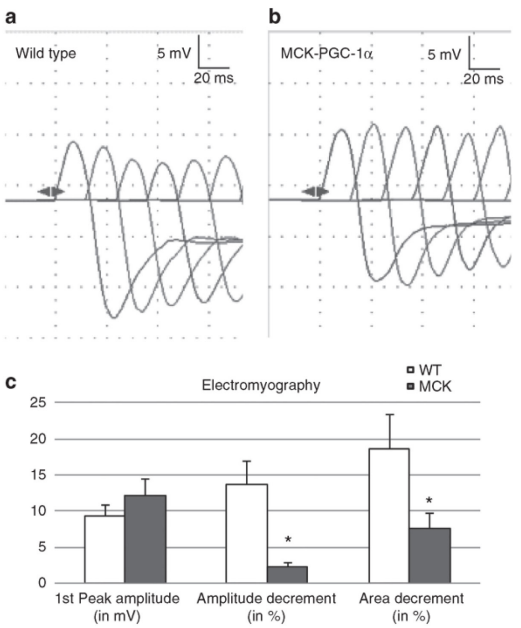

Figure 4 | Electromyographic properties of the gastrocnemius muscles in WT and MCK mice after repetitive nerve stimulation of the sciatic nerve. (**a**,**b**) Representative electromyography traces of WT (**a**) and MCK mice (B). (**c**) Average of the first peak amplitude in mV and the decrement (amplitude and area) calculated between the first and the fourth peaks of the train of action potentials recorded in WT (white bars) and MCK animals (grey bars). Each bar represents mean ± s.e.m. *$P < 0.05$ ($n = 7$, *t*-test two-tailed).

was observed in the MCK mice, as a trend at 20 Hz ($P = 0.054$, *t*-test), and with statistical significance at 50 Hz ($P = 0.014$, *t*-test) and 100 Hz ($P = 0.004$, *t*-test; Fig. 5c). In motor nerve fibres that innervate slow (type I) muscle fibres neurotransmitter release is better sustained than in nerve terminals that innervate fast muscle fibres. This has been attributed to a larger synaptic vesicle pool size and lower quantal content (the number of quanta of neurotransmitter released per action potential) in slow motor neurons[23]. Therefore, we next compared the quantum content in the LAL of MCK and WT animals (Fig. 5b) and found that it was significantly smaller in MCK (34.7 ± a s.d. of 1.8) than in WT (45.8 ± a s.d. of 3.6) terminals ($P = 0.01$, *t*-test). Therefore, the reduction in the number of fused vesicles per action potential, probably, contributed to less depression in MCK LAL terminals. This compensation was further confirmed when the cumulated number of fused synaptic vesicles during 100-Hz stimulation was calculated in both genotypes and not statistical differences were obtained (Supplementary Table 2). Finally, we also estimated size of the readily releasable pool of synaptic vesicles[32] in the nerve terminals of the LAL and found no differences between MCK and WT mice (Supplementary Table 3), suggesting that if the overexpression of PGC-1α induces an increase of synaptic vesicles that should be at the level of the reserve pool and not at the readily releasable pool.

In the oxidative TVA, a significant increase in the amplitude of the spontaneous mEPPs as well as an increase in the decay time constant of the evoked EPPs was found in MCK mice

