The Role of Air Pollution in the Aetiology of Type 2 Diabetes

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Summary

Background. The public health burden of type 2 diabetes cannot be overestimated. Prevalence of type 2 diabetes is continuously increasing and has caused a great number of deaths and economic losses. Optimal prevention measures for type 2 diabetes entail that more risk factors need to be identified. Air pollution is one of the modifiable environmental risk factors causing health problems, most notably respiratory diseases. Recently there have been indications for a spill-over of its effects into the cardio-metabolic systems. Short-term exposure to air pollution may exert acute or sub-acute inflammatory cardio-metabolic responses which on long-term, sustained exposure could lead to overt cardiovascular diseases and type 2 diabetes. However, it is unclear if long-term exposure to pollutants in the air contributes to the development of type 2 diabetes. This work generates evidence to fill knowledge gaps on the impact of air pollutants on the development of type 2 diabetes and on how different susceptibilities in the general population could contribute to the understanding of the mechanisms involved in this relationship.

Methods. First, this work summarized the existing evidence on the possible relationship between long-term exposure to air pollutants and type 2 diabetes. Furthermore, in the framework of the first follow-up of SAPALDIA- the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults, this work used indices for long-term exposure air pollution – 10-year mean particulate matter <10μm in diameter [PM_{10}] and nitrogen dioxide [NO_2] - assigned to participants' residences using a combination of Gaussian dispersion and Land-use regression models, participants residential histories and pollutant trends at monitoring stations. It identified diabetes and metabolic syndrome cases in a comprehensive way considering self-reports, blood tests and other physical measures. It additionally identified genetic variants through genotyping on two different arrays – the Human Illumina610quad Bead Chip and the Taqman PCR assay - for 63 type 2 diabetes genetic

polymorphisms [towards a diabetes gene score] and a functional polymorphism on the *IL6* gene respectively. Based on the above and detailed health socio-demographic and lifestyle characteristics including smoking habits, occupational exposures, alcohol, nutrition, physical activity, body measurements and additional data collected in SAPALDIA, it was ideal to investigate the cross-sectional relationships between air pollutants and diabetes and to explore interactions [based on various susceptibilities] to understand mechanisms involved in the relationship between long-term exposure to air pollutants and type 2 diabetes.

Results. In this work, we found a positive relationship between $PM_{2.5}$ and NO_2 and the risk of T2D in the pooled evidence synthesized from electronic databases. In the frame of SAPALDIA biobank, we found a moderate positive association between long-term exposure to PM_{10} [and NO_2] and prevalent diabetes, and demonstrated a sustained effect of PM_{10} independent of NO_2, while NO_2 lost its association on accounting for PM_{10} in multi-pollutant models. Among the measures of cardio-metabolic function, PM_{10} impacted most on impairment of glucose homeostasis and least on blood lipoproteins and triglycerides. The relationship between PM_{10} and impaired fasting glycaemia was more apparent among the physically active. Age also appeared to influence the relationship between PM_{10} and impaired fasting glycaemia. People at higher polygenic risk for type 2 diabetes were more susceptible to PM_{10}. Genetic risk for insulin resistance and obesity appeared to be more relevant than those for beta-cell function in modifying the effects of PM_{10}, especially among those with some background inflammatory conditions. Carriers of the pro-inflammatory major 'G' allele of IL6-572GC, with allele frequency of 93%, were also more susceptible to PM_{10} in relation to diabetes.

Conclusions. This work has greatly contributed to evidence suggesting the possible role of air pollutants in diabetes aetiology. The reported associations were observed at mean concentrations below current air quality guidelines. PM_{10} may be a good marker for aspects of

air pollution [rather than NO_2] relevant for the development of diabetes. In particular, PM_{10} might act through sub-clinical inflammation and resultant impaired insulin sensitivity. Impairment of insulin secretion may be a less relevant pathway for PM_{10} action. Physical activity, though beneficial, presented another likely pathway for PM_{10} effects. These findings, if confirmed, call for the strengthening of air quality policies and adaptation of physical activity promotion to environmental contrasts. Future studies should explore the totality of environmental exposures – exposomics –in a life-course fashion. The mediating role of DNA methylation influencing genetic expression should be further explored. For global generalizability, there is a strong need for evidence replication in developing countries where outdoor and indoor air pollution is quite high and mostly unregulated, and the burden of non-communicable diseases is rapidly growing.

Zusammenfassung

Einführung. Die Belastung der öffentlichen Gesundheit durch Typ-2-Diabetes kann nicht überschätzt werden. Die Prävalenz von Typ-2-Diabetes steigt kontinuierlich an und hat bislang eine sehr hohe Anzahl an Todesfällen sowie wirtschaftliche Verluste verursacht. Um optimale Präventionsmassnahmen für Typ-2-Diabetes einleiten zu können, müssen noch mehr Risikofaktoren identifiziert werden. Die Umweltverschmutzung gehört zu den beeinflussbaren Umweltrisiken, welche Gesundheitsprobleme verursachen, insbesondere in Bezug auf Atemwegserkrankungen. In jüngster Zeit wurden Hinweise auf Spill Over-Effekte auf das kardio-metabolische System gefunden. Eine Kurzzeitexposition gegenüber Luftverschmutzung kann akute oder subakute entzündliche kardio-metabolische Reaktionen verursachen, welche bei langfristiger, anhaltender Exposition zu einer offenkundigen Herz-Kreislauf-Erkrankung und Typ-2-Diabetes führen können. Es ist jedoch unklar, ob eine Langzeitexposition gegenüber Schadstoffen in der Luft tatsächlich zu Typ-2-Diabetes beiträgt. Diese Arbeit füllt Wissenslücken in Bezug auf den Zusammenhang zwischen Luftverschmutzung und Typ-2-Diabetes und sowie in Bezug auf Wirkmechanismen und individuelle Empfindlichkeiten.

Methoden. Diese Arbeit hat erstens die vorhandene Evidenz zum Zusammenhang zwischen Langzeitexposition gegenüber Luftschadstoffen und Typ-2-Diabetes zusammengefasst. Im Rahmen der ersten Nachuntersuchung von SAPALDIA, der Schweizerischen Kohortenstudie über Luftverschmutzung und Lungen und Herzerkrankungen bei Erwachsenen, hat diese Arbeit darüberhinaus Parameter für Langzeitexpositionen gegenüber Luftverschmutzung genutzt – Feinstaub <10µm in Durchmesser [PM_{10}] und Stickstoffdioxid [NO_2] –, welche den Wohnorten der Studienteilnehmer zugeordnet wurden. Sie hat Diabetes und Fälle mit metabolischem Syndrom in umfassender Weise identifiziert, indem Selbstberichte, Bluttests und andere physische Messungen berücksichtigt wurden. Sie hat zudem Genvarianten durch

Genotypisierung mit zwei unterschiedlichen Methoden identifiziert – der Human Illumina610quad Bead Chip und der Taqman PCR Test: 63 Typ-2-Diabetes genvarianten zur Berechnung eines Risikoscore; ein funktioneller Polymorphismus auf dem *IL6*-Gen. Basierend auf den oben erwähnten und detaillierten Gesundheits- und Lifestyle-Charakteristiken, einschliesslich soziodemografischen Merkmalen, Rauchgewohnheiten, berufsbedingten Expositionen, Alkohol, Ernährung, körperlicher Aktivität, Körpermasse und ergänzenden in SAPALDIA erhobenen Daten, war es möglich die Querschnitts-Zusammenhänge zwischen Luftschadstoffen und Diabetes zu untersuchen und Interaktionen zu erforschen [basierend auf zahlreichen Anfälligkeiten]. Die Resultate helfen die Mechanismen zu verstehen, welche dem Zusammenhang zwischen Langzeitexposition gegenüber Luftschadstoffen und Typ-2-Diabetes zu Grunde liegen.

Resultate. Wir haben in dieser Arbeit einen positiven Zusammenhang zwischen $PM_{2.5}$ und NO_2 und dem T2D-Risiko in gepoolten, aus elektronischen Datenbanken synthetisierten Beweisen gefunden. Im Rahmen von SAPALDIA haben wir einen moderaten positiven Zusammenhang zwischen Langzeitexposition gegenüber PM_{10} und NO_2 gefunden. Die Assoziation mit PM_{10} blieb unabhängig von NO_2 bestellen, während NO_2 seine Wirkung nach Mitenbezug von PM_{10} in Multi-Schadstoff-Modellen verlor. Unter den Parametern zur kardio-metabolischen Funktion wirkte sich PM_{10} am stärksten auf die Beeinträchtigung der Glukosehomöostase und am schwächsten auf Blutlipoproteine und Triglyceride aus. Der Zusammenhang zwischen PM_{10} und Typ-2-Diabetes trat stärker bei den körperlich Aktiven hervor. Auch Alter schien den Zusammenhang mit gestörter Nüchternglukose zu modifizieren. Menschen mit erhöhtem polygenem Risiko für Typ-2-Diabetes waren anfälliger für die glykämischen Wirkungen von PM_{10}. Ein genetisches Risiko für Insulinresistenz und Übergewicht schien dabei relevanter zu sein als jenes für Beta-Zell-Funktion, insbesondere bei Personen mit entzündlichen Erkrankungen. Zusätzlich waren Träger des

entzündungsfördernden „G“-Allels des IL6-572GC Polymorphismus [Allelfrequenz von 93%] empfänglicher für die glykämischen Wirkungen von PM_{10}.

Schlussfolgerungen. Diese Arbeit hat wesentlich zur Evidenz beigetragen, dass Luftschadstoffe eine Rolle spielen konnten in der Entstehung von Diabetes. Die gefundenen Zusammenhänge wurden auch unterhalb Limiten aktueller Luftqualitätrichtlinien beobachtet. PM_{10} scheint dabei ein guter Marker für diabetes-relevante Luftverschmutzung zu sein [besser als NO_2]. Insbesondere könnte PM_{10} über subklinische Entzündungen und die daraus resultierende Insulinsensitivität wirken. Körperliche Aktivität, obschon vorteilhaft in der Diabetesprävention, erhöht möglicherweise die diabetogene Auswirkung von PM_{10}. Sollten sich diese Ergebnisse bestätigen, ist eine Stärkung der Luftqualitätpolitik notwendig sowie auch eine Anpassung der Förderung von körperlicher Betätigung an den Umweltkontext. Zukünftige Studien sollten die Gesamtheit der Umweltexpositionen – das Exposom – in verschiedenen Altersbereichen erforschen. Zudem sollte die vermittelnde Rolle der die Genexpression beeinflussende DNA-Methylierung mit untersucht werden. Für die globale Generalisierbarkeit der Zusammenhänge, ist es dringend nötig die Ergebnisse in Entwicklungsländern zu replizieren, in denen die Verschmutzung der Innen- und Aussenluft sehr hoch und überwiegend ungeregelt ist und wo zudem die Belastung durch nicht übertragbare Krankheiten rasant ansteigt.

Résumé

Background. Le poids du diabète de type 2 sur la santé publique ne peut pas être surestimé. Sa prévalence est en constante augmentation et a causé un grand nombre de décès et de pertes économiques. Les mesures de prévention optimales du diabète de type 2 impliquent une meilleure identification des facteurs de risque. La pollution de l'air est un des facteurs de risque environnemental modifiable causant des problèmes de santé, notamment des maladies respiratoires. Récemment, plus d'intérêt a été porté à l'extension potentielle de ses effets sur les systèmes cardiométaboliques. L'exposition à court terme à la pollution de l'air peut exercer une réponse inflammatoire cardiométabolique aiguë ou subaiguë, qui, accompagnée d'une exposition continue sur le long terme, peut conduire à des maladies cardiovasculaires et au diabète de type 2. Il est cependant encore peu clair si l'exposition à long terme aux polluants dans l'air contribue au développement du diabète de type 2. Cet ouvrage génère des indices comblant les connaissances manquantes sur l'impact des polluants de l'air sur le développement du diabète de type 2 et sur la manière dont les différentes susceptibilités dans la population peuvent contribuer à la compréhension des mécanismes en jeu dans cette relation.

Méthodes. Premièrement, cet ouvrage a rassemblé les indices existants sur la relation possible entre l'exposition à long terme à la pollution de l'air et au diabète de type 2. De plus, dans le cadre du premier suivi de SAPALDIA – la cohorte suisse sur la pollution de l'air et les maladies pulmonaires et cardiaques chez les adultes, cet ouvrage a utilisé des indicateurs pour l'exposition à long terme à la pollution de l'air – particules fines <10um de diamètre [PM_{10}] et dioxyde d'azote [NO_2] – assigné à l'adresse de résidence des participants. Les cas de diabète et de syndrome métabolique ont étés identifiés de manière complète en appliquant des auto-évaluations, des tests sanguins et d'autres mesures physiques. En addition, pour le diabète de type 2, les variantes de 63 polymorphismes génétiques [vers un score de gène du diabète] et

d'un polymorphisme fonctionnel sur le gène *IL6* ont été identifiées par génotyping sur deux plateformes différentes d'analyse – le Human Illumina610quad Bead Chip et le Taqman PCR. Basé sur ce qui précède et les caractéristiques de santé et d'hygiène de vie détaillées, consommation de tabac, exposition liée au travail, consommation d'alcool, nutrition, activité physique, mesures corporelles et autres données collectées dans le cadre de SAPALDIA, il s'est avéré idéal d'investiguer, en usant SAPALDIA, les relations transversales entre les polluants de l'air et le diabète et d'explorer les interactions (basées sur des susceptibilités variées) afin de comprendre les mécanismes en jeu dans la relation entre l'exposition à long terme à la pollution de l'air et le diabète de type 2.

Résultats. Dans cette ouvrage, nous trouvons une relation positive entre $PM_{2.5}$ et NO_2 sur le risque de diabète de type 2 dans les indices collectés dans des banques de données électroniques. Dans le cadre de SAPALDIA biobanque, nous trouvons une association positive modérée entre l'exposition à long terme au PM_{10} et NO_2, et démontrons un effet continu de PM_{10} indépendent du NO_2 qui perd son effet quand le PM_{10} est pris en compte dans les modèles multipolluants. Considérant les mesures de fonction cardiométabolique, le PM_{10} a plus d'impact sur l'homéostase de glucose déficient et moins d'impact sur les protéines sanguines et les trigycérides. La relation entre PM_{10} et la glycémie à jeûn déficiente était plus apparente au sein des sujets pratiquant une activité physique. Age semble également influencer la relation entre les PM_{10} et la glycémie à jeûn déficiente. Les sujets à plus haut risque polygénique pour le diabète de type 2 étaient plus susceptibles aux effets glycémiques des PM_{10}. Les risques génétiques de la résistance à l'insuline et l'obésité étaient plus importants que ceux des fonctions des cellules béta dans la médiation des effets des PM_{10}, en particulier parmi ceux avec un historique de condition inflammatoires. Les porteurs de l'allèle pro-inflammatoire 'G' de IL6-572GC avec une fréquence d'allèle de 93% étaient également plus susceptibles des PM_{10} en relation à diabète.

Conclusions. Cet ouvrage a grandement contribué aux preuves suggérant le rôle possible des polluants de l'air dans l'étiologie du diabète. Ces observations ont également eu lieu à des concentrations moyennes en dessous des directives actuelles de qualité de l'air. PM_{10} pourrait être un marqueur bien plus important [à la place de NO_2] des effets de la pollution de l'air sur le diabète. En effet, $PM_{10,}$ pourrait induire une inflammation sub-clinique et ceci résulterait en une déficience à l'insuline accrue. Les déficiences dans la sécrétion de l'insuline pourraient être un mécanisme moins important de l'action des PM_{10}. L'activité physique, bien que bénéfique, présentait un autre mécanisme probable pour les effets des PM_{10}. Ces résultats, si confirmés, appellent à un renforcement des politiques de qualité de l'air et à une adaptation de la promotion de l'activité physique aux contrastes environnementaux. Les études à venir devraient explorer la totalité des expositions environnementales – exposomics – sur durée d'une vie. Le rôle médiateur de la méthylation de l'ADN influençant l'expression génétique devrait être exploré plus avant. Pour une généralisation globale, un fort besoin d'études de réplication venant des pays en voie de développement où la pollution de l'air à l'intérieur et à l'extérieur est haute et généralement non régulée et où le poids des maladies non transmissibles est en rapide augmentation.

