Original article

MOLECULAR METABOLISM

PGC-1α expression in murine AgRP neurons regulates food intake and energy balance

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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 an 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.

Keywords PGC-1α; Agouti-related protein; Metabolism; Energy homeostasis; Pro-opiomelanocortin; Transcriptional regulation

1. 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 [1–4]. Two major cell populations in the ARC nucleus, namely the orexigenic agouti-related protein (AgRP) and an orexigenic 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 [5,6], leptin, and insulin [7], and nutrients such as glucose [8]. As a result, in the fasted state, AgRP neurons stimulate appetite and decrease energy expenditure while, in the fed state, POMC neuron activation leads to food satiety and enhanced energy production [8–10]. 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 [11,12]. Deletion of the NAD^+-dependent protein deacetylase sirtuin-1 (SIRT1) in AgRP neurons impairs the response to ghrelin and thus affects energy homeostasis [13]. Finally, the forkhead protein FoxO1 mediates leptin inhibition of AgRP expression and food intake [14].

The peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a major coregulator of transcription factors involved in the control of cellular metabolism [15]. 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 induce the expression of gluconeogenic and mitochondrial genes in the liver [16]. Similarly, AMPK activation leads to transcriptional induction and activating phosphorylation events of the PGC-1α gene and protein, respectively [17]. In hepatocytes, Foxo1 engages PGC-1α in the context of insulin-regulated gluconeogenesis [18]. Importantly, global as well as brain specific deletion of PGC-1α protects mice from diet-induced obesity [19,20]. Furthermore, PGC-1α levels in the hypothalamus are increased in response to fasting [20], suggesting that PGC-1α may act as a metabolic integrator of different signaling pathways in the ARC

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Abbreviations: AgRP, Agouti-related protein; AMPK, AMP-activated protein kinase; FOXO1, Forkhead protein 1; PGC-1α, Peroxisome proliferator-activated receptor γ coactivator 1α; POMC, Pro-opiomelanocortin; SIRT1, NAD^+-dependent protein deacetylase sirtuin-1; TBP, TATA-binding protein

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nucleus for the regulation of whole body energy homeostasis [21]. 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 to investigate 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 response to different metabolic challenges.

2. EXPERIMENTAL PROCEDURES

2.1. Animals

Male mice were kept under a 12 h/12 h 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-1$\alpha^{loxP/loxP}$ mice with transgenic AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ mice, respectively. The PGC-1$\alpha^{loxP/loxP}$ mice have been described previously [19]. AgRP$^{Cre/+}$ (Agrp$^{tm1(cre)Lowl}$, Jax #012899) and POMC$^{Cre/+}$ (STOCK tg(Pomc1-cre) 16Lowl/J, Jax #005965) were purchased from Jackson Laboratories. AgRP- and POMC-PGC1α KO or AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ 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-1$\alpha^{loxP/loxP}$ littermates mice without AgRP$^{Cre/+}$ and POMC$^{Cre/+}$ 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 AgRP$^{Cre/+}$, POMC$^{Cre/+}$ 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.

2.2. 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.

2.3. COBAS blood analysis

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

2.4. Glucose tolerance test

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

2.5. Comprehensive laboratory animal monitoring system (CLAMS)

Whole body metabolism was assessed with an indirect calorimetric system (CLAMS, Columbus Instruments). Food intake, locomotor activity, VO_2 and VCO_2 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.

2.6. 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.

2.7. 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 h 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 h later.

2.8. 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% CO_2. 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 h, the medium was exchanged with low glucose DMEM supplemented with 5% FBS to mimic refeeding. Cells were harvested 4 h after starvation and 1 h after refeeding. Cells exposed to normal growth medium were used as a fed state.