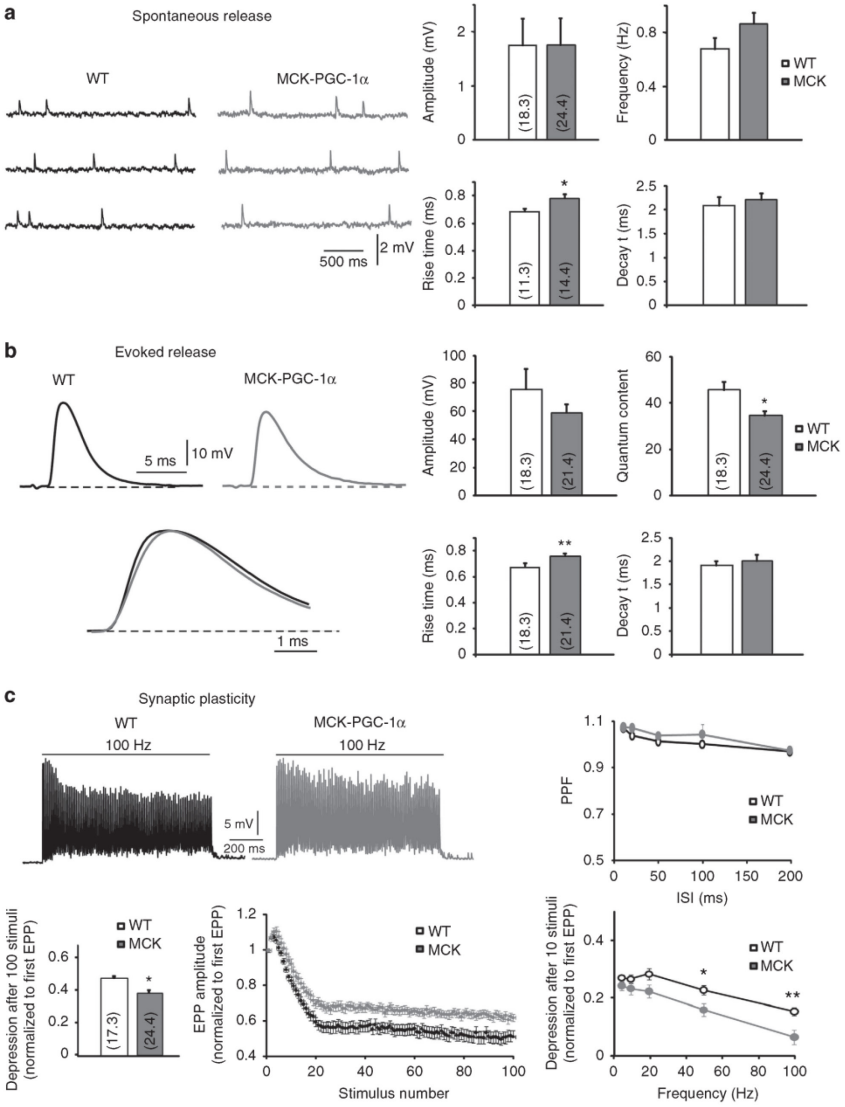

Figure 5 | Electrophysiological properties of LAL muscles from WT or transgenic mice (**a**) The spontaneous neurotransmitter release of LAL muscle resembled the representative traces of mEPP in WT (black) and MCK-PGC-1α (gray) NMJs. Amplitude, frequency of mEPP and their rise and decay times were determined. (**b**) Evoked neurotransmitter release resembled the representative traces of EPP in WT (black) and MCK-PGC-1α (grey) NMJs. Amplitude, quantum content of EPP and their rise and decay times were determined. (**c**) The synaptic plasticity shows a representative trace of steady-state depression in WT (black) and MCK-PGC-1α (grey) mice. The pair-pulse-facilitation (PPF) at inter-stimulus-intervals (ISI) ranging from 10 to 200 ms and the steady-state depression were measured at the end of the train and normalized to the amplitude of the first EPP. The normalized depression after 10 repetitive stimuli was measured between 0 and 100 Hz. The results are represented as mean ± s.e.m. The numbers in the bars are *n*, *N*: number of fibres, number of mice.

(Supplementary Fig. 3). Finally, short-term depression after 10 repetitive stimuli at 50 Hz was reduced in the TVA of MCK animals compared with WT animals, while the rest of the electrophysiological parameters did not differ significantly between the genotypes in this muscle (Supplementary Fig. 3).

Pre-synaptic remodelling of the NMJ by PGC-1α. The functional data obtained by the single motor neuron–muscle fibre-based electrophysiology strongly implies that the nerve endplates in the MCK animals with muscle-specific overexpression of PGC-1α not only undergo a post-synaptic but also a pre-synaptic

Table 1 | Spontaneous neurotransmitter release in NMJs of LAL and TVA muscles.

	WT	MCK	*P*-value
LAL			
Amplitude (mV)	1.75 ± 0.32 (18, 3)	1.76 ± 0.15 (21, 4)	0.97
Frequency (Hz)	0.68 ± 0.08 (18, 3)	0.87 ± 0.08 (24, 4)	0.11
Rise time (ms)	0.68 ± 0.02 (11, 3)	0.78 ± 0.03 (14, 4)	**0.03***
Decay time constant (ms)	2.09 ± 0.18 (11, 3)	2.21 ± 0.13 (14, 4)	0.58
TVA			
Amplitude (mV)	0.67 ± 0.04 (17, 3)	0.98 ± 0.08 (17, 3)	**0.002***
Frequency (Hz)	1.06 ± 0.14 (17, 3)	1.47 ± 0.16 (17, 3)	0.07

LAL, levator auris longus; MCK, muscle creatine kinase; NMJ, neuromuscular junction; TVA, transversus abdominis; WT, wild type.
The results are represented as mean ± s.e.m. The numbers in the parenthesis are *n*, *N*: number of fibres, number of mice.
Statistically significant values are indicated in bold.

Table 2 | Evoked neurotransmitter release in NMJs of LAL and TVA muscles.

	WT	Tg	*P*-value
LAL			
Amplitude (mV)	75.9 ± 14.8 (18, 3)	59.1 ± 6.1 (21, 4)	0.31
Quantum Content	45.8 ± 3.6 (18, 3)	34.7 ± 1.8 (24, 4)	**0.011***
Rise time (ms)	0.66 ± 0.02 (18, 3)	0.75 ± 0.02 (21, 4)	**0.0085***
Decay time constant (ms)	1.84 ± 0.06 (18, 3)	1.96 ± 0.12 (21, 4)	0.38
TVA			
Amplitude (mV)	43.1 ± 3.95 (17, 3)	51.2 ± 3.4 (17, 3)	0.126
Quantum content	64 ± 4.5 (17, 3)	55.4 ± 4.1 (17, 3)	0.167
Rise time (ms)	0.78 ± 0.02 (17, 3)	0.74 ± 0.02 (17, 3)	0.15
Decay time constant (ms)	1.92 ± 0.1 (17, 3)	2.26 ± 0.1 (17, 3)	**0.019***

LAL, levator auris longus; MCK, muscle creatine kinase; NMJ, neuromuscular junction; TVA, transversus abdominis; WT, wild type.
The results are represented as mean ± s.e.m. The numbers in the parenthesis are *n*, *N*: number of fibres, number of mice.
Statistically significant values are indicated in bold.

remodelling. To substantiate this hypothesis, we studied the pre-synapse in more detail. Quantification of the nerve terminal branches within the region of the NMJ revealed a significantly higher number in nerve branches as well as an increased total length and branching complexity in the SCM of MCK animals compared with WT mice (Fig. 6a). These findings indicate a change in the pre-to-post-synaptic coupling in the PGC-1α muscle-specific transgenics. In fact, increased length of branching is usually accompanied by an increase in the number of ACh-containing vesicles in the pre-synaptic part in order to store more neurotransmitter. First, we quantified the number of synaptic vesicles using TEM (Fig. 6b). We observed a significantly higher number of synaptic vesicles in nerve terminals of the glycolytic SCM muscle of MCK mice compared with WT animals but no significant differences in terminals from the oxidative soleus muscle (Fig. 6c). Interestingly, the protein expression of synaptophysin (Syp), a pre-synaptic regulator of vesicle fusion, was likewise differentially expressed in the TA and SCM of MCK animals compared with WT mice (Fig. 6d). It was previously shown that the overexpression of PGC-1α in the muscle increased the frequency of motor neuron expressing the synaptic vesicle protein SV2A[34]. By isolating the synaptic area in the SCM muscle, we could confirm an increase in the expression of this protein in the MCK samples compared with their WT counterparts (Fig. 6e).