PART I: INTRODUCTION

1. Background

1.1 Type 2 diabetes in the context of non-communicable diseases

1.1.1 Epidemiology and Public Health Burden

Diabetes mellitus [DM] constitutes about 25% of the major non-communicable diseases [NCDs], – diseases which are not transmissible from person to person, of slow progression, age-related and of long duration [even life-long] – which also includes cardiovascular diseases [CVD], cancers and chronic respiratory diseases. As there is a global trend towards longer life expectancy, occurrence of NCDs becomes more likely. According to the World Health Organization [WHO], NCDs lead to about 38 million annual deaths, 82% and 5% of which are attributed to these four major NCDs and diabetes respectively (WHO, 2015). The International Diabetes Federation [IDF] estimates that diabetes is the fourth leading cause of death in Europe and about 70% of diabetes cases in Europe die from cardiovascular disease (IDF, 2008). In Switzerland, about 2,500 deaths were attributed to diabetes in 2013. Seventy-five percent of all NCD-related deaths occur in low- and middle income countries (IDF, 2013). Figure 1 shows global deaths from diabetes in 2013.

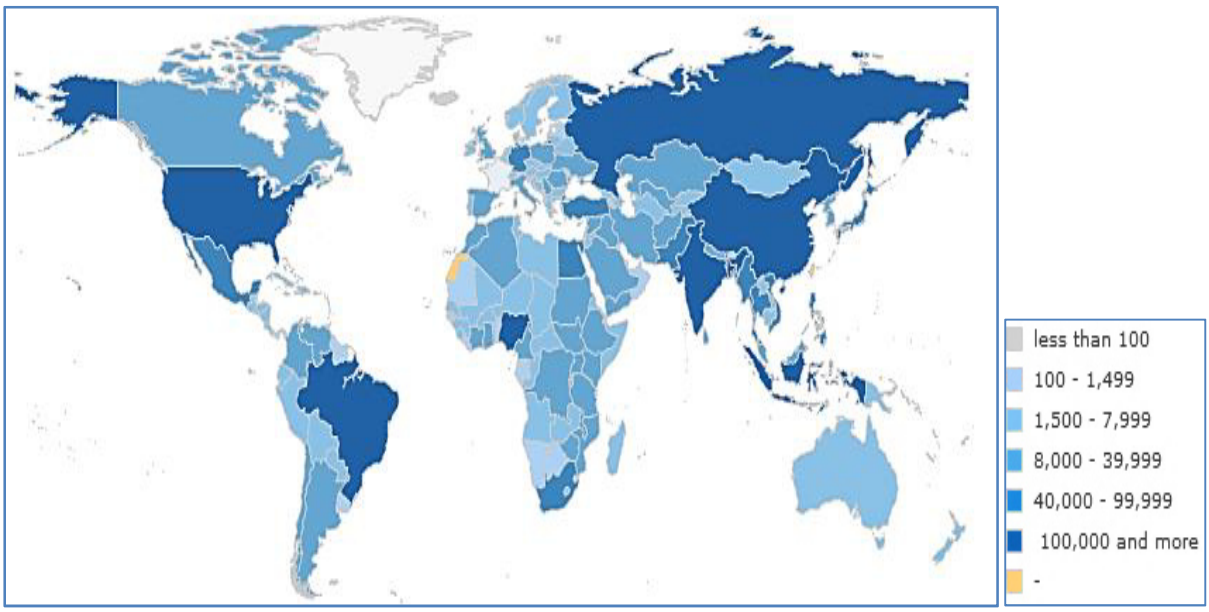

Figure 1: Global distribution of mortality due to diabetes (IDF, 2013)

The number of global diabetes cases in 2013 was put at 317 million. This is expected to increase to 519 million in 2030 if adequate preventive measures are not implemented (IDF, 2013). The global distribution of diabetes prevalence is presented in Figure 2. In 2013, prevalence of diabetes and impaired glucose tolerance in Switzerland were 7.5% and 8.1% respectively (IDF, 2013).

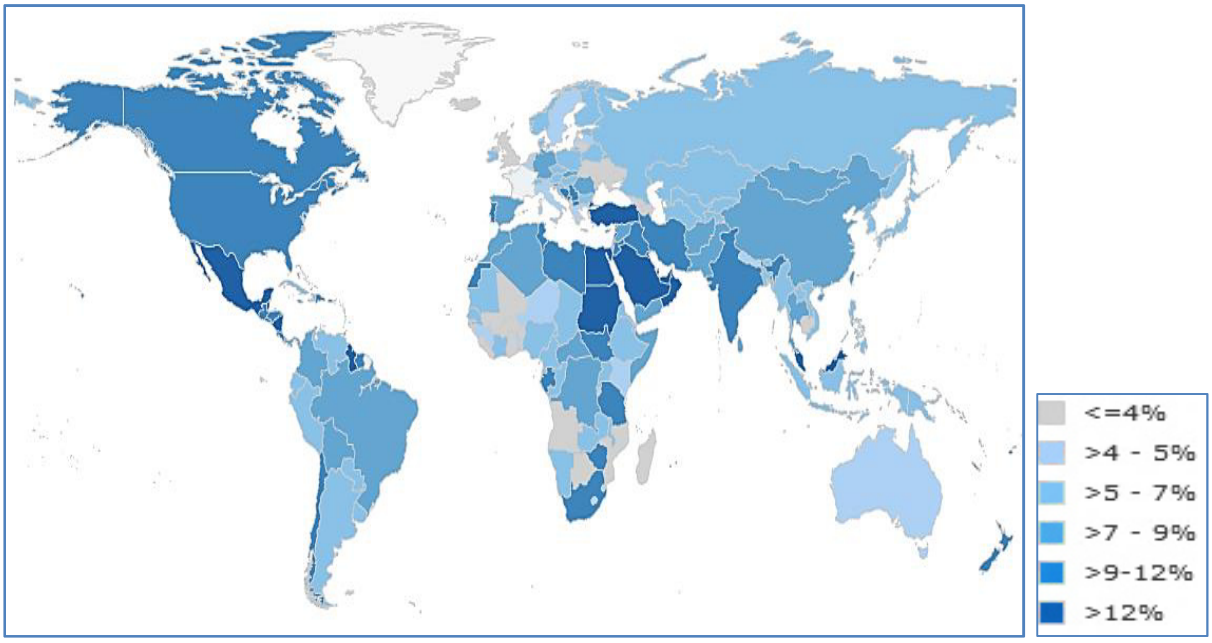

Figure 2: Global distribution of diabetes prevalence (IDF, 2013)

Similar to other NCDs, diabetes is of great economic importance. The direct costs of treatments and the indirect costs due to disability and mortality are quite profound. Presently, most of these costs are borne by the high income countries [Figure 3] in part explained by the substantial degree of under-diagnosis and under-treatment of diabetes and its consequences in low and middle income countries [LMIC], for instance, Switzerland is estimated to have spent about USD 10,000 per diabetes patient in total cost per annum whereas Nigeria spent about USD 150. By 2030 (IDF, 2013), this is expected to change drastically, with the low and middle income countries taking up about 75% of both direct and indirect costs of diabetes.

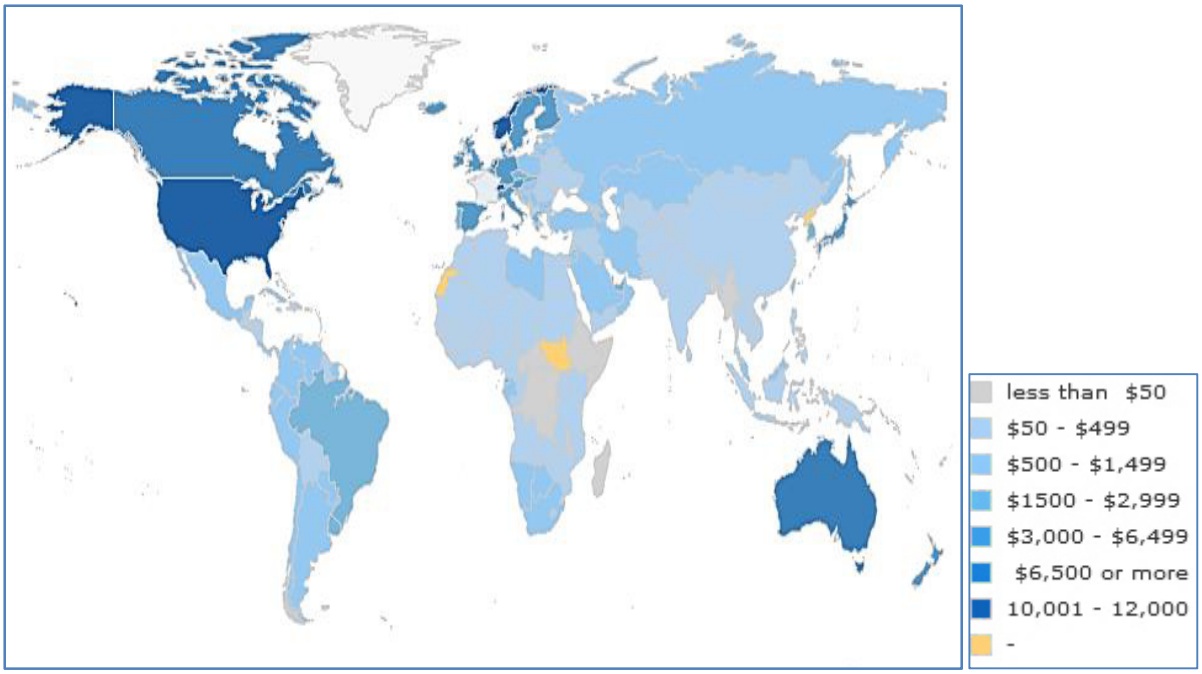

Figure 3: Global distribution of diabetes expenditure (IDF, 2013)

1.1.2. Symptoms, diagnosis and management

Symptoms of type 2 diabetes

Diabetes has several symptoms which may occur mildly in some people, and often go unnoticed for a long time. Symptoms may progress with disease severity. Some of the common symptoms of type 2 diabetes [T2D] according to the American Diabetes Association [ADA] include (ADA, 2015a) polyphagia – excessive eating or appetite, occurring even after eating; polydipsia – excessive thirst; polyuria – frequent urination; extreme fatigue and slow healing of bruises or infections. According to the National Institutes of health [NIH], other symptoms which could be reported on presentation, but are also indicative of on-going complications include blurry vision, tingling sensation or pain in the hands and feet and erectile dysfunction (NIH, 2015). About half of global diabetes cases remain undiagnosed (Harris et al., 1987, IDF, 2008) leading to late presentations and increased complications on presentation.

Diagnoses of T2D

Type 2 diabetes accounts for >90% of all diabetes in adults and reduces life expectancy by 5-10 years (Zimmet et al., 2001, King et al., 1998). A diagnosis of T2D can be made using plasma glucose or glycosylated haemoglobin [HbA1c] levels. Plasma glucose may be measured in a fasting state, 2 hours following an oral 75g glucose load or randomly regardless of when the last meal was had (ADA, 2014, ADA, 2015b, IDF, 2012). Diagnosis using HbA1c requires that the A1c assay be certified by the National Glycohaemoglobin Standardization Programme and standardized to the Diabetes Control and Complications Trial reference assay (ADA, 2015b). Although HbA1c is more convenient, has greater pre-analytic stability and does not fluctuate to daily stress variations and illness compared to plasma glucose measurements, it is more expensive, has limited availability in developing countries and may not correlate well with plasma glucose in some individuals (Nowicka et al., 2011, Garcia de Guadiana Romualdo et al., 2012, Ziemer et al., 2010, Kumar et al., 2010). A HbA1c value of ≥0.065, fasting [no caloric intake for at >=8 hours] plasma glucose of ≥7.0mmol/L and 2-hour post-prandial [75g oral glucose dissolved in water] glucose of ≥11.1 mmol/L are suggestive of diabetes (ADA, 2014, IDF, 2012). In the absence of unequivocal hyperglycemia, confirmation should be done by a repeat testing (ADA, 2015b).

In asymptomatic individuals, a repeat test is necessary following an abnormal glucose test, to confirm diabetes whereas in symptomatic individuals a repeat test may not be necessary to make a diagnosis of diabetes (IDF, 2012). Screening/diagnosis in asymptomatic individuals is recommended using the following criteria (ADA, 2015b): (a) Testing should be considered in all adults who are overweight [BMI≥25kg/m^2 or 23kg/m^2 in Asians] and have additional risk factors; (b) Testing should begin at 45 years especially for obese persons; (c) If results are normal, testing should be repeated at a minimum of 3-year interval. If abnormal, a one-year testing interval may be applied.

Management of type 2 diabetes

Optimal management of T2D entails that patients make necessary lifestyle modifications including improved nutrition, physical activity, quit smoking among other things. Patients are educated on self-management [Diabetes self-management education] and provided necessary support [Diabetes self-management support] to develop and maintain healthy behaviours towards achieving target plasma glucose levels (ADA, 2015b). For pharmacological treatment, Metformin is usually the first drug of choice (ADA, 2015b). Other agents may be added if target glucose levels are not reached. Insulin therapy is always initiated at some point in the course of T2D, especially when complications develop. Lifestyle modifications alongside pharmacological treatment ensure the best outcome in the management of more advanced T2D.

1.1.3. Pathophysiology of type 2 diabetes

Type 2 diabetes mainly results from impairment of insulin secretion by the pancreatic beta-cells or reduction in insulin sensitivity by the muscle, fat and liver cells (Buse JB, 2003).

Insulin secretion

Glucose enters the pancreatic beta cell through facilitated diffusion by the glucose transporter 2 on the beta cell membrane (Buse JB, 2003). Glucose metabolism increases cytosolic ATP, and initiates insulin secretion by blocking the ATP-dependent potassium ion channel on the beta cell membrane. This blockade induces beta cell membrane depolarization, increases cytosolic calcium ions and insulin secretion (Buse JB, 2003). Apart from glucose which is the most important regulator of insulin release (Porte Jr and Pupo, 1969, Chen and Porte, 1976), essential amino acids (Levin et al., 1971, Fajans and Floyd Jr, 1972), gastrointestinal peptide hormones (Creutzfeldt and Ebert, 1985, Dupre et al., 1973) and parasympathetic stimulation through vagal nerve fibres (Nishi et al., 1987, Kurose et al., 1990) also contribute to the

regulation of insulin release. Studies have shown that in any 24-hour period, 50% of insulin secretion occurs under basal conditions and 50% in response to meals (Kruszynska et al., 1987, Polonsky et al., 1988). Some circadian pattern in insulin secretion has been reported, with maximal secretion occurring in the morning following breakfast (Malherbe et al., 1969, Polonsky et al., 1988, Jarrett et al., 1972). Insulin secretion can be measured directly by fasting insulin levels, or indirectly using the C-peptide concentration, a product of proinsulin cleavage within the Golgi apparatus of the beta cell (Melani et al., 1970, Horwitz et al., 1975). C-peptide is released in equimolar concentrations with those of insulin but unlike insulin, is not extracted by the liver (Melani et al., 1970). In addition, C-peptide also has a longer half-life [30 minutes] compared to insulin [4 minutes] (Palmer et al., 2004) making it preferable as a peripherally measurable marker of beta cell function, but under the assumption of constant mean clearance rates in normal physiologic conditions (Polonsky et al., 1983, Polonsky et al., 1986). The secretion of insulin is also influenced by genetic constitution (Perry and Frayling, 2008).