2.9. ARC nucleus punch isolation and imaging

Mice were killed by CO_2 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, 15 μm sections containing the arcuate nucleus of AgRP- or POMC-EGFP-Cre and AgRP- or POMC-EGFP-Cre-PGC1α KO mice

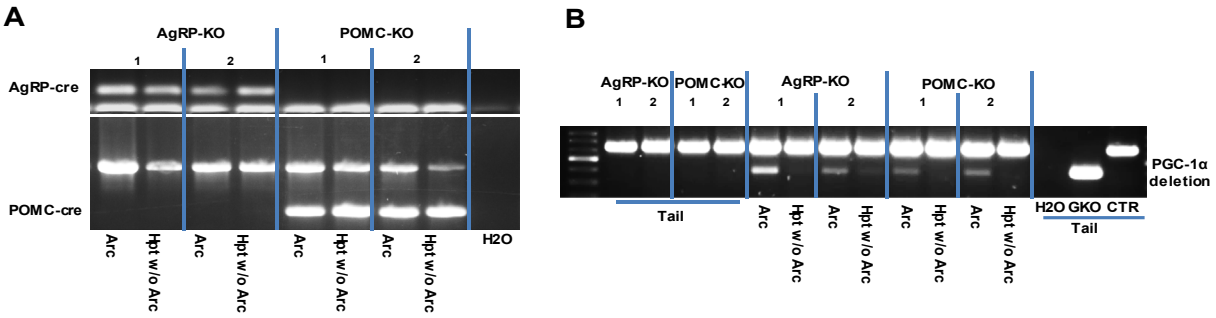

Figure 1: Specific deletion of PGC-1α in the ARC nucleus of AgRP and POMC mice. (**A**) Detection of AgRP-Cre and POMC-Cre expression in punches targeting the ARC nucleus. Genotyping PCR with specific primers was used to detect the presence of the AgRP-cre or POMC-cre allele in isolated hypothalamic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

expressing EGFP in AgRP or POMC neurons were visualized with a Zeiss point scanning confocal microscope.

2.10. 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 s. 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.

2.11. 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.

2.12. 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.

3. RESULTS

3.1. 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 $PGC\text{-}1\alpha^{loxP/loxP}$ with transgenic $AgRP^{Cre/+}$ or $POMC^{Cre/+}$ mice, respectively. The presence of the Cre transgene under the control of either the AgRP or the POMC promoter was identified by PCR (Figure 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 (Figure 1B).

3.2. 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 showed differences in total body mass (Figure 2A,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 (Figure 2B and Figure S1A). In association with elevated fat mass, higher triglycerides levels were detected in AgRP-PGC1α KO mice (Figure 2E), while we observed no significant alteration of blood glucose levels (Figure 2F) or glucose tolerance (Figure 3A,C) even though a trend 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 (Figure 4A). None of these parameters, including body composition, food intake or glucose tolerance, were affected by PGC-1α deletion in POMC neurons (Figure 2B,D, Figure S1B, Figure 3B,D and Figure 4B).

3.3. 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 (Figure 4D), energy substrate utilization (Figure 4F) or locomotor activity (Figure 4H) in fed POMC-PGC1α mice. Similarly, VO_2 levels (Figure 4C) were unchanged in AgRP-PGC1α KO mice. However, we observed a significant reduction in the respiratory exchange ratio (RER) in the AgRP-PGC1α KO mice during nighttime (Figure 4E). In addition, a trend towards overall reduction of locomotor activity was noted in the AgRP-PGC1α KO animals (Figure 4G). To further assess spontaneous locomotor activity in the absence of AgRP specific PGC-1α expression, voluntary wheel running