Importantly, both acetylcholine biosynthesis and synaptic vesicle assembly are energy-consuming processes. To assess whether the structural and functional changes of the pre-synapse are accompanied by a corresponding adaptation of the metabolic properties, mitochondrial size and morphology were quantified with TEM. Intriguingly, a significant increase in the mitochondria volume density was observed in the transgenic animals (Fig. 6f). This was accompanied by a prominent reduction in the number of mitochondria of the smallest volume density range, which was compensated by an increase in the proportion of mitochondria with a high volume density (Fig. 6g). In contrast, no statistically significant difference in average surface (cristae) density was found (Fig. 6h). Strikingly, even in the oxidative soleus muscle, a significant increase in pre-synaptic mitochondrial volume density was observed, while mitochondrial surface density was unchanged in the active zone of the motor neuron innervating Soleus (Supplementary Fig. 4). After pre-synaptic acetylcholine release, relative acetylcholine esterase activity in the neuromuscular cleft determines the rate of neurotransmitter breakdown and hence removal from the cleft. In contrast to the clear pre- and post-synaptic differences, acetylcholine esterase activity in the synaptic cleft is statistically indistinguishable between the two genotypes in any of the muscles studied (Fig. 6i and Supplementary Fig. 5).

Discussion

In the mature skeletal muscle tissue, activity-driven plasticity is the most important cause for NMJ remodelling[12]. Nevertheless, despite the well-documented modulation of NMJ structure and morphology in response to altered neuromuscular activity, very little is known about the molecular processes that control this type of NMJ plasticity. Historically, altered firing patterns of the motor neuron have been implicated as an exclusive source of the remodelling of the neuromuscular interface and the ensuing adaptations in the muscle fibre[35]—that is, a switch towards oxidative and glycolytic muscle fibres caused by altered intramyocellular calcium transients in endurance and resistance

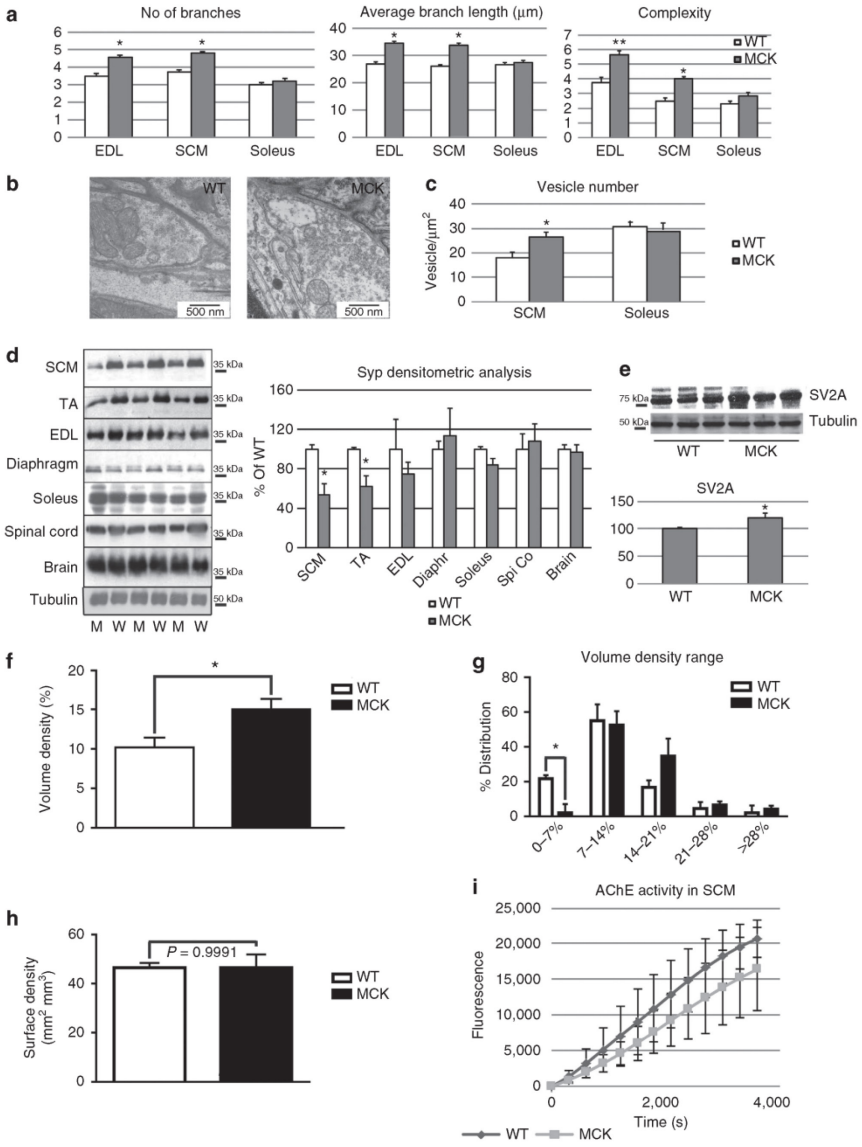

Figure 6 | Pre-synaptic remodelling of the NMJ by PGC-1α. (**a**) The number of branches per AChR cluster and the average length of the branches were determined on confocal images stack using Image J in arbitrary units on WT (white bars) and MCK (grey bars) mice. The complexity is defined as the branch number × the total branch length divided by 100. Each bar represents mean ± s.e.m. from at least 100 NMJ from three mice $^{*}P<0.05$ $^{**}P<0.01$ ($N=3$, $n>100$, t-test two-tailed). (**b**) Representative TEM picture illustrating the synaptic vesicle number in the SCM muscle from WT and MCK mice. (**c**) Quantification of synaptic vesicle number per µm^2 determined by Image J on WT (white bars) and MCK (grey bars) mice. Each bar represents mean ± s.e.m. from at least 10 NMJ from three mice $^{*}P<0.05$ ($N=3$, $n>10$, t-test two-tailed. (**d**) Western blot detection of synaptophysin on protein extracts from different tissues of WT (W, white bars) and MCK (M, grey bars) animals. The relative band intensity was analyzed by Image J. The WT/tubulin ratio was set as 100%. $^{*}P<0.05$ ($N=3$). (**e**) Western blot detection of the synaptic vesicle 2A on SCM protein extract in WT and MCK mice. (**f**,**g**) Volume density of mitochondria within the NMJ was calculated according to Weibel [33] using a D64 grid ($q^2=16$, $P_T=64$, $P'_T=1024$) at × 8,500 magnification. Individual NMJ data ($N=9$–16 per mouse) were averaged per mouse and subsequently between mice at the same genotype (WT = 4, MCK = 3). Unpaired t-test. (**h**) The surface density of mitochondria (*right*) was calculated according to Weibel using a customized D576 Grid ($q^2=16$, $P_T=576$, $P'_T=9216$) at × 8500 magnification. Individual NMJ data ($N=9$–16 per mouse) were averaged per mouse and subsequently between mice at the same genotype (WT = 4, MCK = 3). Unpaired t-test. (**i**) For SCM muscle, a kinetic of AChE activity was measured indirectly by detecting the fluorescence emitted at 590 nm for 3,600 s. Each point represents mean ± s.e.m. ($N=3$).