Insulin sensitivity

An impairment in insulin sensitivity results in insulin resistance. This is manifested by a reduction in insulin-stimulated glucose transport and metabolism in target cells including adipocytes, hepatocytes and skeletal muscle (Buse JB, 2003). Age, abdominal fat, ethnicity, physical inactivity and certain medications influence insulin sensitivity (Paolisso et al., 1999). Like beta-cell function, insulin resistance also has a genetic component. First-degree relatives of T2D patients have insulin resistance even without being obese (Groop, 2000, Lehtovirta et al., 2000). Hyperinsulinaemia is another determinant of insulin resistance. High levels of insulin down-regulate insulin receptors and desensitizes post-receptor pathways (Olefsky et al., 1985) and suppression of insulin secretion in insulin resistant people results in improved

insulin sensitivity. In many cases, T2D manifests when insulin resistance occurs in a background of some degree of impairment in beta-cell function.

1.1.4 Determinants of type 2 diabetes

Non-genetic determinants

The traditional risk factors include demographic characteristics such as age and sex. T2D mostly occurs in older age groups (Harris et al., 1997, Harris et al., 1987, Mokdad et al., 2001) although there has been an increasing occurrence in children, reaching up to 50% of childhood diabetes (Fagot-Campagna et al., 2000, Fagot-Campagna et al., 1998, Willi et al., 1997). T2D prevalence may vary by sex, depending on the age group and ethnicity. There is roughly equal sex ratio in diagnoses made before 15 years of age (Gale and Gillespie, 2001). In Europeans between 15-40 years, there is a male excess whereas in non-Europeans, there is some female excess (Gale and Gillespie, 2001). Some lifestyle-related characteristics including obesity (Shai et al., 2006), physical inactivity (Sigal et al., 2006), dietary patterns (Shai et al., 2006, Hu et al., 2001, Salmeron et al., 2001) and stress (Heraclides et al., 2009, Novak et al., 2013, Siddiqui et al., 2015) have been epidemiologically identified to be important predictors of T2D.

Intermediate risk phenotypes for T2D including impaired glucose tolerance (Soderberg et al., 2004, Shaw et al., 1999, de Vegt et al., 2001) and insulin resistance (Meigs et al., 2007, McKeigue et al., 1991, Everson-Rose et al., 2004) are also predictors of T2D. Pregnancy-related determinants of T2D include parity (Nicholson et al., 2006, Araneta and Barrett-Connor, 2010), gestational diabetes (Kim et al., 2002), and intrauterine malnutrition (Iliadou et al., 2004, Meier, 2009).

Genetic determinants

There is ample evidence for a strong genetic basis for T2D. At the level of ethnicity, non-European populations including Africans, Asians and South Americans are at greater risk for T2D (Fagot-Campagna et al., 1998, Everson-Rose et al., 2004, McKeigue et al., 1991, Shai et al., 2006). According to Neel (1962), in his thrifty gene hypothesis, the positive selection of genes that promote energy storage necessary for survival in periods of famine is now detrimental for survival since food is plenty, with limited physical activity. This holds true for the populations which have been shown to be at high risk for diabetes after undergoing genetic selection following a history of famine and transition into Western lifestyle (Kilpelainen and Franks, 2014).

At the family level, concordance for T2D was observed in both monozygotic [~70%] and dizygotic twins [~25%] (Kaprio et al., 1992). Other familial evidence include a 40% lifetime risk of developing T2D in an offspring of a parent with T2D, and almost 70% if both parents are affected (Groop et al., 1996). A two-fold risk of T2D has been associated with a first degree family history of T2D (Lyssenko et al., 2005, Lyssenko et al., 2008).

Following developments in high throughput genotyping, [with arrays producing an excess of 1 million polymorphisms] (Ragoussis, 2009), and genome-wide association studies (GWAS), about 65 T2D genetic variants [Table 1] have been identified (Morris et al., 2012) which taken together, explain about 10% of the heritability of T2D (Talmud et al., 2015). The low heritability attributed to T2D genetic variants may imply that more partially rare genetic loci are likely awaiting discovery with continuing advances in genotyping and GWAS. So far, studies on quantitative glycaemic traits (Dimas et al., 2014, Scott et al., 2012, Manning et al., 2012, Harder et al., 2013, Perry and Frayling, 2008) have shown that many of these variants regulate beta-cell function whereas fewer regulate insulin sensitivity, in a ratio of 3:1 [Table

1] possibly signifying a stronger genetic component in insulin secretion compared to insulin resistance (Hong et al., 2001, Mills et al., 2004, Rich et al., 2004).

Table 1: Genetic variants associated with type 2 diabetes and the risk allele frequencies in European population

RS number	CHR	Gene$_{[pathway]}$	Risk/ other allele	Risk allele frequency
rs10923931	1	*NOTCH2*	T/G	0.12
rs2075423	1	*PROX1*$_{[BCF]}$	G/T	0.62
rs780094	2	*GCKR*$_{[IR]}$	C/T	0.61
rs10203174	2	*THADA*$_{[BCF]}$	C/T	0.89
rs243088	2	*BCL11A*	T/A	0.45
rs7569522	2	*RBMS1*	A/G	0.44
rs13389219	2	*GRB14*$_{[IR]}$	C/T	0.60
rs2943640	2	*IRS1*$_{[IR]}$	C/A	0.63
rs1801282	3	*PPARG*$_{[IR]}$	C/G	0.86
rs1496653	3	*UBE2E2*$_{[BCF]}$	A/G	0.75
rs12497268	3	*PSMD6*	G/C	0.80
rs6795735	3	*ADAMTS9*	C/T	0.59
rs11717195	3	*ADCY5*$_{[BCF]}$	T/C	0.77
rs4402960	3	*IGF2BP2*$_{[BCF]}$	T/G	0.33
rs17301514	3	*ST64GAL1*	A/G	0.13
rs4458523	4	*MAEA*	T/C	0.96
rs459193	4	*WFS1*	G/T	0.57
rs459193	5	*ANKRD55*$_{[IR]}$	G/A	0.70
rs6878122	5	*ZBED3*	G/A	0.28
rs7756992	6	*CDKAL1*$_{[BCF]}$	G/A	0.29
rs4299828	6	*ZFAND3*	A/G	0.79
rs3734621	6	*KCNK16*	C/A	0.03
rs17168486	7	*DGKB*$_{[BCF]}$	T/C	0.19
rs849135	7	*JAZF1*	G/A	0.52
rs10278336	7	*GCK*$_{[BCF]}$	A/G	0.50
rs17867832	7	*GCC1*	T/G	0.91
rs13233731	7	*KLF14*$_{[IR]}$	G/A	0.51
rs516946	8	*ANK1*$_{[BCF]}$	C/T	0.76
rs7845219	8	*TP53INP1*	T/C	0.52
rs3802177	8	*SLC30A8*$_{[BCF]}$	G/A	0.66
rs10758593	9	*GLIS3*$_{[BCF]}$	A/G	0.42
rs16927668	9	*PTPRD*	T/C	0.24
rs10811661	9	*CDKN2A/B*$_{[BCF]}$	T/C	0.82
rs17791513	9	*TLE4*	A/G	0.91
rs2796441	9	*TLE1*	G/A	0.57
rs11257655	10	*CDC123*$_{[BCF]}$	T/C	0.23
rs12242953	10	*VPS26A*	G/A	0.93
rs12571751	10	*ZMIZ1*	A/G	0.52
rs1111875	10	*HHEX/IDE*$_{[BCF]}$	C/T	0.58

rs7903146	10	*TCF7L2*$_{[BCF]}$	T/C	0.27
rs2334499	11	*DUSP8*	T/C	0.43
rs163184	11	*KCNQ1*$_{[BCF]}$	G/T	0.50
rs5215	11	*KCNJ11*$_{[BCF]}$	C/T	0.41
rs1552224	11	*ARAP1*$_{[BCF]}$	A/C	0.81
rs10830963	11	*MTNR1B*$_{[BCF]}$	G/C	0.31
rs11063069	12	*CCND2*	G/A	0.21
rs10842994	12	*KLHDC5*	C/T	0.80
rs2261181	12	*HMGA2*$_{[IR]}$	T/C	0.10
rs7955901	12	*TSPAN8*	C/T	0.45
rs12427353	12	*HNF1A [TCF1]*	G/C	0.79
rs1359790	13	*SPRY2*	G/A	0.72
rs4502156	15	*C2CD4A*$_{[BCF]}$	T/C	0.52
rs7177055	15	*HMG20A*	A/G	0.68
rs11634397	15	*ZFAND6*	G/A	0.64
rs2007084	15	*AP3S2*	G/A	0.92
rs12899811	15	*PRC1*	G/A	0.31
rs9936385	16	*FTO*$_{[IR]}$	C/T	0.41
rs7202877	16	*BCAR1*$_{[BCF]}$	T/G	0.89
rs2447090	17	*SRR*	A/G	0.62
rs11651052	17	*HNF1B [TCF2]*	G/A	0.44
rs12970134	18	*MC4R*$_{[IR]}$	A/G	0.27
rs10401969	19	*CILP2*	C/T	0.08
rs8182584	19	*PEPD*$_{[IR]}$	T/G	0.38
rs8108269	19	*GIPR*	G/T	0.31
rs4812829	20	*HNF4A*	A/G	0.19

CHR: chromosome; BCF: beta cell function; IR: insulin resistance.

Gene - environment interactions in type 2 diabetes

A gene-environment interaction [GEI] occurs when there is a mutual dependency between a genetic variant and an environmental factor contributing to the development of a trait. T2D is a complex disease involving the interplay of genetic and environmental factors. T2D may develop if genetically predisposed individuals are exposed to diabetes-promoting exogenous factors. This is demonstrated by the fact T2D is best predicted using a combination of genetic variation clinical/environmental components (Lyssenko and Laakso, 2013, Talmud et al., 2015).

Physical activity and variants near the *FTO* gene are one of the most studied GEI in T2D (Kilpelainen and Franks, 2014). A Danish study reported the attenuation of the obesogenic

effect of *FTO* risk allele by physical activity (Andreasen et al., 2008). This followed previous evidence on the mediating effect of the *FTO* variant on T2D-BMI association (Frayling et al., 2007). A subsequent meta-analysis of 45 inconsistent studies demonstrated an attenuation of the effect of *FTO* variant on BMI among the physically active compared to the inactive (Kilpelainen et al., 2011). The Pro12Ala variant of *PPARG* was shown to modify the association between physical activity and glucose regulation in people with (Adamo et al., 2005) and without diabetes (Franks et al., 2004, Kahara et al., 2003).

Evidence from GEI studies on nutrition and T2D also demonstrated that the carriers of this *PPARG* variant are more responsive to the beneficial effects of unsaturated fat and less susceptible to the adverse effects of saturated fat on glucose regulation and/or body mass index (Lamri et al., 2012, Luan et al., 2001, Memisoglu et al., 2003, Cornelis and Hu, 2012). Carriers of a *TCF7L2* risk variant had a lower T2D risk when they were on low glycemic diet (Cornelis et al., 2009a, Fisher et al., 2009) and following some intensive lifestyle changes (Florez et al., 2006, Haupt et al., 2010). A large meta-analysis identified the modifying effect of *SLC30A8* on the cross-sectional relationship between zinc intake and glucose homeostasis, observing a stronger negative relationship between zinc and glucose among carriers of the fasting glucose-raising allele (Kanoni et al., 2011). In another study, a higher whole grain intake was associated with smaller reductions in fasting insulin among carriers of the insulin raising allele of *GCKR* variant (Nettleton et al., 2010).

GEI studies on T2D have also taken a wider, better-powered genetic risk score [GRS] approach, pooling variants into a composite score and identifying population subsets at greater genetic risk for T2D (Cornelis and Hu, 2012, Talmud et al., 2015). Using a GRS of 10 variants, Cornelis et al. (2009b) found the relationship between GRS and T2D to be greater with increasing BMI. This finding was subsequently replicated by studies using a GRS of 49 (Langenberg et al., 2014) and 46 variants (Andersson et al., 2013). A more western dietary

lifestyle, in another study using a GRS of 10 variants, increased T2D risk only among people with high GRS (Qi et al., 2009).

1.2. Air pollution as an emerging environmental risk factor for type 2 diabetes

Ambient air pollution ranks high among the risk factors for the global burden of disease (Lim et al., 2012). There has been growing interests in the health effects of air pollution following the deaths that occurred from the great smog in London in 1952. Evidence has been growing on the relationships between exposure to classical air pollutants- particulate matter [PM], nitrogen dioxide [NO_2], nitrogen oxides [NOx] and Ozone [O_3] - and respiratory and cardiovascular mortality and morbidity (Schikowski et al., 2014, Brook et al., 2010). It is suggested that toxicological properties of combustion products from traffic sources may be most detrimental to health (Brook et al., 2010, Schlesinger et al., 2006)

1.2.1. Sources, composition and assessment

Common sources and composition of air pollution

Air pollution represents a complex mixture including gases, liquids and solid state components. The gaseous pollutants are usually products of local combustion processes [traffic and power plants] and include NO_2, NOx, and sulphates. Other gaseous pollutants like O_3 are produced as a second-stage reaction- reaction of NO_2 with hydrocarbons under sunlight exposure- and exhibit a short half-life (Koike et al., 2005).

The solid or liquid droplet fraction of air pollutants comprise the PM. Apart from combustion, these particles can also be generated from natural sources including molds, spores, soil and metals. Thus, the composition of PM is varied and depends on source and location (Eeftens et al., 2012). Following production, PM is suspended in air and travel long distances, long distances being inversely related to the size of the particles. Their size also determines their respirability, with the smaller ones going further into the respiratory tract. Based on their size,

PM are grouped into three categories - diameter < 10um [PM_{10} or coarse particles], diameter < 2.5um [$PM_{2.5}$ or fine particles] and diameter <0.1um [ultra-fine particles] (Brook et al., 2004). PM_{10} may deposit in the trachea and pulmonary bronchi while $PM_{2.5}$ and UFP may reach the alveoli and pulmonary circulation respectively (Oberdorster et al., 2000, Oberdorster et al., 2002).

Assessment of air pollution

Due to their stability, particulate matter and nitrogen oxides are the more commonly measured pollutants. These pollutants' concentrations in the air can be measured using devices that either measure on the spot or sample the air over long spans of time using various methods. Measurement of individual exposures to these pollutants can be achieved through the use of personal exposure measurement devices (Clench-Aas et al., 1999, Kramer et al., 2000).

For big epidemiological studies, it becomes difficult to directly measure pollutants for each participant on both short and long terms. In these situations, values from the nearest air pollution monitoring station can be used as a proxy for an individual's exposure (Kramer et al., 2010). More refined modeling techniques that incorporate several measurements from various devices, among other variables, can be applied to estimate an individual's air pollution exposure over a long period of time. Common air pollution modeling techniques include proximity models, spatial interpolation models, dispersion models, land-use regression [LUR] models, integrated meteorological-emission models and hybrid models (Jerrett et al., 2005).

Proximity models represent the most basic approach in assigning air pollution exposures using the assumption that distance from emission source proxies for actual exposure (Dijkema et al., 2011, Jerrett et al., 2005). Buffers can also be used to assign exposures where those within the buffer are exposed and those outside the buffers are non-exposed (Venn et al.,

2000, Kramer et al., 2010). Interpolation models attempt to estimate air pollution at sites other than the locations of monitoring stations. Estimates are usually obtained at a grid centre, imposed over the study area and used to establish a continuous surface pollutant concentration (Jerrett et al., 2005). A commonly applied geostatistical technique is kriging (Jerrett et al., 2001) which supplies the best linear unbiased estimate of the variable's value at any point in the study area (Pikhart et al., 2001, Coogan et al., 2012).