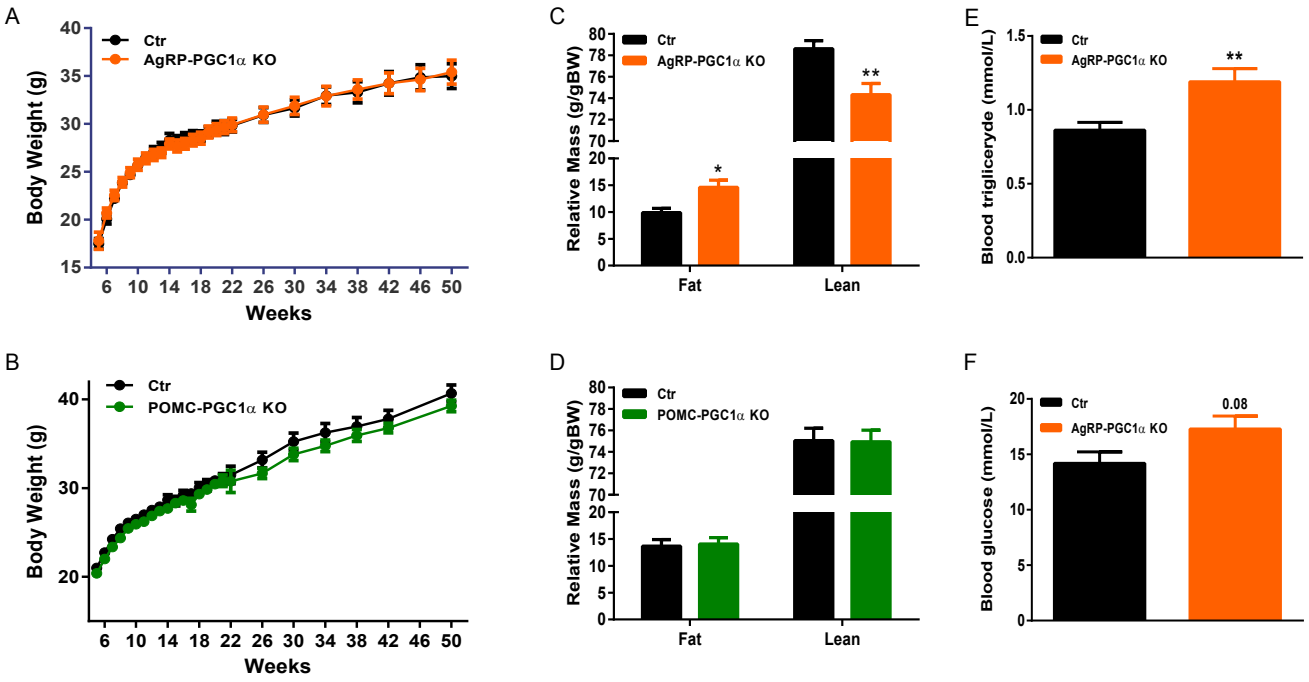

Figure 2: PGC-1α deletion in AgRP, but not in POMC neurons promotes fat storage. (**A** and **B**) Body weight curves (n = 10–14) (**C** and **D**) body composition (n = 7–8) and (**E** and **F**) blood triglycerides and glucose levels (n = 5–7) in AgRP-, POMC-PGC1α KO and Ctr mice. Values and error bars represent the mean ± SEM. *p < 0.05; **p < 0.01.

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 (Figure 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 (Figure 5B).

3.4. 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

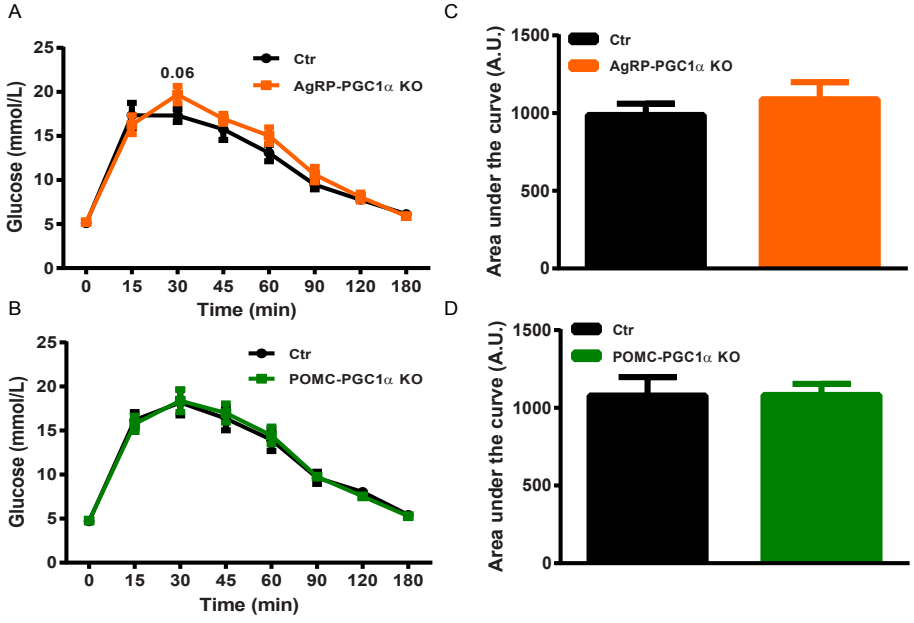

Figure 3: Glucose homeostasis is not altered in AgRP- and POMC-PGC1α KO mice. (**A** and **B**) Blood glucose curve during glucose tolerance test and (**C** and **D**) calculated area under the curves in AgRP-, POMC-PGC1α KO and Ctr mice (n = 7–8). Values and error bars represent the mean ± SEM. *p 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