training, respectively[15]. In contrast to the developmental aspects of NMJ installation, the contribution of skeletal muscle fibres to these adaptive processes has been only rudimentarily studied. In recent years however, our understanding of the molecular mechanisms that underlie skeletal muscle cell plasticity has been tremendously expanded. For example, skeletal muscle is now widely recognized as an endocrine tissue that releases auto-, para- and endocrine hormones, so-called myokines[36]. Furthermore, we could show that intramyocellular calcium handling is not only controlled by motor neuron input but also cell autonomously modulated by PGC-1α[30]. In the present study, we now provide strong evidence of a significant contribution of skeletal muscle to pre- and postsynaptic remodelling of the NMJ through elevated activity of the coactivator PGC-1α. In mice with skeletal muscle-specific overexpression of PGC-1α, the NMJ is altered morphologically, structurally and functionally despite unchanged locomotion and hence motor neuron-mediated activation of muscle fibres. Moreover, these mice exhibit a similar muscle fibre size distribution as control animals[37]: a change in fibre size thus is not the underlying cause of altered NMJ properties. Importantly, the extent of the plastic changes correlates with the relative overexpression of PGC-1α in muscles with different fibre-type characteristics. For example, major rearrangements of the NMJs are found in the glycolytic SCM with high transgenic overexpression of PGC-1α compared with the minor alterations in the oxidative soleus where transgenic elevation of PGC-1α is minimal.

Morphology and function of the NMJs in the MCK mice resemble those of slow-type NMJs innervating oxidative muscle beds—for example, as indicated by the smaller endplate size[38]. Functionally, this switch towards slow-type NMJs allows a more sustained firing pattern that is typical for the high endurance contractions in slow muscles, at least in part by a higher number of synaptic vesicles and increased mitochondrial volume density in the active zone combined with a smaller quantum content. This modulation in synaptic vesicle handling in the active zone of the motor neuron is further underlined by the distinct expression of proteins involved in synaptic vesicle function such as synaptophysin and the slow-type NMJ-enriched synaptic vesicle protein 2A (SV2A), which has previously been reported to be increased in NMJs of MCK animals[34]. Similarly, the changes in kinetics of the mEPPs and EPPs, in particular the increase in rise time, are typical for slow-type NMJs[31,39]. Finally, our data also reveal the increase in pre-synaptic nerve terminal branching that is linked to activity-driven adaptations of the NMJ[12,17]. Importantly, the reduction in quantum content is not indicative of failure in neurotransmission, given the high safety factor in the NMJ, the unchanged high amplitude of the post-synaptic end-plate potential, the reduced depression in the amplitude of the responses following repetitive nerve stimulation in the EMG and in the intracellular recordings and finally illustrate the fatigue resistance in PGC-1α-overexpressing muscles *in* and *ex vivo*. Thus, muscle PGC-1α induces many post- and, importantly, pre-synaptic changes that have been linked to activity-driven NMJ plasticity in the adult muscle.

The large amplitude of the EPP that we observe may be related with the mouse background. In our experience, and in that of others, EPP amplitude in mice with a C57BL/6 background have larger EPP than FVB mice[40]. This large amplitude was also confirmed in a number of fibres in which two-electrode voltage clamp recordings were obtained. Typical peak currents in WT mice were 70–80 nA, while the mean input resistance was 0.84 MΩ. This results in EPP amplitudes of 58–68 mV.

Intriguingly, PGC-1α-mediated modulation of NMJ morphology also closely resembles the beneficial effects of caloric restriction and exercise on age-related deterioration of the neuromuscular interface[18]. Thus, we observed an improvement in morphological NMJ integrity both in post-synaptic features such as AChR density and pretzel fragmentation, as well as pre-synaptically in terms of innervation and sprouting. On the ultrastructural level, the increase in synaptic fold number and length in the MCK animals indicates a structural stabilization as well as an enhancement of the synaptic transmission triggered by overexpressed muscle PGC-1α[41]. Interestingly, exercise affects only the NMJs of a subgroup of aged muscles whereas the effect of caloric restriction is more ubiquitous[18]. Similarly, muscle-specific overexpression of PGC-1α had distinctive outcomes on NMJ morphology and function in different muscles. In our experimental context of sedentary mice, these differences could be caused by the relative usage of the muscles that were characterized in the animals. It thus might be interesting in future studies whether *bona fide* exercise of the MCK mice extends the remodelling of the NMJs beyond those that were observed in the sedentary animals. A corresponding amplification of muscle-specific transgenic expression of PGC-1α by treadmill running has previously been reported in regard to diet-induced peripheral insulin resistance[42].

Our finding that muscle PGC-1α not only remodels the post-synapse, but also triggers a significant pre-synaptic adaptation of the NMJ obviously suggest the production of a PGC-1α-dependent neurotrophic factor that mediates a local, retrograde signal from muscle to the motor neuron. While a number of retrograde signals have been identified in the formation of NMJs during development, very little about the role of skeletal muscle and hence about putative retrograde signaling mediators is known in physiological NMJ plasticity in the adult muscle. We have tested some candidate retrograde signaling molecules that have been described in the context of developmental NMJ differentiation[2], including several extracellular matrix components, but did not observe any differences in gene expression between MCK and WT mice (Supplementary Fig. 6). Therefore, it is conceivable that different factors convey a retrograde signal in the mature compared with the developing NMJ. However, while we would expect a transcriptional response due to overexpression of the transcriptional coactivator PGC-1α, our findings obviously do not exclude post-transcriptional mechanisms to contribute to retrograde signalling in our experimental context. Similarly, the increase in PGC-1α-mediated AChR expression and clustering, MuSK or some other post-synaptic protein might contribute to the retrograde signaling. Nevertheless, it is also possible that so far uncharacterized neurotrophic factors could be involved in physiological NMJ plasticity and future studies will aim at identifying those.