Dispersion models estimate spatial air pollutant concentrations relying on Gaussian equations and using data on air pollution concentrations, meteorology and emissions. Air pollution data used for model calibration are usually obtained from monitoring stations; meteorological data provide information on wind speed, direction and temperature; emissions data include local-source emissions and traffic-based emissions (Clench-Aas et al., 1999, Liu et al., 2007, Andersen et al., 2012). Land-use regression models predict air pollution levels based on the land use and traffic characteristics at a given site. This method provides a practical approach to estimating traffic-related air pollution, using measured pollution concentration at a specific location as the response variable and land use types around the same location as the predictors of the measured locations (Hoek et al., 2008, Kramer et al., 2010, Puett et al., 2011). Land-use regression models thus rely on additional measurement campaigns.

Integrated meteorological-emission models use chemical modules to simulate the dynamics of atmospheric pollutants. These models have high implementation costs, are useful for areas lacking comprehensive meteorological data and combine information on meteorology, chemistry transport, visualization and analysis to estimate air pollutant concentrations (Chen and Dudhia, 2001). Hybrid models combine personal or regional exposure monitoring data with other exposure modeling data (Liu et al., 1997, Zmirou et al., 2002) or combine different exposure models to optimize individual exposure estimates (Liu et al., 2012).

1.2.2. Health effects of air pollution

Inhalation of air pollutants induces local pulmonary inflammation and oxidative stress and may generate reactive oxygen species at the airway (Ghio and Cohen, 2005, Moller et al., 2010). Sustained exposure could lead to pulmonary inflammation resulting in the activation of pro-inflammatory cytokines, transcription factors and chemokines through various signaling pathways (Brook et al., 2010). Pulmonary oxidative stress and inflammation resulting from exposure to air pollutants have been shown to extend into the cardiovascular system (Ghio et al., 2000, Gurgueira et al., 2002, Brook et al., 2010).

Health impacts of short-term exposure to air pollution

Epidemiological short-term impact health studies relying on relationships between hourly or daily air pollution measures and health outcomes measured at comparable intervals have demonstrated positive relationships between air pollution exposure and daily hospitalizations (due to symptom exacerbations) and mortality due to pulmonary and cardiovascular morbidities (Brook et al., 2010, Ruckerl et al., 2011). Short-term exposure to air pollution was shown to increase blood pressure and triggering strokes and myocardial infarctions (Brook et al., 2010, Ruckerl et al., 2011). There is strong evidence for an increase in serum inflammatory markers [which may mediate air pollution-related morbidity] on short-term exposure to air pollution (Li et al., 2012, Tsai et al., 2012).

Health impacts on long-term exposure to air pollution

Long-term exposure to air pollution has been similarly associated with all-cause mortality and mortality due to cardiopulmonary causes (Brunekreef et al., 2015, Beelen et al., 2014). Chronic exposure to air pollution was associated with worsening of lung function in apparently healthy individuals, and individuals with asthma (Ruckerl et al., 2011, Gehring et al., 2013, Adam et al., 2015). In adults, a positive association between chronic exposure to air

pollution and incident asthma was reported (Perez et al., 2010). In children, air pollution exposure was positively associated with impaired lung function improvement that led to attenuation of age-dependent improvements in lung function (Gauderman et al., 2004, Gehring et al., 2013) and in neonates, an inverse relationship was observed between birth weight and long-term PM exposure (Pedersen et al., 2013). Incidence of coronary artery events and lung cancer were also linked to long-term exposure to PM in adults (Cesaroni et al., 2014, Raaschou-Nielsen et al., 2013). Long-term exposure to air pollutants was also shown to impact on several inflammatory biomarkers (Li et al., 2012, Mostafavi et al., 2015).

Modifying effects of genetic variations on the health impacts of air pollution

Functional genetic variants regulating the pathways through which air pollutants exert their effects on the cardiopulmonary system can modify an individual's susceptibility. Most of the available evidence is on the modifying effect of oxidative stress-related variants on the relationship between air pollutants and cardiovascular (Zanobetti et al., 2011) and respiratory outcomes (Minelli et al., 2011, Curjuric et al., 2012, Curjuric et al., 2010).

The degree of reduction in markers of heart rate variability, in relation to air pollutants, was associated with deletions in *GSTM1* (Chahine et al., 2007), and long GT repeats of *HMOX-1* (Schwartz et al., 2005). This modification was also observed for carriers of wild type *HFE* (Park et al., 2006), *cSHMT* (CC) (Baccarelli et al., 2008) and IL6-572G>C (GG) (Adam et al., 2014). GEI studies examining the modifying effect of variants [in *APOE*, *VEGF* and *LPL*] acting on autonomic function through lipid/endothelial metabolism pathway also reported a modifying effect of these variants on the relationship between $PM_{2.5}$ and heart rate variability (Ren et al., 2010). Other reported GEI on air pollutants and cardiovascular outcomes include a modifying effect of variants of *PHF11*, *MMP1*, *ITRP2* (Wilker et al., 2009), *DICER*, *GEMIN3, GEMIN4* and DGCR8 (Mordukhovich et al., 2009) on the association of diastolic and systolic blood pressure with $PM_{2.5}$; variants of *GSS* on the association of QT interval with

NO_2, black carbon and carbon monoxide (Baja et al., 2010); and variants of *AGTR1* and *ALOX5* on the association of left ventricular mass with residential proximity to major highways (Van Hee et al., 2010).

Similarly, in the respiratory system, a stronger effect on lung function in adults was observed with ozone among carriers of combined *NQOI* wild-type/*GSTM1* null genotype (Bergamaschi et al., 2001, Chen et al., 2007). Carriers of the *GSTP1* (Ile105Val) and long GT repeats on *HMOX-1* also had stronger reductions in lung function in response to O_3 exposure (Chen et al., 2007, Alexeeff et al., 2008). In another study, homozygotes of wild-type *NQOI* showed higher susceptibility to NO_2 in relation to asthma (Castro-Giner et al., 2009). In children, homozygotes of *GSTP1* (Ile105Val) showed higher susceptibility to NOx and sulphates for prevalent asthma (Lee et al., 2004); to O_3, NOx and diesel exhaust particles for wheezing (Romieu et al., 2006, Melen et al., 2008, Schroer et al., 2009); to proximity to major road for asthma (Salam et al., 2007), and to ozone and PM for incident asthma (Islam et al., 2009). Also, *GSTM1* variants were reported to modify relationships between traffic proximity and asthma symptoms in children (Salam et al., 2007). The Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults [SAPALDIA] provided first evidence on genome-wide interactions between air pollution and lung function decline. The strongest interaction signal was found for a SNP in *CDH13*, a gene involved in adiponectin metabolism (Imboden et al., 2015).

There is lack of evidence on the interactions between exposure to air pollution and genetic variations on metabolic outcomes including T2D and its intermediate phenotypes.

1.3. Air pollution and Type 2 diabetes: state of knowledge

Following the evidence supporting the inflammatory role of air pollution exposure on cardiopulmonary morbidities, it was thought that there could be an extension to the metabolic

morbidities including obesity and T2D. Cardiovascular morbidities and T2D share risk factors and constitute the metabolic syndrome, a pro-inflammatory condition characterized by a background of insulin resistance. These interrelationships led to the hypothesis that exposure to air pollution may contribute to the development of T2D. The following sections summarize the state of knowledge at the commencement of this work

1.3.1. Potential mechanisms: Evidence from experimental studies

Experimental evidence suggests that PM activates the innate immunity leading to production of inflammatory cytokines and oxidative products which may get into systemic circulation and create an inflammatory state (Rajagopalan and Brook, 2012, Liu et al., 2013). $PM_{2.5}$ was shown to mediate endothelial dysfunction in both humans and animals (Mills et al., 2005, Sun et al., 2005) through impairments in the phosphatidyl inositol 3-kinase-Akt-endothelial nitric oxide synthase signaling (Sun et al., 2005). In conjunction with a high fat diet over 24 weeks, $PM_{2.5}$ increased fasting postprandial glucose levels, insulin levels and induced insulin resistance in animal models (Sun et al., 2009). Similar derangements in glucose and insulin sensitivity measures were observed in mice models exposed to $PM_{2.5}$ with or without high fat diet (Xu et al., 2010).

$PM_{2.5}$ exposure also led to increase in visceral adipose tissue macrophages characterized by increase in tumor necrosis factor-alpha, IL6 and decreased IL-10 gene expression (Sun et al., 2009), which are characteristic of type 2 diabetes (Rajagopalan and Brook, 2012). $PM_{2.5}$ exposure was also associated with oxidative stress in the visceral adipose tissues and increased phosphorylation of a key cytosolic subunit of NADPH oxidase, and p47 (Xu et al., 2010, Kampfrath et al., 2011). $PM_{2.5}$ exposure was associated with defective insulin signaling in the liver and decreased gluconeogenesis (Zheng et al., 2013). Pathophysiologic stress in the endoplasmic reticulum, as a result of exposure to $PM_{2.5}$ was also shown to be associated with abnormalities in glucose homeostasis and insulin resistance (Zheng et al., 2013, Laing et al.,

2010). A 10-moth exposure to $PM_{2.5}$ was associated with decreases in inter-scapular brown adipose tissue and mitochondrial size, accompanied by excess oxidative and nitrosative stress in brown adipose tissue. The expression of peroxisome proliferator-activated receptor gamma [PPARG] coactivator 1-alpha and uncoupling protein 1 in brown adipose tissue was decreased, suggesting a down-regulation of insulin sensitivity in adipose tissue (Xu et al., 2011b, Xu et al., 2011a).

1.3.2. Evidence from epidemiological studies

Short-term exposure to pollutants in Chile including PM_{10}, NO_2 among others, was associated with an increase in hospitalization risk due to acute diabetes complications (Dales et al., 2012). In Canadian mortality studies, short- and long-term exposures to PM and gases were associated with diabetes-related mortality (Goldberg et al., 2006, Brook et al., 2013). O'Neill et al. (2007) demonstrated a decreased vascular reactivity among diabetes patients compared to those without diabetes following increasing levels of $PM_{2.5}$, particle number, black carbon and sulphates over 6 days. Chuang et al. (2011) reported an increase in blood glucose and HbA1c levels in association with long-term exposure to PM_{10} among Taiwanese adults. In a study of children living in Iranian cities, a seven-day mean PM_{10} exposure was positively associated with homeostatic model assessment of insulin resistance (Kelishadi et al., 2009).

Lockwood (2002) presented one of the first evidence, through an ecological correlation study, for a potential direct link between industrial emissions and diabetes prevalence in the US. Since then, epidemiological evidence from various study designs and populations has continued to increase, however, with inconsistent findings. Existing epidemiological evidence comprises five longitudinal studies (Kramer et al., 2010, Andersen et al., 2012, Puett et al., 2011, Chen et al., 2013, Coogan et al., 2012), two cross-sectional studies (Brook et al., 2008, Dijkema et al., 2011) and one ecologic study (Pearson et al., 2010), with varying study

population, settings, markers and range of air pollution exposures, models for individual air pollution exposure assignment and analytic models.

1.3.3. Research Needs

More evidence is needed to confirm/ refute the hypothesis of a possible role of exposure to ambient air pollution in the development of T2D. The inconsistency in the epidemiological evidence summarized in section 1.3.2 warrants that a systematic quantitative synthesis is done, providing a precise summary estimate of the type 2 diabetes risk associated with exposure to air pollutants. This summary would also identify knowledge gaps for recommendation to future studies.

In addition, more well-designed studies with adequate control of potential confounders from better representative population samples, which have not been studied, are needed for the generalizability of any evidence. There is also need to understand the mechanism of air pollution effects on a population level, and confirming the translation of observed effects in animal models presented in section 1.3.1 into the human population. Because of the complex interrelationships between non-communicable diseases and their risk factors, well-designed population-based epidemiologic studies may uncover other pathways that will guide the understanding of the potential diabetogenic effects of air pollutants.

As demonstrated in sections 1.1.3 and 1.2.3, genotypes are important research instruments in understanding causalities and susceptibilities to environmental stressors. Until now, there is very limited evidence on the role of genetic variation in modifying the diabetogenic effects of exposure to air pollutants. The stability of genetic variation to confounders and the increasing availability of genetic information due to advancements in genotyping and reduced cost make the study of gene-environment interactions an ideal tool towards mechanistic understanding.

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PART II OBJECTIVES, METHODS & IMPLICATIONS FOR CURRENT RESEARCH

2. Objectives

2.1. General objective

In the light of the state of knowledge at the commencement of this work, on the possible role of air pollution in the development of T2D, and the need for more population-based epidemiologic evidence in establishing a direct link and contributing to mechanistic understanding of involved pathways, this work sets out to contribute to scientific knowledge on these relationships using various epidemiologic and statistical methods, making optimal use of the research potential of the well-characterized Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults [SAPALDIA] in answering the research questions.

2.2. Specific objectives

A. Investigating the relationship between long-term exposures to markers of ambient air pollution and T2D on a population level

1. To systematically synthesize published population-based epidemiologic evidence on the relationship between long-term exposure to air pollution and T2D. Results are presented in chapter 4

2. To assess the relationship between long-term exposure to air pollution and T2D within the SAPALDIA cohort. Results are presented in chapter 5

B. Understanding the mechanisms involved in the cardio-metabolic impacts of long-term exposure to ambient air pollution on a population level

3. To assess the relationship between long-term exposure to air pollution and metabolic syndrome, taking into account, the different pathway-dependent phenotypes. Results are presented in chapter 6

4. To assess the modifying effect of type 2 diabetes genetic variants - in a polygenic risk score approach, also reflecting the pathophysiologic pathways of T2D-on the relationship between long-term exposure to air pollutants and diabetes, in the SAPALDIA cohort. Results are presented in chapter 7

5. To assess the modifying effect of a functional variant on the pro-inflammatory *IL6* gene on the relationship between long-term exposure to air pollutants and type 2 diabetes, in the SAPALDIA cohort. Results are presented in chapter 8.

3. Methodology

This work answered the research questions presented in chapter 2 in two broad methodological parts which include a systematic review and meta-analysis and cross-sectional analyses using the SAPALDIA cohort. A general overview of the systematic review and meta-analysis, and the SAPALDIA cohort are presented in this chapter whereas the detailed objective-specific descriptions are presented in chapters 4-8.

3.1. Systematic review and meta-analysis

At the beginning of this work, we conducted a systematic review of the published literature for existing epidemiologic evidence on the relationship between exposure to ambient air pollution and T2D. This was done to identify existing knowledge and gaps, and to guide further research questions. We searched electronic databases- Medline, Embase and ISI web of knowledge- up to 29 April 2014 and hand-searched reference lists of identified relevant articles (Eze et al., 2015). This work included only studies in English reporting an association between any air pollutant and T2D or adult diabetes, and excluded studies not reporting an association with adult diabetes, or reporting association with diabetes mortality or intermediate end-points (Eze et al., 2015).

We synthesized the study characteristics, diabetes and air pollution definitions, and the reported crude and adjusted estimates of association between air pollutants and T2D. We pooled the effect estimates of similar pollutants, from the included population-based studies in meta-analysis, using both random- and fixed-effects after converting the exposures to a uniform scale to facilitate comparison (Eze et al., 2015). This work adhered to the preferred reporting items for systematic reviews and meta-analyses (Moher et al., 2010) and the meta-analysis of observational studies in epidemiology (Stroup et al., 2000) guidelines at every stage of the methodology and reporting of this work.