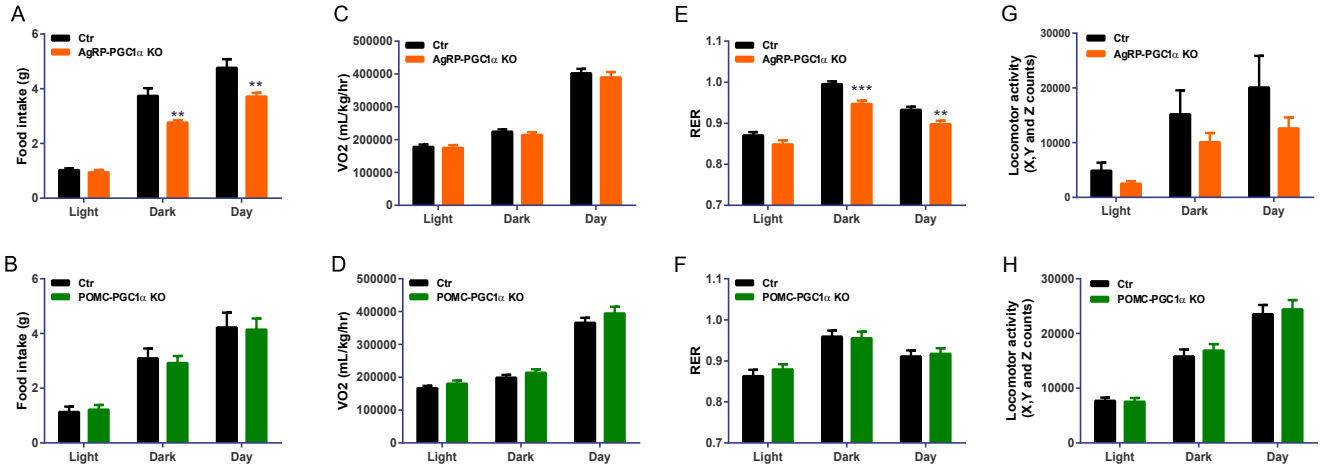

Figure 4: PGC-1α is required by AgRP but not POMC neurons to control basal metabolism. (**A** and **B**) Food intake, (**C** and **D**) oxygen consumption (**E** and **F**) respiratory exchange ratio and (**G** and **H**) spontaneous locomotion of AgRP-, POMC-PGC1α KO and Ctr mice (n = 7–8). Values and error bars represent the mean ± SEM. **p < 0.01; ***p < 0.001.

promotes food intake via AgRP neuronal activation [22]. A strong increase of food intake 1, 2, and 3 h after ghrelin injections in both ctr and AgRP-PGC1α KO animals was observed (Figure 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 [8]. 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 (Figure 6B). Interestingly, leptin injections significantly lowered food intake and body mass changes (Figure 6B,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.

3.5. 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 (Figure 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 (Figure 7B,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 an orexigenic gene expression levels were detected in the absence of PGC-1α (Figure 7D). In stark contrast, the induction of AgRP gene expression by fasting was significantly blunted in AgRP-PGC1α KO mice (Figure 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 (Figure 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 (Figure 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 (Figure 7F). Taken together, our *in vivo* and *in vitro* data indicate that PGC-1α regulates the levels of AgRP expression in response to fasting.