In summary, we now for the first time provide strong evidence for a significant contribution of skeletal muscle to physiological NMJ plasticity in adult muscle, both on the post- as well as on the pre-synaptic side. In particular, it is conceivable that PGC-1α, a regulatory nexus in metabolic and myofibrillar endurance exercise adaptation in muscle, also controls activity-dependent remodeling of the NMJ and contributes to the attenuation of the age-related deterioration of neuromuscular morphology and function by exercise. PGC-1α has previously been described to ameliorate muscle atrophy and fibre damage in different pathological contexts, including Duchenne muscular dystrophy[20]. In Duchenne muscular dystrophy, NMJ dysfunction is thought to contribute to the disease pathology. While the exact mechanism by which skeletal muscle-specific overexpression of PGC-1α improves muscle fibre integrity and function in mdx animals, the mouse model for Duchenne muscular dystrophy, PGC-1α-mediated stabilization of the NMJ could certainly be a part of this therapeutic effect. Similarly, the prevention of sarcopenia in

muscle-specific PGC-1α transgenic mice could be due to the functional improvement in the NMJ as has been shown in old animals[27]. In contrast, skeletal-muscle-specific overexpression of PGC-1α ameliorated the muscle pathology of an amyotrophic lateral sclerosis mouse model, but was insufficient to extend survival[29]. These findings indicate that the effect of muscle PGC-1α on NMJ stability might be insufficient in very severe motor neuron diseases, but could be therapeutically beneficial in pathologies with a milder motor neuron phenotype or with secondary deterioration of the NMJ. Therefore, identification of pharmacological agents that activate PGC-1α in human muscle or that target the muscle-derived factors, which mediate the retrograde signaling in this context, is of high clinical importance. Thus, similar to our current understanding of the potential deployment of "exercise mimetics" in the treatment of metabolic diseases, such compounds might also be excellent adjuvant interventions to facilitate and *bona fide* exercise in patients with impaired exercise tolerance.

Methods

Animals. All experiments were performed on male, 6- to 8-week-old wild-type (WT) and transgenic mice (all mice obtained from in-house breeding) where the PGC-1α expression is driven by a muscle creative kinase (MCK) promoter (designated MCK mice, as opposed to the WT littermates)[22]. The animals were kept in a room with a 12/12 light/dark cycle, were fed a standard laboratory chow diet and had *ad libitum* access to water. All animal experiments were approved by the institutional as well as Swiss cantonal veterinary authorities according to the guidelines of the European Council Directive for the Care of Laboratory Animals.

Tissue preparation. Isolated muscles were directly frozen in liquid nitrogen. Total RNA was extracted using TriReagent according to the the manufacturer's protocol and was treated with RNase-free DNase (Invitrogen). The concentration was adjusted and 1 μg of RNA was reverse-transcribed into cDNA using Superscript RT (Invitrogen) with random hexamer primers (Roche). Real-time PCR was performed with the Power Sybr Green Master Mix (Applied Biosystems) using the StepOne Plus Lightcycler (Applied Biosystems). The relative expression level for each gene of interest was estimated according to the $\Delta\Delta C_t$ method, using the WT samples as reference and the expression of the TATA-binding protein as a calibrator. Real-time PCR primers are shown in Supplementary Table 4.

For protein extraction, the frozen muscles or tissues were digested with lysis buffer (Tris HCl 20 mM, NaCl 138 mM, KCl 2.7 mM, Glycerol 5% v/v, NP40 1% v/v) supplemented with a cocktail of protease inhibitors (Roche) and the protein concentration was quantified with the BCA protein assay kit (Pierce, Thermofisher). Proteins were resolved by SDS-PAGE, transferred to a polyvinylidene difluoride membrane and incubated with an anti-synaptophysin antibody (Abcam, Ref. ab53166, 45 kD), anti-SCN4A antibody (Novus Biological, Ref NBP1–19008, 208 kDa) and SV2A antibody (Novus Biological, Ref NBP1-46367, 88 kD). Tubulin expression (Upstate Cell Signaling, Ref 05–829, 55 kDa) was used as loading control. The relative intensity of the bands of interest was analysed using Image J and was expressed relative to the tubulin band intensity. The WT/tubulin level was set as 100%.

To be able to measure the expression of pre-synaptic markers from the muscle samples, the synaptic part was stained with Alexa Fluor 488-coupled α-bungarotoxin and micro-dissected under a binocular microscope. The isolated synaptic part was then processed as other samples for protein or RNA extraction.

For immunofluorescence, soleus, EDL, diaphragm and SCM were isolated. The collected muscles were fixed for 10 min at − 20 °C in methanol, washed in phosphate-buffered saline and incubated for 2 h at room temperature in a blocking solution (phosphate-buffered saline supplemented with 1% bovine serum albumin, 5% Horse serum, 1% Triton-X100, 0.1% NaN3). The whole muscles were then incubated overnight at 4 °C with a mixture of primary antibody and Alexa Fluor 488-coupled α-bungarotoxin (Molecular Probe, Ref B13422) diluted 1/10,000 in blocking solution. The polyclonal antibody against the neurofilament (Chemiconmillipore, Ref AB1987) was used at a 1/1,000 dilution and the polyclonal anti-synaptophysin (Dakocytomation Ref M7315) at 1/200. After 4 h of incubation with the adequate secondary antibodies, the muscles were whole-mounted on slides with a fluorescent mounting medium (Dako). The samples were observed under a confocal microscope (DMI6000, Leica) and maximum intensity projections of stacks were used to study the NMJ structure. The same settings were kept for all the samples (thickness of the scanned area, number of layers, gain and offset).

The NMJ architecture characterization was based on the definitions given by Sanes and Lichtman[18]. More than 100 NMJs (n) from at least three animals (N) were counted. Pre-synaptic variables of NMJ included the number of branches identified at the nerve terminal, the total length of those branches, the average length per branch and the branching complexity obtained by multiplying the number of branches by the total length of those branches, and dividing that figure by 100 as described previously[43].