3.2. Description of the Swiss Cohort Study of Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA)

3.2.1. Study design

SAPALDIA was initiated in 1991 to study the health impacts of air pollution exposure in Swiss adults, randomly sampled from the general population (Martin et al., 1997). Participants were drawn from eight Swiss communities with varying degrees of urbanization and representing the diverse geographical and climatic characteristics of Switzerland. About 10,000 participants were enrolled in the baseline survey that focused on respiratory outcomes (Martin et al., 1997). At the first follow-up in 2001/2002, about 8000 participants participated (Ackermann-Liebrich et al., 2005). At this point, the survey was expanded to also include cardio-metabolic outcomes and consenting participants' blood was sampled into a bio bank for blood marker and genetic assays (Ackermann-Liebrich et al., 2005). Figure 4 shows the location of participants of the first follow-up survey, clustered around the eight SAPALDIA study areas- Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne and Wald.

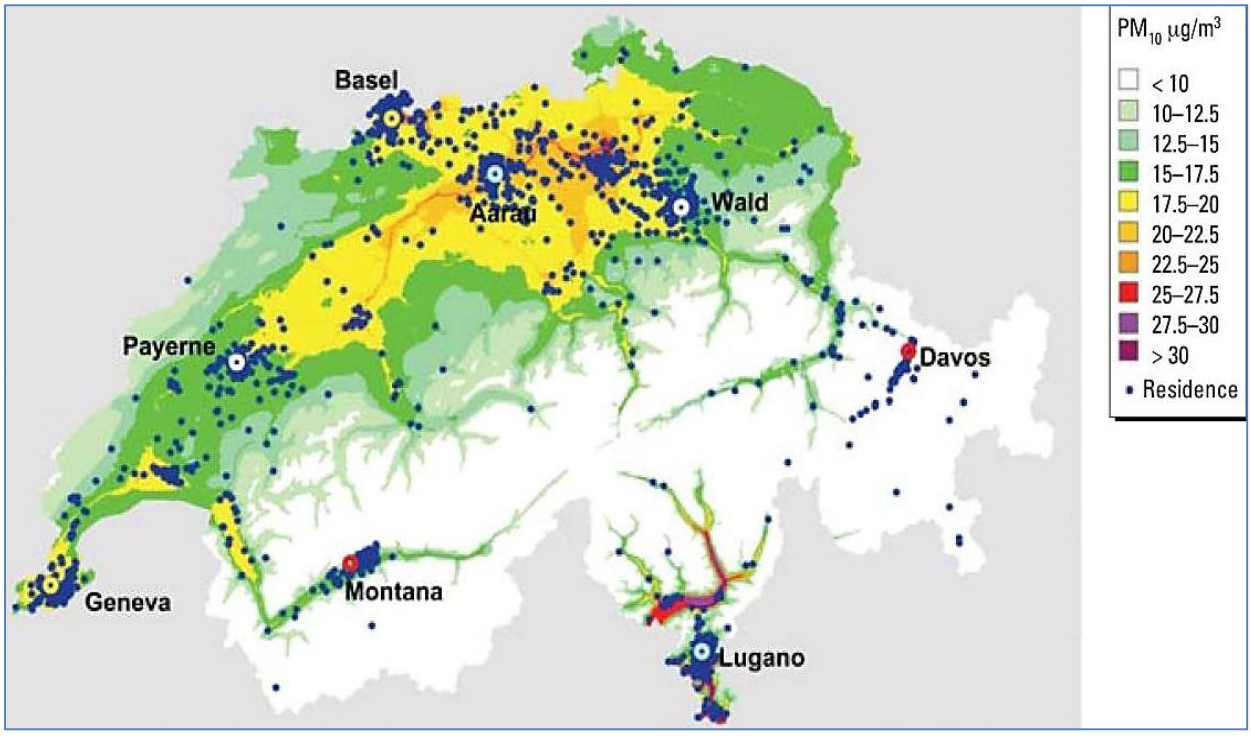

Figure 4: Location of first follow-up SAPALDIA participants in a background of PM_{10} (Liu et al., 2007)

The second follow-up survey took place in 2011/2012 with 60% of the baseline participants taking part and further contributing to the bio bank. In over 20 years of the SAPALDIA study, the main health focus has been to understand the development and progression of non-communicable diseases. Participants provided informed consent and ethical clearance was obtained from the Swiss national ethics committee and the ethics committees of the eight SAPALDIA areas. The SAPALDIA study flow is summarized in Figure 5.

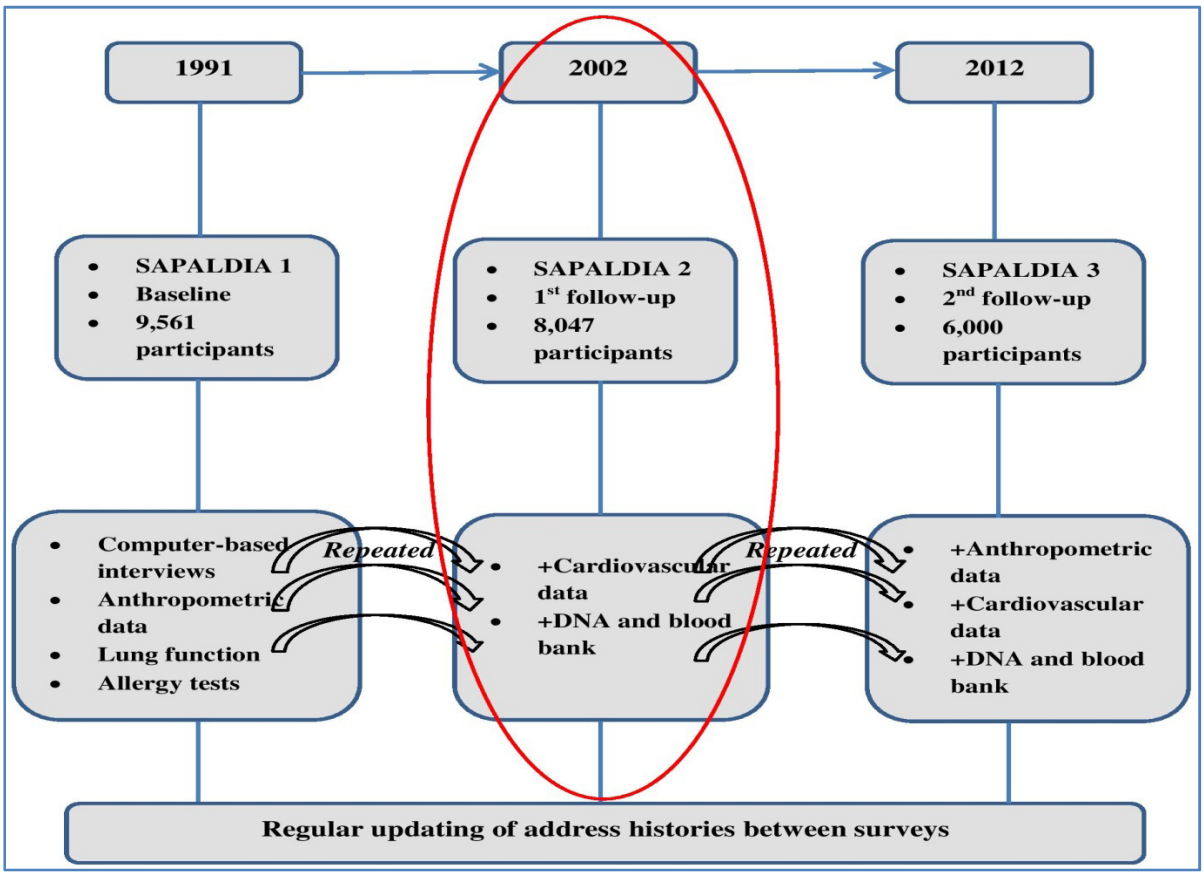

Figure 5: Basic characteristics of SAPALDIA cohort across 3 surveys

At the commencement of this work, data from SAPALDIA 3 were still being collected and cleaned, thus it was not feasible to use the second follow-up data. This study therefore focused on the first follow-up survey, indicated by the red circle, where all necessary information for answering the research questions was available.

3.2.2. Questionnaires and physical examination

At the first follow-up survey, participants had computer-assisted interviews on their socio-demographic, health and lifestyle characteristics. Questions included age, sex, educational attainment, occupation, detailed smoking history, and occupational exposure to vapours, dusts, gases and fumes, dietary history, alcohol consumption, detailed physical activity questions. Participants responded to questions about physician-diagnoses of several medical conditions including diabetes, hypertension, asthma, dyslipidaemia and their medication use. An overview of the questionnaires used in this survey can be found in Appendix 3. Physical examination included height, weight, blood pressure, lung function among others. Non-fasting glucose, HbA1c [in a subset of participants] and lipids were among the measured blood markers (Ackermann-Liebrich et al., 2005).

3.2.3. Air pollution modelling for assignment of exposures

PM_{10} and NO_2 were modelled to participants' residences in 1990 and 2000 using PolluMap, a Gaussian dispersion model which predicted mean annual pollutant concentration in both years. This model applied emission inventories [from traffic, agriculture, construction, household, commercial and industrial sources] topography, height of source and meteorological data (SAEFL, 2003). Air pollutant maps of the two years were temporally interpolated based on temporal trends at monitoring stations across Switzerland. Estimates of individual residential exposure for each intermediate year were obtained by combining interpolated pollutant maps and residential histories of participants (Liu et al., 2007). Since the dispersion model did not predict NO_2 as well as it predicted PM_{10} in non-traffic sites, a hybrid model, incorporating land-use regression was applied to optimize the prediction of NO_2 (Liu et al., 2012). Individual long-term pollutant exposure assignment is schematically depicted in Figure 6 below

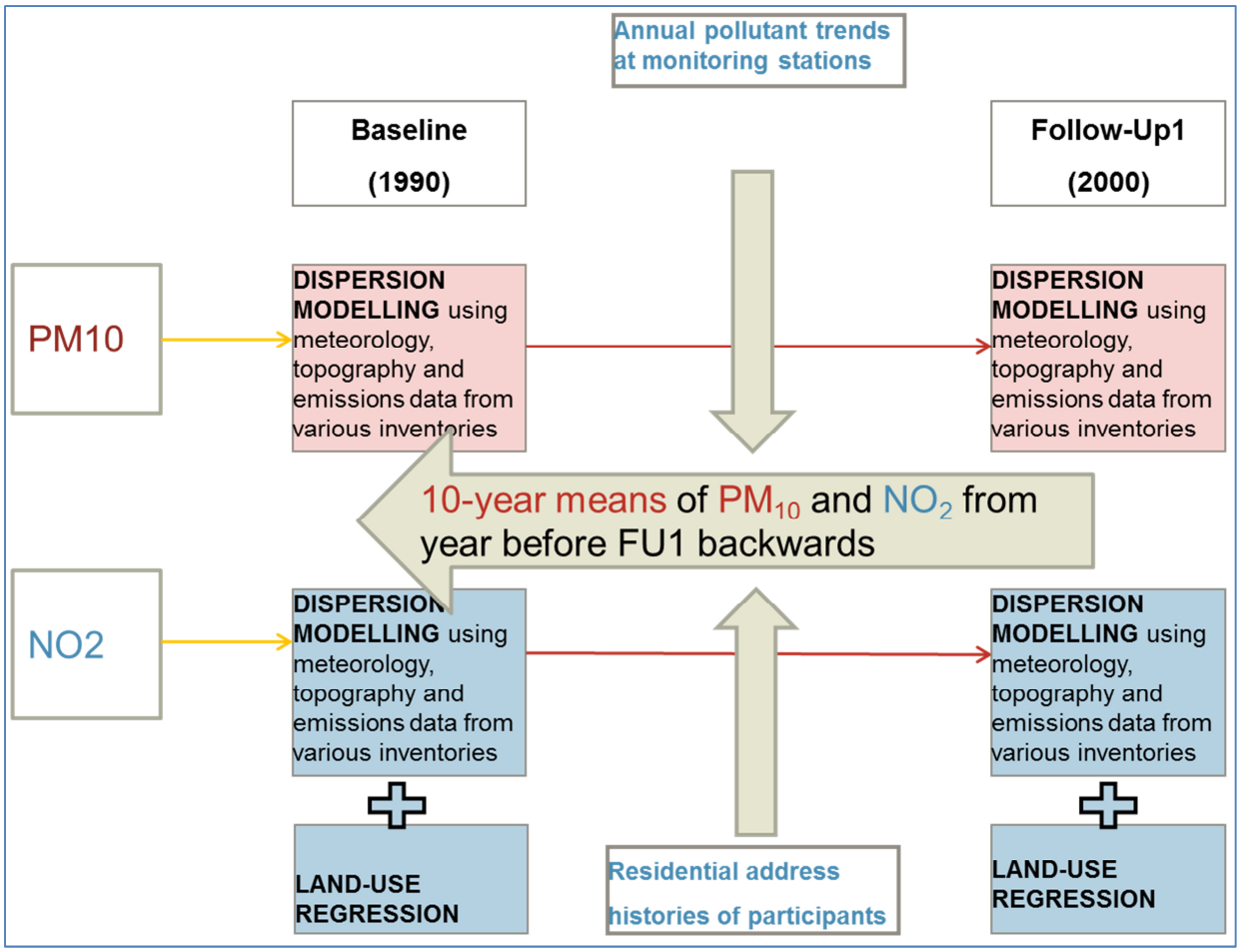

Figure 6: Schematic representation of individual long-term exposure assignment for present study

3.2.4. Genotyping and genetic data

Genotyping of some candidate genes was done on DNA extracted from EDTA-buffered blood samples by 5' nuclease real-time PCR in dedicated laboratories in Zurich, Pavia and Innsbruck in about 6000 participants who consented to genetic testing (Adam et al., 2014).

In the framework of the GABRIEL study, a large collaborative study to investigate gene and environment interactions in asthma, about 1600 participants (asthma cases and controls) had whole genome genotyping using Human Illumina610-Quad bead chip (Moffatt et al., 2010). This yielded ~570,000 polymorphisms following quality control which included tests on Hardy-Weinberg equilibrium, genotyping call rate and minor allele frequencies (Moffatt et

al., 2010). The genotyping data was subjected to 1000 genome imputation resulting in the imputation of >29 million SNPs (Artigas et al., 2015).

3.2.5. Implications for present research

- As previously stated in section 3.2.1, this work focused only on SAPALDIA 2, the first follow-up survey due to the availability, at this survey, of all necessary data to contribute to scientific knowledge on the relationship between long-term exposure to air pollution and type 2 diabetes

- This implies that this work could not study incident type 2 diabetes

- All diabetes cases were assumed to be type 2 since >90% of adult diabetes is of type 2 (Alberti and Zimmet, 1998).

- Participants were identified as having diabetes if they responded 'yes' to a physician diagnosis of diabetes or reported use of diabetes medication. We identified undiagnosed cases if they had a non-fasting glucose ≥11.1 mmol/L or HbA1c≥0.065.

- Clusters of cardio-metabolic symptoms (diabetes or impaired fasting glycaemia; hypertension; dyslipidaemias and central obesity) were identified among participants and metabolic syndrome was diagnosed according to WHO, IDF and NCEP-ATPIII criteria. Since waist circumference was not measured at first follow-up survey, but measured at second follow-up, we developed a validated prediction model among participants at the second follow-up and applied the residuals to the first follow-up survey to predict their waist circumference at first follow-up. This was to enable us to better capture central obesity instead of using only body mass index in identifying the cases.

- The markers of long-term exposure to air pollution were 10-year means of PM_{10} and NO_2 up to the year before the first follow-up survey. $PM_{2.5}$ and ultra-fine particles, which may exert stronger health effects due to their physical properties, were only measured at the second follow-up survey. $PM_{2.5}$ currently constitutes ~80% of the fraction of PM_{10} across SAPALDIA areas, so we would expect very similar findings with $PM_{2.5}$.

- This work applied mixed logistic regression models with random intercepts for the study areas across all research questions. This was to enable us generalize our findings to the whole of Switzerland. Random slopes by study areas were always tested as sensitivity analyses.