4. 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 [23], hepatic gluconeogenesis [18] and endurance-training adaptation of skeletal muscle [24,25]. Interestingly, PGC-1α expression is important in hippocampus for dendritic spines maintenance [17], in hypothalamic cells to protect against high fat diet-induced pathological changes [26], and exhibits daily oscillations in the hypothalamus [27]. Moreover, brain-specific PGC-1α deletion in mice induces a hypermetabolic state [20], pointing towards a key role for PGC-1α in the neuronal network controlling energy balance.

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

We thus 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). Thus, in our experimental conditions, PGC-1α might be dispensable for POMC neurons, which are less sensitive to change in food intake than AgRP neurons [28]. Therefore, different physiological contexts might need 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 important

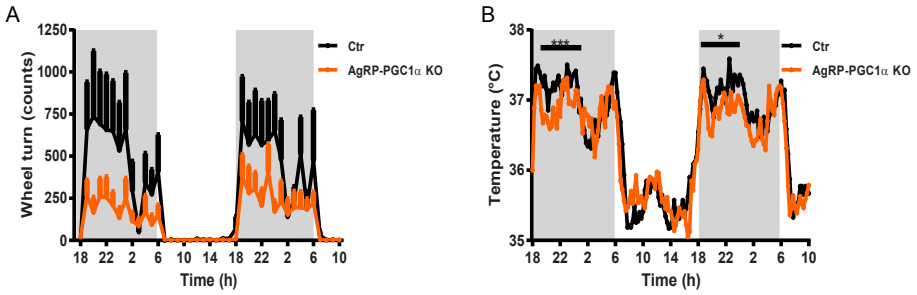

Figure 5: PGC-1α deletion in AgRP neurons reduces energy expenditure. (**A**) Voluntary activity measured by running wheel revolutions in AgRP-PGC1α KO and Ctr mice (n = 5–6). Values represent 2 weeks of measurements. (**B**) Basal body temperature measured during 48-h in AgRP-PGC1α KO and Ctr mice (n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

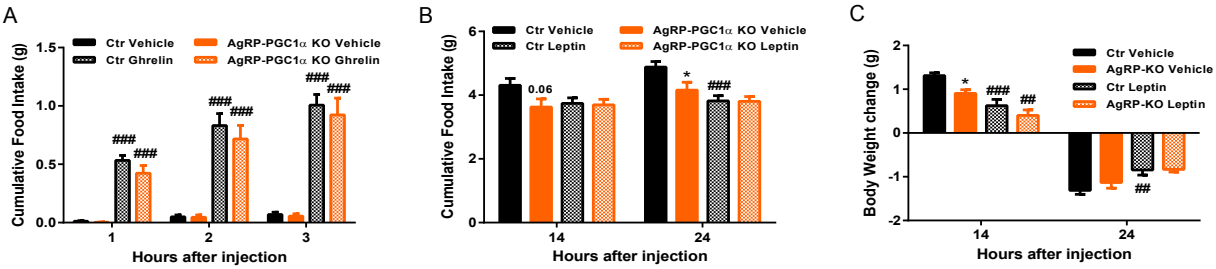

Figure 6: PGC-1α deletion in AgRP neurons alters response to leptin. (**A**) Food intake of AgRP-PGC1α KO and Ctr mice 1, 2 and 3 h after ghrelin or vehicle injection (n = 8). (**B** and **C**) Feeding response and body weight changes of 8 weeks old AgRP-PGC1α KO and Ctr individually housed-mice 16 and 24 h after leptin or vehicle injection (n = 8). A second injection was performed 16 h 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.

not only for inducing satiety but also for 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) [29]. 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