For electrophysiology recordings, the LAL and TVA muscles were dissected with their nerve branches intact and pinned to the bottom of a 2-ml chamber, over a bed of cured silicon rubber (Sylgard, Dow Corning. Preparations were continuously perfused with a solution of 125 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM $NaHCO_3$ and 15 mM glucose under a continuous flow of a 5% CO_2/95% O_2 gas mixture.

Synaptic fold quantification. Synaptic fold lengths were determined for individual NMJs using the Image J software and TEM images at × 8,500 magnification. Lengths were measured cursively from the edge of the synapse to the end of each fold using a Wacom CTH-470 pen tablet. Synaptic fold lengths and synaptic fold number per NMJ (N=9–16 per mouse) were averaged per mouse and subsequently between mice at the same genotype (WT = 4, MCK = 4 in SCM muscle).

Volume and surface density of pre-synapse. Volume and surface density of mitochondria within the NMJ were calculated according to methods previously described by Weibel[33] and digitally adapted to Adobe Photoshop. Mitochondria and cristae structures within the NMJ were identified and outlined manually using a Wacom CTH-470 pen tablet. Volume density was determined in a test point system utilizing a D64 grid ($q^2 = 16$, $P_T = 64$, $P'_T = 1024$) using TEM images of × 8,500 magnification. Volume density (V_V) is defined in the equation 1 as the ratio of test points residing within mitochondria (P_M) and the total amount of test points within the NMJ (P_{NMJ}).

$$V_v = P_M / P_{NMJ} \quad (1)$$

Cristae density of mitochondria was measured at equal magnification in a test point system using a D576 grid ($q^2 = 16$, $P_T = 576$, $P'_T = 9216$). Cristae density of individual mitochondria ($S_{V(cr,mt)}$) is defined in equation 2 as the total amount of intersections between inner mitochondrial membrane and test lines (I_{cr}) divided by the product of the amount of points within the mitochondria (P_{mt}) and the actual length of fine test lines in μm (d).

$$S_{V(cr,mt)} = I_{cr}/(P_{mt} \cdot d) \quad (2)$$

Cristae density was determined in one mitochondrion randomly selected in each NMJ for a total of 9–16 NMJ per mouse. NMJ data for cristae and volume density were averaged per mouse and subsequently between mice at the same genotype (WT = 4, MCK = 3 in SCM muscle).

AChE enzymatic activity measurement. The AChE activity was measured using the Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (Molecular Probes). Each reaction contained 200 μM Amplex Red Reagent containing 1 U ml^{-1} HRP, 0.1 U ml^{-1} choline oxidase and 100 μM acetylcholine. The choline generated from AChE activity is oxidized by choline oxidase to betaine and H_2O_2 that reacts with the Amplex Red reagent to generate a fluorescent product detected at 590 nm (N=3 for each experimental group).

Electromyography. Mice were anesthetized using sevofluorane. The electromyographic properties of the gastrocnemius were recorded using a Keypoint EMG machine (Meridian, Neurolite AG, Switzerland). Briefly, the sciatic nerve was directly repetitively stimulated by a train of 15 stimulations (0.04 ms of duration, 10 mA of amplitude) in a supramaximal conditions at 50 Hz with two monopolar needle electrodes and the action potentials in the gastrocnemius muscle response were recorded using a needle electrode placed directly in the muscle belly. The decrement percentage in terms of amplitude and area was averaged for WT and MCK mice (N=4 for each experimental group).

Electrophysiology. The electrical stimulation and intracellular recording were performed as previously described[44]. Briefly, the nerve was stimulated by means of a suction electrode. The stimulation consisted of square-wave pulses at variable frequencies. A glass microelectrode filled with 3 M KCl was connected to an intracellular recording amplifier (Neuro Data IR283, Cygnus technology) and used to impale single muscle fibres near the motor nerve endings. EPP and mEPPs were recorded from different NMJs within the muscle as described previously. Muscle contraction was prevented by including in the bath 3–4 μM μ-conotoxin GIIIB (Alomone Laboratories), a specific blocker of muscular voltage-gated sodium channels. The data were analysed as previously described[44]. EPP amplitudes were normalized to − 70 mV and corrected for nonlinear summation.

Transmission electronic microscopy. The samples were prepared and processed by the Center for Microscopy of the Basel University (ZMB, Basel, Switzerland). The muscles were fixed in a 3% paraformaldehyde, 0.5% glutaraldehyde-buffered solution for 1 h and subsequently incubated in a 1% osmium tetroxide-buffered solution. The slides were dehydrated in a graded EtOH series (50–100%), infiltrated in 100% acetone, embedded in Epon and serially thin-sectioned before staining with uranyl acetate and lead acetate. The samples were analysed on a transmission electronic microscopy Moragni 268D (Philips) at 80 kV.

Statistical analysis. The results are represented as mean ± s.e.m., unless otherwise stated. The MCK and WT samples were compared using the Student's *t*-test (two-tailed) and a *P*-value < 0.05 was considered statistically significant. $N =$ number of mice, $n =$ number of pretzels or number of fibres.