- There were variations in the studied populations for the various research questions answered in SAPALDIA. These variations were due to the requirements of the research questions. For instance, metabolic syndrome required the use of fasting samples, so we had to exclude participants who reported less than 4 hours fasting time before blood draw; the modifying effect of polygenic risk score required that participants come from the ~1600 who had whole genome genotyping whereas the *IL6* gene study required participants to come from the ~6,000 who were genotyped for some candidate genes. We applied inverse probability weighting and multiple imputations to assess potential selection bias in our findings.

- The above points and some additional points, specific to each research question, were discussed in great length in the individual articles. Sensitivity analyses were applied to explore the robustness of our findings, given any of the limitations.

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PART III INVESTIGATING THE RELATIONSHIP BETWEEN AMBIENT AIR POLLUTION AND TYPE 2 DIABETES

4. Article: Association between ambient air pollution and diabetes mellitus in Europe and North America: systematic review and meta-analysis.

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Association between Ambient Air Pollution and Diabetes Mellitus in Europe and North America: Systematic Review and Meta-Analysis

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Background: Air pollution is hypothesized to be a risk factor for diabetes. Epidemiological evidence is inconsistent and has not been systematically evaluated.

Objectives: We systematically reviewed epidemiological evidence on the association between air pollution and diabetes, and synthesized results of studies on type 2 diabetes mellitus (T2DM).

Methods: We systematically searched electronic literature databases (last search, 29 April 2014) for studies reporting the association between air pollution (particle concentration or traffic exposure) and diabetes (type 1, type 2, or gestational). We systematically evaluated risk of bias and role of potential confounders in all studies. We synthesized reported associations with T2DM in meta-analyses using random-effects models and conducted various sensitivity analyses.

Results: We included 13 studies (8 on T2DM, 2 on type 1, 3 on gestational diabetes), all conducted in Europe or North America. Five studies were longitudinal, 5 cross-sectional, 2 case–control, and 1 ecologic. Risk of bias, air pollution assessment, and confounder control varied across studies. Dose–response effects were not reported. Meta-analyses of 3 studies on $PM_{2.5}$ (particulate matter ≤ 2.5 μm in diameter) and 4 studies on NO_2 (nitrogen dioxide) showed increased risk of T2DM by 8–10% per 10-μg/m^3 increase in exposure [$PM_{2.5}$: 1.10 (95% CI: 1.02, 1.18); NO_2: 1.08 (95% CI: 1.00, 1.17)]. Associations were stronger in females. Sensitivity analyses showed similar results.

Conclusion: Existing evidence indicates a positive association of air pollution and T2DM risk, albeit there is high risk of bias. High-quality studies assessing dose–response effects are needed. Research should be expanded to developing countries where outdoor and indoor air pollution are high.

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Introduction

Ambient air pollution ranks high among risk factors for the global burden of disease (Lim et al. 2012), and is linked to several chronic noncommunicable conditions such as cardiovascular diseases (Bauer et al. 2010; Brook et al. 2010; Künzli et al. 2010), asthma (Bui et al. 2013; Jacquemin et al. 2012; Künzli et al. 2009), chronic obstructive pulmonary diseases (COPD) (Andersen et al. 2011; Schikowski et al. 2014; Zanobetti et al. 2008), and cancers including lung (Raaschou-Nielsen et al. 2013a), cervical, and brain cancers (Raaschou-Nielsen et al. 2011). Persons with type 2 diabetes mellitus (T2DM) are at increased risk to develop micro- and macrovascular diseases and reduced lung function (Jones et al. 2014; Kinney et al. 2014). Air pollution has also been shown to be more detrimental to diabetic patients, worsening their clinical outcomes (O'Neill et al. 2005; Raaschou-Nielsen et al. 2013b; Whitsel et al. 2009; Zanobetti and Schwartz 2001).

More recent evidence is supportive of an air pollution effect on diabetes risk. Experimental evidence show that possible pathways may include endothelial dysfunction, overactivity of the sympathetic nervous system (Rajagopalan and Brook 2012), immune response alterations in visceral adipose tissues; endoplasmic reticulum stress resulting in alterations in insulin transduction (Sun et al. 2009), insulin sensitivity, and glucose metabolism; and alterations in mitochondria and brown adipocytes (Liu et al. 2013; Rajagopalan and Brook 2012).

Papazafiropoulou et al. (2011) systematically reviewed the etiologic association between environmental pollution and diabetes, taking into account studies on organic pollutants and secondary effects of air pollution on diabetic patients published up to November 2010. They described a positive association between environmental pollution and prevalent diabetes, as well as increased morbidity and mortality among diabetic patients. A number of pertinent studies have been published since this review, and thus far there is, to the best of our knowledge, no meta-analysis of the available evidence. We therefore systematically identified and reviewed the epidemiological evidence on the association between air pollution and diabetes mellitus, and synthesized the results of studies on the association with T2DM.

Methods

Search strategy. We systematically searched electronic literature databases [MEDLINE (http://www.nlm.nih.gov/bsd/pmresources.html), EMBASE (https://www.embase.com), and ISI Web of Science (http://www.webofknowledge.com)] for pertinent literature published up to 3 February 2014. Terms used in this search included "air pollution," "air pollutants," "particulate matter," "PM_{10}," "$PM_{2.5}$," "nitrogen dioxide," "NO_2," "NO_x," "ozone," "soot," "smog," "diabetes mellitus," "diabetes," "T1DM," "T2DM," "type 1 DM," "type 2 DM," "IDDM," "NIDDM," alone and in combination. We applied no filters for study designs. Reference lists of eligible articles were searched for further pertinent articles. After de-duplication, titles and abstracts were screened for eligibility and potentially relevant articles were retrieved as full texts. Screening was performed independently by two reviewers and any discrepancies were resolved by discussion.

Inclusion and exclusion criteria. We included only original research published in English as a full publication in a peer-reviewed journal. We accepted any type of study design. In eligible studies, the definition of air pollution and diabetes mellitus

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had to be clearly stated. Air pollution had to be outdoor (ambient, including traffic-related), and we accepted any type of assessment including particle concentration in the air or indicators of long-term traffic exposure. Diabetes mellitus had to be physician diagnosed or based on the use of antidiabetic medications. We included any type of diabetes mellitus (type 1, type 2, and gestational). Eligible studies had to report quantitative measures of association between air pollution and diabetes mellitus, and their 95% confidence intervals (CIs) (or enough data to allow derivation of this association). We excluded studies that were based on the effect of blood markers, and not clearly defining clinical outcomes. Studies testing only whether diabetes status would modify the association between air pollution and health outcomes were not considered in this review. Animal studies were excluded.

For the meta-analysis, only studies on individual type 2 diabetes risk were included. We included all studies that quantified particle concentrations as "per ... μg/m^3" or "ppb." If the diabetes type was not clearly stated, we considered diagnoses of diabetes in nonpregnant adults (≥ 18 years age) as diagnoses of T2DM because > 90% of new diagnoses of adult diabetes is type 2 diabetes (Alberti and Zimmet 1998).

Data extraction. We extracted the following data from the eligible studies: year of study, study setting, study design, year of publication, population demographics, study definition of diabetes and assessment of air pollution exposure, confounder adjustments, and effect modification assessments. We extracted data on the effect estimates (unadjusted and final model) of the association (and their 95% CIs) between air pollution and diabetes.

Data were extracted independently by two reviewers and disagreements were resolved by discussion.

Meta-analysis. We used random-effects models to synthesize the associations between air pollution and T2DM (Lau et al. 1997). Random-effect models give more weight to smaller studies and have typically wider CIs because in addition to the within-study variance, they also consider potential variation between the true effects that all included studies estimate. We used fixed-effects models (which assume that all studies share a common true effect) in a sensitivity analysis.

We used risk ratios as measure of association across all studies. When hazard ratios and incidence risk ratios were reported, we directly considered them as risk ratios. Because diabetes is not very common, we considered reported odds ratios as equivalent to risk ratios. For studies with estimates of association from multiple particle concentration sources, we chose the estimates modelled at participants' residences (land-use regression, kriging, or satellite-based estimates). We used the effect estimates reported by the study authors as "main model" or "fully adjusted model." We used estimates of association and their standard errors reported as "per 10 μg/m^3" of exposure and we converted other reported quantities or units where necessary.

We described the between-study heterogeneity using the I^2 metric and the between-studies' variance using Tau^2. We assessed publication bias using the Egger's test for asymmetry (Egger et al. 1997). We conducted sensitivity analyses including only studies that *a*) measured air pollution exposure before DM diagnosis, *b*) comprised both males and females, and *c*) were longitudinal, and we applied a fixed-effects analysis. All analyses were performed with Stata version 12 (StataCorp, College Station, TX, USA) using the "metan" command. p-Values were two-tailed and $p < 0.05$ was considered nominally statistically significant.

For reporting, we followed the Meta-analysis Of Observational Studies in Epidemiology (Stroup et al. 2000) and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (Moher et al. 2010) guidelines.

Results

The database search yielded 636 records after de-duplication, which were screened on title/abstract level for eligibility (Figure 1). Sixteen potentially eligible articles were screened on full-text level, and 3 were excluded (Figure 1). Thirteen studies were included (Table 1). There were 5 longitudinal studies (Andersen et al. 2012; Chen et al. 2013; Coogan et al. 2012; Krämer et al. 2010; Puett et al. 2011), 5 cross-sectional studies (Brook et al. 2008; Dijkema et al. 2011; Fleisch et al. 2014; Malmqvist et al. 2013; van den Hooven et al. 2009), 2 case–control studies (Hathout et al. 2002, 2006), and 1 ecologic study (Pearson et al. 2010). Two studies were on type 1 diabetes (Hathout et al. 2002, 2006); 3 studies on gestational diabetes (GDM) (Fleisch et al. 2014; Malmqvist et al. 2013; van den Hooven et al. 2009), and 8 studies on T2DM (Andersen et al. 2012; Brook et al. 2008; Chen et al. 2013; Coogan et al. 2012; Dijkema et al. 2011; Krämer et al. 2010; Pearson et al. 2010; Puett et al. 2011). Seven non-ecological studies on T2DM were selected for quantitative synthesis (with the exclusion of Pearson et al. 2010). Air pollution estimates from these studies were based on land-use regression (Andersen et al. 2012; Brook et al. 2008; Dijkema et al. 2011; Krämer et al. 2010; Puett et al. 2011), kriging (Coogan et al. 2012), and satellite-derived estimates (Chen et al. 2013). All studies were conducted in Europe or North

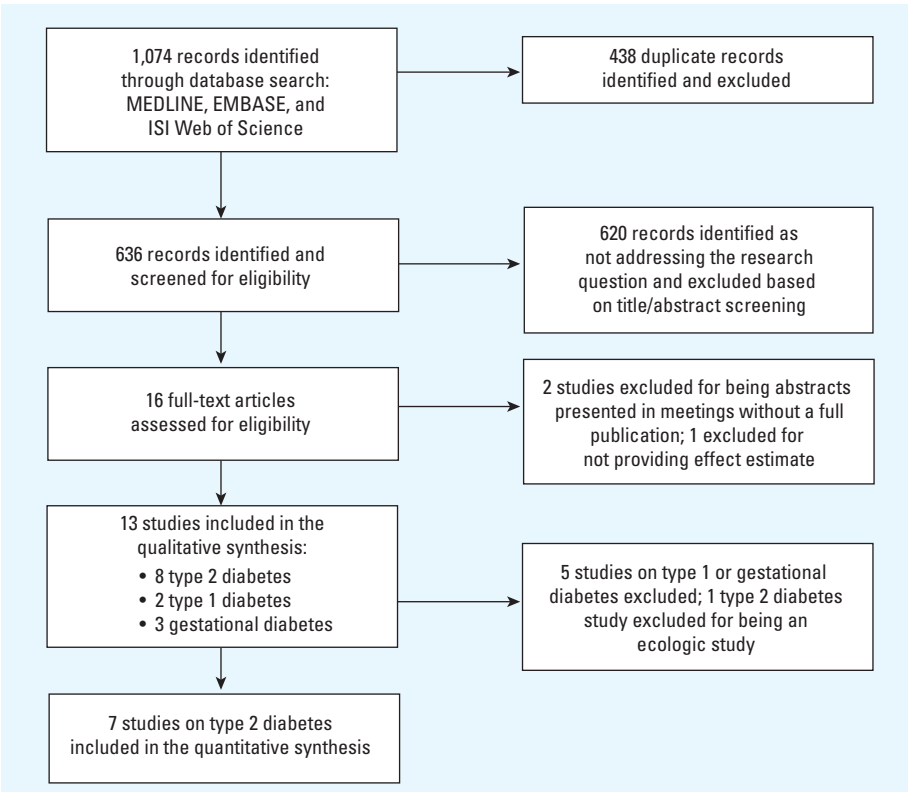

Figure 1. Results of systematic literature search.

America. Tables 1 and 2 and Supplemental Material, Table S1, provide an overview of the 13 eligible studies. Table 3 summarizes the data reported in the studies synthesized in meta-analyses.

In the Supplemental Material, Table S2 provides an overview of potential sources of bias and how they were assessed by the 13 studies. These are discussed in detail below.

Bias due to outcome assessment. As shown in Table 2, some studies relied on self-reported, physician-diagnosed DM (Coogan et al. 2012; Dijkema et al. 2011; Krämer et al. 2010), whereas others linked participants to established databases to identify cases (Andersen et al. 2012; Brook et al. 2008; Chen et al. 2013; Hathout et al. 2002, 2006; Malmqvist et al. 2013). Additional steps were taken by some studies with self-reported outcomes to test the validity of the DM diagnosis. These steps included sending a follow-up questionnaire with the same questions about diabetes (Krämer et al. 2010) and confirmation from medical records provided by physicians (Coogan et al. 2012). Dijkema et al. (2011) further tested participants who did not report physician-diagnosed diabetes, to identify undiagnosed cases.

Bias due to exposure assessment. The reviewed studies used different approaches to assess exposure of participants to air pollution, including modeled concentrations of various particulate matters, nitrogen oxides (NO_x), sulfates, ozone, and various proxies to estimate traffic-related pollution, with varying buffer levels. The studies are also heterogeneous with regard to the lag time considered for exposure assessment. Only the Danish cohort (Andersen et al. 2012) assessed the impact of different lag times, albeit with little evidence for substantial differences in effects (see Supplemental Material, Table S1). In the absence of a biological basis for the latency between exposure and diagnosis of diabetes, different lag times should be tested. Overall, the diversity of exposure measurement makes it difficult to compare the reported effect estimates across these studies.

Bias due to confounder adjustment. **Indoor air pollution and smoking.** Beyond adjustment for basic DM risk factors at baseline (see Supplemental Material, Table S2), Krämer et al. (2010) also adjusted for environmental tobacco smoke (ETS), indoor heating with fossil fuels, as well as occupational exposure to dust, fumes and extreme temperatures; Andersen et al. (2012) also adjusted for ETS. One study done in children considered ETS exposure (Hathout et al. 2006).

Demographics, physical activity, and dietary factors. The longitudinal studies uniformly adjusted for age, body mass index (BMI), and sex (when study population includes both sexes). The studies on women did not adjust for dietary factors, and all longitudinal studies but one adjusted for alcohol consumption and physical activity (see Supplemental Material, Table S1). The other studies assessed confounding by age and BMI except the case–control studies, which did not consider the children's BMI in their models. The GDM studies mostly considered maternal alcohol consumption (but not dietary factors) whereas the cross-sectional T2DM studies did not consider either factor (see Supplemental Material, Table S1).

Socioeconomic status. There was a uniform adjustment for socioeconomic status in all studies, although on different scales. At the individual level, educational attainment as a socioeconomic determinant was most commonly used across studies, and a few studies additionally considered household income and ethnicity (see Supplemental

Table 1. Characteristics of the studies on the relationship between air pollution and diabetes mellitus.