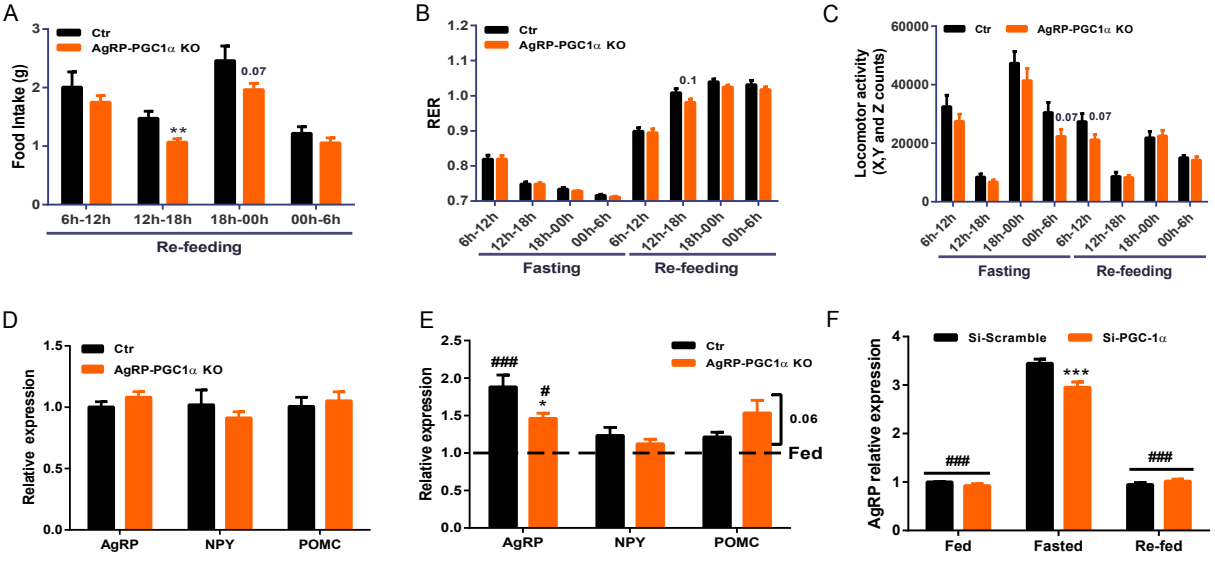

Figure 7: PGC-1α deletion in AgRP neurons impairs energy homeostasis and hypothalamic signaling in response to fasting. (**A**) Food intake, (**B**) respiratory exchange ratio and (**C**) spontaneous locomotion in AgRP-PGC1α KO and Ctr mice measured with CLAMS during fasting and refeeding (n = 7–8). (**D** and **E**) AgRP, NPY and POMC mRNA levels in the hypothalamus of AgRP-PGC1α KO and Ctr 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.

could be compensated by PGC-1β in POMC neurons as both PGC1 coactivators are active and share redundant functions in different cell types [30,31]. 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 [32]. In addition, pre- and postnatal ablation of AgRP neurons leads to different feeding behavior phenotypes [33] 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 [5,34] to allow the deletion of PGC-1α in adult neurons might be of interest to assess the different behavioral phenotypes.

4.2. 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 [35–37]. Thus, PGC-1α could be 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, in which neuronal degeneration in the striatum is thought to trigger a Huntington's-like phenotype [19,20]. 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 [38]. Mice with ablated AgRP neurons also showed a reduced fat gain upon HFD feeding [38], reminiscent of the AgRP-PGC1α KO animals where a HFD normalized the differences in body weight between the genotypes (Figure S3A,B). In contrast, however, that RER was observed to be reduced during the whole dark period in the AgRP-PGC1α KO mice and correlated with the change in locomotor activity is different from the early drop in RER in the AgRP-neuron-ablated mice [38] (Figure S3C,D). Collectively, even though the absence of PGC-1α did not affect the integrity of AgRP neurons (Figure 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 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 [38]. Moreover, since recent studies have shown that AgRP neurons are also important for non-feeding behavioral responses linked to motivation and stereotypic behaviors [39–41], 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.

4.3. 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 [20]. 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 co-localize with AgGP neurons [42], and its expression is elevated in the hypothalamus [20] and AgRP cell line upon fasting (Figure S1) further supporting a direct role for PGC-1α in AgRP neurons for their response to fasting. Therefore, importantly, the work described herein 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 [43–45]. Taken together, these results suggest that PGC-1α in AgPR neurons might participate in the integration of the collective output of peripheral hormonal signals through AMPK [11], SIRT1 [13], and signaling cascades [46–48] and engage various transcription factors, including those of the FoxO family [14,35] as in other cell types [16–18]. Moreover, PGC-1α-dependent remodeling of mitochondrial number and dynamics, known to be crucial for AgRP neurons control of energy balance [49–51], 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.

4.4. 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 [52].

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molmet.2016.05.008.

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