References

1. Lin, S., Landmann, L., Ruegg, M. A. & Brenner, H. R. The role of nerve- versus muscle-derived factors in mammalian neuromuscular junction formation. *J. Neurosci.* **28,** 3333–3340 (2008).
2. Wu, H., Xiong, W. C. & Mei, L. To build a synapse: signaling pathways in neuromuscular junction assembly. *Development* **137,** 1017–1033 (2010).
3. Sanes, J. R. & Lichtman, J. W. Development of the vertebrate neuromuscular junction. *Annu. Rev. Neurosci.* **22,** 389–442 (1999).
4. Farrugia, M. E. & Vincent, A. Autoimmune mediated neuromuscular junction defects. *Curr. Opin. Neurol.* **23,** 489–495 (2010).
5. Hirsch, N. P. Neuromuscular junction in health and disease. *Br. J. Anaesth.* **99,** 132–138 (2007).
6. Lin, W. *et al.* Distinct roles of nerve and muscle in postsynaptic differentiation of the neuromuscular synapse. *Nature* **410,** 1057–1064 (2001).
7. Kim, N. & Burden, S. J. MuSK controls where motor axons grow and form synapses. *Nat. Neurosci.* **11,** 19–27 (2008).
8. Shi, L., Fu, A. K. & Ip, N. Y. Molecular mechanisms underlying maturation and maintenance of the vertebrate neuromuscular junction. *Trends Neurosci.* **35,** 441–453 (2012).
9. Yang, X. *et al.* Patterning of muscle acetylcholine receptor gene expression in the absence of motor innervation. *Neuron* **30,** 399–410 (2001).
10. Yumoto, N., Kim, N. & Burden, S. J. Lrp4 is a retrograde signal for presynaptic differentiation at neuromuscular synapses. *Nature* **489,** 438–442 (2012).
11. Frank, C. A. Homeostatic plasticity at the *Drosophila* neuromuscular junction. *Neuropharmacology* **78,** 63–74 (2013).
12. Deschenes, M. R., Tenny, K. A. & Wilson, M. H. Increased and decreased activity elicits specific morphological adaptations of the neuromuscular junction. *Neuroscience* **137,** 1277–1283 (2006).
13. Desaulniers, P., Lavoie, P. A. & Gardiner, P. F. Habitual exercise enhances neuromuscular transmission efficacy of rat soleus muscle in situ. *J. Appl. Physiol.* **90,** 1041–1048 (2001).
14. Froemming, G. R., Murray, B. E., Harmon, S., Pette, D. & Ohlendieck, K. Comparative analysis of the isoform expression pattern of Ca(2+)-regulatory membrane proteins in fast-twitch, slow-twitch, cardiac, neonatal and chronic low-frequency stimulated muscle fibers. *Biochim. Biophys. Acta* **1466,** 151–168 (2000).
15. Baylor, S. M. & Hollingworth, S. Sarcoplasmic reticulum calcium release compared in slow-twitch and fast-twitch fibres of mouse muscle. *J. Physiol.* **551,** 125–138 (2003).
16. Sanes, J. R. & Yamagata, M. Many paths to synaptic specificity. *Annu. Rev. Cell Dev. Biol.* **25,** 161–195 (2009).
17. Deschenes, M. R., Roby, M. A. & Glass, E. K. Aging influences adaptations of the neuromuscular junction to endurance training. *Neuroscience* **190,** 56–66 (2011).
18. Valdez, G. *et al.* Attenuation of age-related changes in mouse neuromuscular synapses by caloric restriction and exercise. *Proc. Natl Acad. Sci. USA* **107,** 14863–14868 (2010).
19. Vatine, J. J. *et al.* Comparison of the electrophysiological pattern of fatigue between athletes required to perform explosive and endurance sports. *Electromyogr. Clin. Neurophysiol.* **30,** 19–25 (1990).
20. Handschin, C. *et al.* PGC-1alpha regulates the neuromuscular junction program and ameliorates Duchenne muscular dystrophy. *Genes Dev.* **21,** 770–783 (2007).
21. Russell, A. P. *et al.* Endurance training in humans leads to fiber type-specific increases in levels of peroxisome proliferator-activated receptor-gamma coactivator-1 and peroxisome proliferator-activated receptor-alpha in skeletal muscle. *Diabetes* **52,** 2874–2881 (2003).
22. Lin, J. *et al.* Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* **418,** 797–801 (2002).
23. Handschin, C. & Spiegelman, B. M. PGC-1 coactivators and the regulation of skeletal muscle fiber-type determination. *Cell Metab.* **13,** 351 author reply 352 (2011).
24. Handschin, C. & Spiegelman, B. M. The role of exercise and PGC1alpha in inflammation and chronic disease. *Nature* **454,** 463–469 (2008).
25. Handschin, C. *et al.* Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J. Clin. Invest.* **117,** 3463–3474 (2007).
26. Handschin, C. *et al.* Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. *J. Biol. Chem.* **282,** 30014–30021 (2007).
27. Wenz, T., Rossi, S. G., Rotundo, R. L., Spiegelman, B. M. & Moraes, C. T. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. *Proc. Natl Acad. Sci. USA* **106,** 20405–20410 (2009).
28. Song, W., Song, Y., Kincaid, B., Bossy, B. & Bossy-Wetzel, E. Mutant SOD1(G93A) triggers mitochondrial fragmentation in spinal cord motor neurons: neuroprotection by SIRT3 and PGC-1alpha. *Neurobiol. Dis.* **51,** 72–81 (2012).
29. Da Cruz, S. *et al.* Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS. *Cell Metab.* **15,** 778–786 (2012).
30. Summermatter, S. *et al.* Remodeling of calcium handling in skeletal muscle through PGC-1alpha: impact on force, fatigability, and fiber type. *Am. J. Physiol. Cell Physiol.* **302,** C88–C99 (2012).
31. Wood, S. J. & Slater, C. R. The contribution of postsynaptic folds to the safety factor for neuromuscular transmission in rat fast- and slow-twitch muscles. *J. Physiol.* **500**(Pt 1): 165–176 (1997).
32. Ruiz, R. *et al.* Active zones and the readily releasable pool of synaptic vesicles at the neuromuscular junction of the mouse. *J. Neurosci.* **31,** 2000–2008 (2011).
33. Weibel, E. *Stereological Methods: Practical Methods for Biological Morphometry* (1979).
34. Chakkalakal, J. V., Nishimune, H., Ruas, J. L., Spiegelman, B. M. & Sanes, J. R. Retrograde influence of muscle fibers on their innervation revealed by a novel marker for slow motoneurons. *Development* **137,** 3489–3499 (2010).
35. Kanning, K. C., Kaplan, A. & Henderson, C. E. Motor neuron diversity in development and disease. *Annu. Rev. Neurosci.* **33,** 409–440 (2010).
36. Arnold, A. S., Egger, A. & Handschin, C. PGC-1alpha and myokines in the aging muscle - a mini-review. *Gerontology* **57,** 37–43 (2011).
37. Perez-Schindler, J., Summermatter, S., Santos, G., Zorzato, F. & Handschin, C. The transcriptional coactivator PGC-1alpha is dispensable for chronic overload-induced skeletal muscle hypertrophy and metabolic remodeling. *Proc. Natl Acad. Sci. USA* **110,** 20314–20319 (2013).
38. Reid, B., Slater, C. R. & Bewick, G. S. Synaptic vesicle dynamics in rat fast and slow motor nerve terminals. *J. Neurosci.* **19,** 2511–2521 (1999).
39. Bewick, G. S., Reid, B., Jawaid, S., Hatcher, T. & Shanley, L. Postnatal emergence of mature release properties in terminals of rat fast- and slow-twitch muscles. *Eur. J. Neurosci.* **19,** 2967–2976 (2004).
40. Krieger, F. *et al.* Fast motor axon loss in SMARD1 does not correspond to morphological and functional alterations of the NMJ. *Neurobiol. Dis.* **54,** 169–182 (2013).
41. Slater, C. R. Structural factors influencing the efficacy of neuromuscular transmission. *Ann. N. Y. Acad. Sci.* **1132,** 1–12 (2008).
42. Summermatter, S. *et al.* PGC-1α improves glucose homeostasis in skeletal muscle in an activity-dependent manner. *Diabetes* **62,** 85–95 (2013).
43. Tomas, J., Penoll, R., Mayayo, E. & Santafe, M. Branching pattern of the motor nerve endings in a skeletal muscle of the adult rat. *J. Anat.* **168,** 123–135 (1990).
44. Ruiz, R., Casanas, J. J., Torres-Benito, L., Cano, R. & Tabares, L. Altered intracellular Ca2+ homeostasis in nerve terminals of severe spinal muscular atrophy mice. *J. Neurosci.* **30,** 849–857 (2010).