Source	Location	Years of study	Study design and duration of follow-up	Population (*n*) and age (years) of participants
Krämer et al. 2010[a]	Ruhrgebiet, Germany	1990–2006	Longitudinal: Study on the Influence of Air Pollution on Lung Inflammation and Aging Follow-up: 16 years	*n* = 1,775 Caucasian women without T2DM at baseline, 54–55 years
Andersen et al. 2012[a]	Copenhagen and Aarhus, Denmark	(1993–1997) –2006	Longitudinal: Danish Diet, Cancer and Health cohort Follow-up: 9.7 years	*n* = 51,818 Caucasians without DM at baseline, 50–65 years
Puett et al. 2011[a]	Metropolitan Statistical Areas (MSA) in north-eastern and midwestern states of USA	1989–2009	Longitudinal, with 2 cohorts: Nurses' Health Study and Health Professionals Follow-up Study Follow-up: 20 years	*n* = 74,412 female nurses 30–55 years and 15,048 male health professionals 40–75 years, without T2DM at baseline
Coogan et al. 2012[a]	Los Angeles, California, USA	1995–2005	Longitudinal: Black Women's Health Study Follow-up: 10 years	*n* = 3,992 African-American women, without DM at baseline and 21–69 years
Chen et al. 2013[a]	Ontario, Canada	(1996–2005) –2010	Longitudinal Follow-up: 8 years	*n* = 62,012 Canadians without DM, ≥ 35 years
Brook et al. 2008[a]	Hamilton and Toronto, Ontario, Canada	1992–1999	Cross-sectional	*n* = 7,634 patients who attended two respiratory clinics in Hamilton and Toronto, ≥ 40 years
van den Hooven et al. 2009	Rotterdam, Netherlands	2002–2006	Cross-sectional: Generation R study	*n* = 7,399 pregnant women, who had delivery date in the study period, 21–38 years
Dijkema et al. 2011	Westfriesland, Netherlands	1998–2000	Cross-sectional: Hoorn Screening Study for T2DM	*n* = 8,018 Caucasian residents, 50–75 years
Malmqvist et al. 2013	Scania, Sweden	1999–2005	Cross-sectional: The Swedish Medical Birth Registry.	*n* = 81,110 women who had singleton deliveries during the study period
Hathout et al. 2006	California, USA	2002–2003	Case–control Follow-up: retrospectively from birth until diagnosis of T1DM	*n* = 402 children (102 with T1DM and 300 age-matched controls), 1–12 years, receiving care at Loma Linda University Pediatric Center
Hathout et al. 2002	California, USA	2002	Case–control Follow-up: retrospectively from birth until diagnosis of T1DM	*n* = 100 children (61 cases: 30 had onset ≤ 5 years and 31 > 5 years) (39 age-matched controls: 19 were ≤ 5 years and 20 were > 5 years) receiving care at Loma Linda University Pediatric Center
Fleisch et al. 2014	Boston, Massachusetts, USA	1999–2002	Cross-sectional: Project Viva Cohort	*n* = 2,093 second-trimester pregnant women without known diabetes
Pearson et al. 2010	USA	2004–2005	Ecologic	*n* = 3,082 counties of USA

Abbreviations: T2DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.
[a]Included in meta-analysis.

Material, Table S1). Few studies considered spatial socioeconomic confounding in forms of unemployment rate, urban/rural residence, neighborhood income and neighborhood socioeconomic status score (see Supplemental Material, Table S1). Overall, there was sufficient consideration for individual-level socioeconomic status, but the insufficient control of area-level socioeconomic status may increase the risk of bias.

Co-morbidities. Some co-morbidities associated with diabetes may also be associated

Table 2. Exposure and outcome definitions.

Source	Outcome	Definition of outcome	Exposure	Definition of exposure	Exposure estimates
Krämer et al. 2010[a]	Incident T2DM	Self-reported, physician-diagnosed T2DM	PM_{10}, PM, $PM_{2.5}$, NO_2, and traffic exposure	5-year means of PM_{10} and NO_2 in an 8-km grid from monitoring stations, before baseline	Median (25th–75th percentile) Monitoring stations (μg/m^3): PM_{10}: 46.9 (44–54.1) NO_2: 41.7 (23.3–48.2)
				Traffic PM and NO_2 in a 1-km grid, in 1 year, from emission inventory	Traffic emission inventory (tons/year/km^2): PM: 0.54 (0.22–1.09)
				Traffic $PM_{2.5}$ and NO_2[b] from a (1-year measurement) LUR model. Distance from the next major road with > 10,000 cars per day	NO_2: 12 (5.4–24.4) LUR soot (10^{-5} m): 1.89 (1.67–2.06) NO_2 (μg/m^3): 34.5 (23.8–38.8) % participants living < 100 m from busy road: 15.8
Andersen et al. 2012[a]	Incident DM	Confirmed DM cases from the Danish National Diabetes Register	NO_2, NO_x, traffic exposure	35[b]- and 15-year mean levels of NO_2 and NO_x, from the Danish AirGIS model before baseline	Median (IQR) 35-year NO_2 and NO_x (μg/m^3): 14.5 (4.9) and 20.9 (11.4) 15-year NO_2 and NO_x (μg/m^3): 15.3 (5.6) and 22.1 (12)
				1-year mean NO_2 and NO_x at baseline	1-year NO_2 and NO_x at baseline (μg/m^3): 15.4 (5.6) and 20.3 (10.9)
				1-year mean NO_2 and NO_x at follow-up	1-year NO_2 and NO_x at follow-up (μg/m^3): 15.2 (5.7) and 21.5 (12)
				Major road (with annual traffic density of ≥ 10,000) within 50 m of residence.	% major road within 50 m: 8.1
				Traffic load within 100 m of residence (10^3 vehicles/km/day)	Traffic load within 100 m (10^3 vehicles/km/day): 0.34 (1.3)
Puett et al. 2011[a]	Incident T2DM	DM according to the National Diabetes Data Group Criteria[c]	$PM_{2.5}$, PM_{10}, $PM_{10-2.5}$	Average $PM_{2.5}$[b], PM_{10}, and $PM_{10-2.5}$ concentrations, from LUR model, 12 months before diagnosis	Mean ± SD $PM_{2.5}$ (μg/m^3): 18.3 ± 3.1 for HPFS and 17.5 ± 2.7 for NHS PM_{10} (μg/m^3): 28.5 ± 5.5 for HPFS and 26.9 ± 4.8 for NHS $PM_{10-2.5}$ (μg/m^3): 10.3 ± 3.3 for HPFS and 9.4 ± 2.9 for NHS
Coogan et al. 2012[a]	Incident T2DM	Self-reported, physician-diagnosed T2DM	$PM_{2.5}$, NO_x, traffic exposure	1-year mean $PM_{2.5}$[b] during follow-up, assigned by kriging model	Mean ± SD $PM_{2.5}$ (μg/m^3): 20.7 ± 2.1 Median (25th–75th percentile) $PM_{2.5}$ (μg/m^3): 21.1 (20.3–21.6)
				1-year mean NO_x the year after follow-up, assigned by LUR model	Mean ± SD NO_x (ppb): 43.3 ± 11 Median (25th–75th percentile) NO_x (ppb): 41.6 (36.9–49.2)
Chen et al. 2013[a]	Incident DM	Physician-diagnosed DM from Ontario database	$PM_{2.5}$	6-year mean $PM_{2.5}$[b] during baseline/follow-up, obtained from satellite-based estimates at 10 x 10 km resolution	Mean (range) $PM_{2.5}$ (μg/m^3): 10.6 (2.6–19.1)
Brook et al. 2008[a]	Prevalent DM	Physician-diagnosed DM from Ontario Health Insurance Plan and Ontario Health Discharge Database	NO_2	NO_2[b] assigned by LUR models developed from mean field measurements within 3 years, from Hamilton and Toronto, Ontario, Canada	Median (25th–75th percentile) NO_2 (ppb) Males: Hamilton: 15.2 (13.9–17.1); Toronto: 23 (20.8–25) Females: Hamilton: 15.3 (14–17); Toronto: 22.9 (20.8–24.7)
van den Hooven et al. 2009	Prevalent gestational DM (GDM)	GDM diagnosed according to the Dutch midwifery and obstetric guidelines	Traffic exposure	Distance-weighted traffic density (DWTD) within a 150-m radius around residence (vehicles/24 hr × m)	Median (P25–P75) DWTD (vehicles/24 hr × m): 5.5×10^5 ($1.6 \times 10^5 - 1.2 \times 10^6$)
				Proximity to a major road (> 10,000 vehicles/day)	Proximity to a major road (m): 143 (74–225)
Dijkema et al. 2011	Prevalent T2DM	Self-reported physician-diagnosed T2DM. Laboratory-based diagnosis for undetected cases	NO_2, traffic exposure	1-year mean NO_2 assigned by LUR model	Median (25th–75th percentile) NO_2 (μg/m^3): 15.2 (14.2–16.5)
				Distance to the nearest main road (≥ 5,000 vehicles/day)	Distance to nearest main road (m): 140 (74–220)
				Traffic flow at the nearest main road (vehicles/24 hr)	Traffic flow at the nearest main road (10^3 vehicles/24 hr): 7.31 (5.87–9.67)
				Total traffic per 24 hr on all roads within a 250-m circular buffer around the address	Traffic within 250-m buffer (10^3 vehicles/24 hr): 680 (516–882)
Malmqvist et al. 2013	Prevalent GDM	GDM as defined in the Swedish Medical Birth Registry	NO_x, traffic exposure	Monthly and trimester means of NO_x assigned by dispersion modeling at a spatial resolution of 500 × 500 m over the duration of the pregnancy	Quartiles of NO_x exposure (μg/m^3): Q1: 2.5–8.9 Q2: 9.0–14.1 Q3: 14.2–22.6 Q4: > 22.7
				Traffic density within a 200-m radius	Categories of traffic density within 200 m (vehicles/min): 1: no road 2: < 2 3: 2–5 4: 5–10 5: > 10

Table continued

with air pollution. These co-morbidities may include hypertension, myocardial infarction, stroke, asthma, and chronic obstructive pulmonary disease (Brook et al. 2010; Pelle et al. 2012; Vojtková et al. 2012). The longitudinal studies considered some of these co-morbidities (see Supplemental Material, Table S1). Participants with co-morbidities were not excluded from any T2DM study.

Effect modification. Several studies reported stronger effects in women compared with men (Andersen et al. 2012; Brook et al. 2008; Chen et al. 2013; Dijkema et al. 2011). Other subgroups reported with potentially increased susceptibility include subjects with low education (Andersen et al. 2012; Chen et al. 2013; Krämer et al. 2010), COPD (Andersen et al. 2012; Chen et al. 2013), asthma (Andersen et al. 2012), higher waist-to-hip ratio (Andersen et al. 2012), and higher level of subclinical inflammation (Krämer et al. 2010), nonsmokers (Andersen et al. 2012), and subjects < 50 years or > 65 years of age (Chen et al. 2013) (see Supplemental Material, Table S1). No study assessed interaction between different air pollutants, air pollutants and noise, or interaction between air pollutants and genetic polymorphisms.

Loss to follow-up. Losses to follow-up and healthy survivor bias present common problems in epidemiological studies. Puett et al. (2011) reported a loss of < 10% in both studied cohorts over 20 years of follow-up, and Coogan et al. (2012) reported < 20% loss of cohort over 10 years of follow-up. The other longitudinal studies did not report losses to follow-up. None of the studies included sensitivity analyses to estimate the effect of the healthy survivor bias.

Publication bias. Although selective reporting and publication bias cannot be ruled out, considering a high probability that negative findings will not be published, we found no indication for such sources of bias (p-value of Egger's test > 0.2). Some studies reported negative findings. However, most studies had several markers of air pollution available, and it remains unclear if some markers have been measured but not reported, so some selective reporting may have occurred.

Table 2. *Continued.*

Source	Outcome	Definition of outcome	Exposure	Definition of exposure	Exposure estimates
Hathout et al. 2006	Prevalent T1DM	Physician-diagnosed T1DM from the database of Loma Linda University Pediatric Center	O_3, NO_2, SO_2, SO_4, and PM_{10}	Average monthly pollutant exposure (obtained from the U.S. EPA and California Air Resources Board) from birth until diagnosis for cases and until enrollment for controls, assigned to residential ZIP codes	Mean (95% CI) For cases: O_3: 29.4 (28, 30.8) ppb SO_4: 3.6 (3.4, 3.87) μg/m^3 SO_2: 1.6 (1.41, 1.75) ppb NO_2: 30.3 (28.4, 32.3) ppb PM_{10}: 48.6 (45.9, 51.3) μg/m^3 For controls: O_3: 25.8 (25.2, 26.3) ppb SO_4: 3.3 (3.2, 3.36) μg/m^3 SO_2: 1.5 (1.42, 1.5) ppb NO_2: 29.7 (29.1, 30.4) ppb PM_{10}: 47.4 (46.3, 48.5) μg/m^3
Hathout et al. 2002	Prevalent T1DM	Physician-diagnosed T1DM from the database of Loma Linda University Pediatric Center	O_3, NO_2, SO_2, SO_4, and PM_{10}	Average monthly pollutant exposure (obtained from the U.S. EPA and California Air Resources Board) from birth until diagnosis for cases and until enrollment for controls, assigned to residential ZIP codes	Mean ± SD For cases: O_3: 32.5 ± 5.22 ppb SO_4: 5.52 ± 0.75 μg/m^3 SO_2: 0.67 ± 0.55 pphm NO_2: 23.7 ± 7.91 ppb PM_{10}: 59.3 ± 12.9 μg/m^3 For controls: O_3: 26.7 ± 9.6 ppb SO_4: 5.88 ± 1.04 μg/m^3 SO_2: 1.29 ± 0.92 pphm NO_2: 24.7 ± 7.26 ppb PM_{10}: 49.6 ± 14.7 μg/m^3
Fleisch et al. 2014	Prevalent GDM	Failed GCT[d] with ≥ 2 high values on the OGTT[e]		$PM_{2.5}$ and black carbon from central sites within 40 km of residence $PM_{2.5}$ and black carbon from spatiotemporal models Neighborhood traffic density [(vehicles/day) × km] within 100 m Home roadway proximity (≤ 200 m)	Mean ± SD From central sites: $PM_{2.5}$: 10.9 ± 1.4 μg/m^3 Black carbon: 0.9 ± 0.1 μg/m^3 From spatiotemporal models: $PM_{2.5}$: 11.9 ± 1.4 μg/m^3 Black carbon: 0.7 ± 0.2 μg/m^3 Traffic density: 1,621 ± 2,234 (vehicles/day × km) Roadway proximity: 281 ± 13
Pearson et al. 2010	Prevalent DM	County-level DM prevalence from the Centers for Disease Control and Prevention	$PM_{2.5}$	County annual mean level of $PM_{2.5}$ obtained from the U.S. EPA as 36-km model, 12-km model, and surface monitor data	$PM_{2.5}$ (μg/m^3): 2004: 36-km model: Q1 mean = 7.71; Q4 mean = 12.11 12-km model: Q1 mean = 7.78; Q4 mean = 11.77 Ground data: Q1 mean = 9.43; Q4 mean = 12.69 2005: 36-km model: Q1 mean = 7.69; Q4 mean = 12.75 12-km model: Q1 mean = 8.41; Q4 mean = 12.38 Ground data: Q1 mean = 9.51; Q4 mean = 13.65

Abbreviations: AirGIS, Air geographic information system; DM, diabetes mellitus; DWTD, distance-weighted traffic density; EPA, Environmental Protection Agency; GDM, gestational diabetes mellitus; HPFS, Health Professionals Follow-up Study; LUR, land-use regression; NHS, Nurses' Health Study; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; O_3, ozone; OGTT, oral glucose tolerance test; PM, particulate matter; PM_{10}, particulate matter ≤ 10 μm in diameter; $PM_{10-2.5}$, particulate matter between 2.5 and 10 μm in diameter; $PM_{2.5}$, particulate matter ≤ 2.5 μm in diameter; SO_2, sulfur dioxide; SO_4, sulfate; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

[a]Studies included in meta-analysis. [b]Air pollution estimates pooled in the meta-analysis. [c]Elevated plasma glucose concentration on at least two different occasions, one or more DM symptoms and a single elevated plasma glucose concentration, or treatment with hypoglycemic medication. [d]Glucose challenge test: serum glucose 1 hr after a non-fasting 50-g oral glucose load. [e]Oral glucose tolerance test: serum glucose 3 hr after a fasting 100-g glucose load.