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Author contributions

A.-S.A. designed and performed research, analysed data and wrote the paper, M.C., R.R., S.Mc.G. performed research and analysed data, J.G. performed research, J.St.-P., L.T. and C.H. designed research, analysed data and wrote the paper.

Additional information

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CV

Jonathan Gill
20 bis Avenue de Bâle
68300 Saint Louis
Mobile : 00.33.6.28.21.11.16
Mail: gill.jonathan.f@gmail.com
Born: 21.05.1988 28 years old

EDUCATION

- *2012-today PhD degree* - Biozentrum - Basel **Switzerland**
- *2008-2011 Biotechnology Engineer degree and master in biotechnology and therapeutic innovation* - ESBS Illkirch **France**

ESBS: Engineering School with a multi-disciplinary tri-national program led by Basel, Freiburg and Strasbourg

- *2006-2008 Biology student* - University Louis Pasteur - Strasbourg - **France** (pre-ESBS recruitment)

SKILLS

Technical:

- Molecular analysis (gene/protein expression, immunostaining, mitochondrial assay, NMJ staining and analysis)
- Mice in vivo test (motor function, endurance, injection, calorimetry, electromyography)
- Cell culture (viral infection, transfection, cell death assessment, muscle-spinal cord co-culture)
- Proteins isolation and purification
- Biotechnology (genetic engineering, immunology, bioproduction)

Microscopy and Computer:

- Bright field and confocal microscopy
- Imaging analysis (Imaris and FIJI)
- Statistics (Graphpad)
- Presentation (Microsoft office, Adobe Illustrator)

Languages:

- French (mother tong)
- English (fluent)
- German (beginner-Intermediate)

AWARDS

- Fellowship for excellence – Biozentrum (Basel)
- Presentation price at the Biozentrum PhD retreat

VOLUNTARY/LEADERSHIP ACTIVITIES

- PhD representative and PhD retreat organizer – 2012-2015
  - Sponsor and financial partners research
  - Organization of event for a group of 60 persons
  - Management of a budget of 30 000 chf
- Leader in a recreation camp – Summer 2007-2009
- Volunteer Handball Coach - 2004-2006
- Handball player - 1999-Today

BIOTECHNOLOGY ENGINEER and PhD CANDIDATE

Obtain a job position that can use my technical skills and experience in animal and molecular biology together with my expertise in cell culture to develop new therapeutic strategies against metabolic and muscle diseases

RESEARCH WORK EXPERIENCE AND ACHIEVEMENT

Position

- **Fellowship For Excellence PhD Candidate** -April 2012-Today
  C. Handschin Laboratory - **Biozentrum**- Basel-SWITZERLAND

"Establishing and leading a study investigating the role of PGC-1α in mice muscle aging and in an in vitro model for cell death induction"

- Summarize literature information to design and construct innovative projects
- Strategic Planning to fit the constraints of an extensive study (timeline, large number of animal group, broad number of assays)
- Adapt and implement the study according to obtained results and literature advances to preserve project novelty
- Perform in an independent manner simultaneous experiments with large amount of samples
- Overview the project and synthetize results in a concise manner
- Confirm previous studies and identify 2 new functions for the protein of interest in the context of muscle aging
- Research and building of new collaborations with other laboratories
- Supervise and drive a technician and a master student on a daily basis
- Write scientific publications and give effective oral presentation to small and large groups

"Generation of knock-out mice to study the function of PGC-1α in a brain region"

- Develop internal and multidisciplinary communication to optimize the success of the project
- Establish/update new methods/protocols for the laboratory
- Identify and solve problems
- Develop and work with new genetic mouse models
- Determination of a novel function for PGC-1 α in a specific brain area

Placements

- **Fellowship For Excellence Rotation** - December 2011-April 2012
  C. Handschin, P. Scheiffele laboratories - **Biozentrum**- Basel-SWITZERLAND

"Development of a FRET sensor using cloning technic to produce a plasmid flanked with YFP and CFP"

- **Harvard Medical School Trainee** – January-September 2011
  J. Cohen laboratory- **Harvard Medical School** – Boston –USA

"Modeling the binding site of propofol in the Gloeobacter violaceus pentameric ligand-gated ion channel (GLIC)"

- Worked with biohazard products in a highly controlled environment
- Optimize and scale up experiments
- Identification of drug binding sites using a combination of protein photolabeling, sequencing and molecular interaction modeling

- **Biozentrum trainee** - June 2009-August 2009 P. Phillipsen laboratoy
  Biozentrum- Basel-SWITZERLAND

"Genetic deletion of AgBSP1 and AgCHC1 genes to investigate the endocytosis process in fungus"

- **INCI Trainee** – June-July 2008
  Institut des Neurosciences Cellulaires et Intégratives – Strasbourg –FRANCE

  "Study the effects of HIV-Tat protein on regulated exocytosis in neuroendocrine cells"
  - Drive projects to meet short agreed timelines
  - Fast integration in a new working environment and methods

References, conferences and publications are available upon request

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