Meta-analysis of studies reporting the association of air pollution and risk of T2DM. Results of seven studies reporting on risk of T2DM [three on particulate matter with diameter ≤ 2.5 μm ($PM_{2.5}$) and four on nitrogen dioxide (NO_2)] were considered for quantitative synthesis. All studies synthesized for $PM_{2.5}$ were longitudinal. For NO_2, two were longitudinal and two were cross-sectional.

The pooled relative risks of T2DM per 10-μg/m^3 increase in exposure to $PM_{2.5}$ (Figure 2) and NO_2 (Figure 3) were 1.10 (95% CI: 1.02, 1.18) and 1.08 (95% CI: 1.00, 1.17), respectively. The effect was more pronounced in females than in males [NO_2: 1.15 (95% CI: 1.05, 1.27) vs. 0.99 (95% CI: 0.93, 1.07); $PM_{2.5}$: 1.14 (95% CI: 1.03, 1.26) vs. 1.04 (95% CI: 0.93, 1.17), respectively] per 10-μg/m^3 increase in exposure. The relative risks were similar across all sensitivity analyses (Table 4). We observed substantial statistical heterogeneity with NO_2 studies (Table 4). Egger's test was consistently > 0.2 (p-value) in all cases.

Discussion

In this systematic review, we considered 13 studies on different types of diabetes. The identified epidemiological evidence is highly diverse: Levels, timing, and assessment of exposure varied, as did the outcome definitions, measures of association, and degree of confounder control. The studies included persons with different age ranges and settings, and some populations included only women. Although there is a risk of bias, the results of the meta-analyses indicate a positive association between traffic-related air pollution and T2DM.

Pathophysiologic mechanisms of DM–air pollution association. There is strong evidence supporting the role of inflammation in T2DM (Donath and Shoelson 2011; Sjöholm and Nyström 2006). Chronic activation of inflammatory mechanisms can contribute to chronic insulin resistance and subsequent T2DM. Air pollution has been shown to be inflammatory (Liu et al. 2013; Rajagopalan and Brook 2012). Its potential mechanisms in mediating T2DM include pulmonary and systemic inflammation, directly releasing cytokines, alterations in glucose homeostasis through defective insulin signaling in tissues, immune cells activation in visceral adipose tissues potentiating inflammation (Sun et al. 2009; Xu et al. 2010; Yan et al. 2011), and endoplasmic reticulum stress in the lung and liver in relation with hepatocyte and alveolar cells (Liu et al. 2013; Rajagopalan and Brook 2012). $PM_{2.5}$ also acts as a hypothalamic stressor, inducing peripheral inflammation and abnormalities in glucose metabolism (Liu et al. 2013; Purkayastha et al. 2011). $PM_{2.5}$ was also shown to mediate dysfunctional brown adipose and mitochondrial tissues (Liu et al. 2013; Rajagopalan and Brook 2012), which is one of the systemic pathologies in T2DM (Lowell and Shulman 2005).

Chuang et al. (2010) demonstrated that exposure to air pollution [PM ≤ 10 μm (PM_{10}) and ozone] exposure leads to alteration in blood pressure, blood lipids, and hemoglobin A1c, a marker of blood glucose control. Kelishadi et al. (2009) found positive associations between exposure to PM_{10}, NO_2, and insulin resistance among children in Iran. Thiering et al. (2013) later found a positive association between residential proximity to traffic, particulate matter (PM_{10}), NO_2, and risk of insulin resistance [homeostatic model assessment (HOMA-IR)] among children who were part of a birth cohort in Germany. Exposure to traffic-related air pollution is also associated with impaired glucose tolerance in pregnancy (Fleisch et al. 2014). Experimental evidence also exists for the association of

Table 3. Data synthesized for meta-analysis.

Source	Population	Pollutant	Assignment of individual exposure	Reported fully adjusted estimate (95% CI)[a]
Krämer et al. 2010	Females	NO_2	LUR model	1.42 (1.16, 1.73) per 15 μg/m^3 of exposure
Andersen et al. 2012	Females	NO_2	LUR model	1.07 (1.01, 1.13) per 4.9 μg/m^3 of exposure
	Males	NO_2	LUR model	1.01 (0.97, 1.07) per 4.9 μg/m^3 of exposure
	Both	NO_2	LUR model	1.04 (1.00, 1.08) per 4.9 μg/m^3 of exposure
Brook et al. 2008	Females	NO_2	LUR model	1.04 (1.00, 1.08) per 1 ppb of exposure
	Males	NO_2	LUR model	0.99 (0.95, 1.03) per 1 ppb of exposure
	Both	NO_2	LUR model	1.015 (0.98, 1.049) per 1 ppb of exposure
Puett et al. 2011	Females	$PM_{2.5}$	LUR model	1.02 (0.94, 1.09) per 4 μg/m^3 of exposure
	Males	$PM_{2.5}$	LUR model	1.07 (0.92, 1.24) per 4 μg/m^3 of exposure
	Both	$PM_{2.5}$	LUR model	1.03 (0.96, 1.10) per 4 μg/m^3 of exposure
Chen et al. 2013	Females	$PM_{2.5}$	Satellite-based estimates	1.17 (1.03, 1.32) per 10 μg/m^3 of exposure
	Males	$PM_{2.5}$	Satellite-based estimates	1.03 (0.91, 1.16) per 10 μg/m^3 of exposure
	Both	$PM_{2.5}$	Satellite-based estimates	1.11 (1.02, 1.21) per 10 μg/m^3 of exposure
Coogan et al. 2012	Females	$PM_{2.5}$	Kriging model	1.63 (0.78, 3.44) per 10 μg/m^3 of exposure
Dijkema et al. 2011	Females	NO_2	LUR model	1.03 (0.90, 1.16) per 10 μg/m^3 of exposure
	Males	NO_2	LUR model	0.97 (0.87, 1.09) per 10 μg/m^3 of exposure
	Both	NO_2	LUR model	1.00 (0.94, 1.06) per 10 μg/m^3 of exposure

Abbreviations: LUR, land-use regression; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter ≤ 2.5 μm in diameter.
[a]All odds ratio, hazard ratio, and incident risk ratio estimates were converted to per 10 μg/m^3 of exposure for meta-analysis. Estimates from Dijkema et al. (2011) were derived from reported nonlinear estimates.

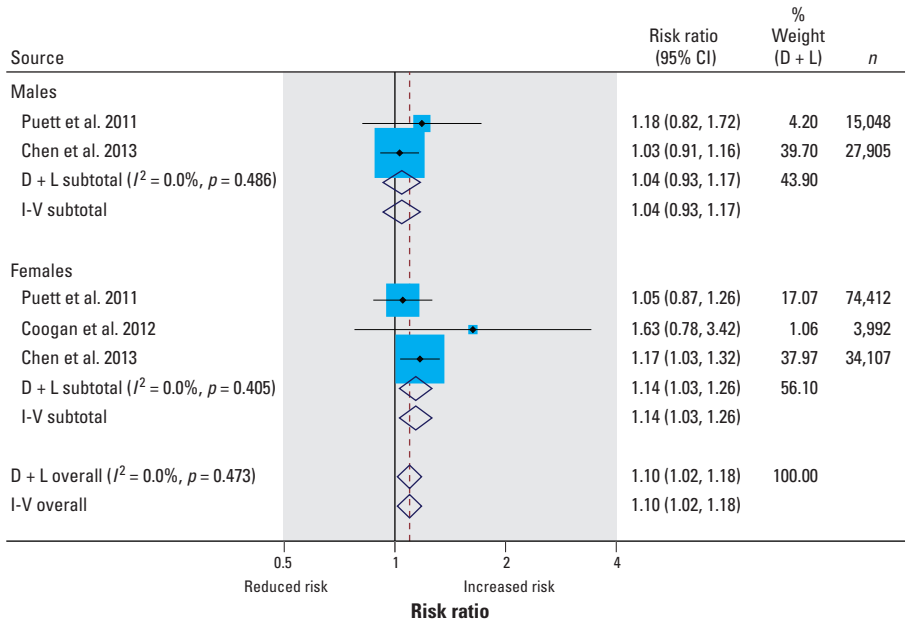

Figure 2. $PM_{2.5}$ and risk of T2DM. Where I^2 is the variation in effect estimates attributable to heterogeneity, D + L (DerSimonian and Laird) overall is the pooled random effect estimate of all studies. I-V (inverse variance) overall is the pooled fixed effects estimate of all studies. Weights are from random-effects analysis. %Weight (D + L) is the weight assigned to each study, based on the inverse of the within- and between-study variance. The size of the blue boxes around the point estimates reflects the weight assigned to each study. The summarized studies were adjusted for age, sex, BMI, smoking, alcohol consumption, and socioeconomic status.

air pollution and type 1 diabetes (T1DM). Ozone is known to alter T-cell dependent immune response, predisposing to autoimmune diseases (Krishna et al. 1998). It may also damage the beta cells of the pancreas possibly as a result of pulmonary reactive oxidative species production and oxidative stress, leading to reduced insulin secretion (Brenner et al. 1993; Kelishadi et al. 2009). Together with sulfate, ozone may have apoptotic properties on the beta cells (Hathout et al. 2006). The use of antioxidant prophylaxis for T1DM also points to the possibility of oxidative or inflammatory mechanisms in T1DM (Albright and Goldstein 1996).

Strengths and limitations. Although we applied a very broad search strategy and accepted any study design, there are few published studies on the association of air pollution with T1DM or GDM. In addition, some studies did not allow distinguishing adult T1DM from T2DM. Only three of the seven synthesized studies explicitly analyzed the T2DM risk (Coogan et al. 2012; Dijkema et al. 2011; Krämer et al. 2010). However, because > 90% of adult diabetes diagnoses are T2DM, this is unlikely to substantially affect the conclusions. Overall, the available data are not sufficient to evaluate associations with these diabetes types.

Our analysis on the association with T2DM was based on results from primary studies with unclear to high risk of bias and high diversity among the included studies. We took this into account by using effect estimates modeled to participants' residences, converting all effect estimates to a comparable unit (per 10 μg/m^3 of exposure), stratifying analyses by sex, including only longitudinal studies, and performing other sensitivity analyses.

The high diversity among the studies was reflected in our observation of substantial heterogeneity in the meta-analysis for NO_2 (Table 4), which synthesized longitudinal and cross-sectional data. This was not observed for $PM_{2.5}$, for which all studies were longitudinal. However, the number of studies was too small to further analyze this heterogeneity.

Prospects. Future studies should report scales of exposure assessment (pollutant quantification and traffic exposure proxies) that allow direct comparisons with existing evidence. It would be important to apply comparable models in assigning exposure to participants. Ideally, traffic distance measures should be replaced by objective particle concentration measures and models of near-road traffic-related pollutants such as ultrafine particles of elemental carbon. Also, it would be important to consider various time lags for exposure.

The studies on T1DM found associations with ozone and sulfates. These pollutants can be included in the future models for T2DM, because pollutants usually occur together in different proportions. Carbon monoxide, lead, oxidative metals, volatile organic compounds, and polycyclic aromatic hydrocarbons are other traffic-related pollutants that may be more deleterious to health but have been given less consideration.

Adjusting for noise exposure is also essential because air pollution and noise can be correlated (Foraster 2013; Kim et al. 2012; Ross et al. 2011; Tétreault et al. 2013) and share health effects. Sørensen et al. (2013) recently reported a positive association between road-traffic noise and incident diabetes, and another large meta-analysis of 10 epidemiologic studies by Cappuccio et al. (2010) found that both quality and quantity of sleep, which are related to noise, were significant predictors of the risk of T2DM. Consideration of noise is thus necessary in assessing the health effects of air pollution.

Also, socioeconomic variables should be adjusted on the spatial scale, apart from individual-level adjustment. Consideration for this spatial confounding is necessary when individual differences in health outcome are associated with neighborhood characteristics such as neighborhood socioeconomic status (Sheppard et al. 2012). It is crucial that studies on diabetes risk consider established diabetes risk factors including obesity, physical activity, and nutrition. Active and passive smoking should be considered when assessing the effect of air pollution. Lack of information on these creates a high risk for bias.

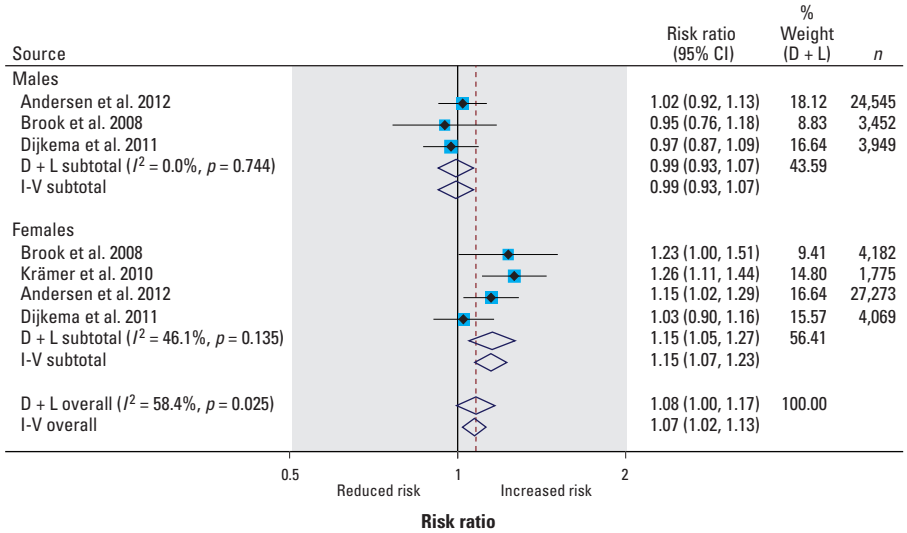

Figure 3. NO_2 and risk of T2DM. Where I^2 is the variation in effect estimates attributable to heterogeneity, D + L (DerSimonian and Laird) overall is the pooled random-effects estimate of all studies. I-V (inverse variance) overall is the pooled fixed-effects estimate of all studies. Weights are from random-effects analysis. %Weight (D + L) is the weight assigned to each study, based on the inverse of the within- and between-study variance. The size of the blue boxes around the point estimates reflects the weight assigned to each study. The summarized studies were adjusted for age, sex, BMI, smoking, and socioeconomic status.

Table 4. Sensitivity analyses and heterogeneity measures.

Analyses	Population	NO_2 OR (95% CI)	Heterogeneity measures [I^2 (%); p-value; Tau^2]	$PM_{2.5}$ OR (95% CI)	Heterogeneity measures [I^2 (%); p-value; Tau^2]
Main model (random effects)	Males	0.99 (0.93, 1.07)	0; 0.744; 0	1.04 (0.93, 1.17)	0; 0.486; 0
	Females	1.15 (1.05, 1.27)	46.1; 0.135; 0.0042	1.14 (1.03, 1.26)	0; 0.405; 0
	Overall	1.08 (1.00, 1.17)	58.4; 0.025; 0.0063	1.10 (1.02, 1.18)	0; 0.473; 0
Studies assessing air pollution before DM diagnosis	Males	1.02 (0.92, 1.13)	NA; NA; 0	1.04 (0.93, 1.17)	0; 0.486; 0
	Females	1.20 (1.10, 1.30)	12.5; 0.285; 0.0006	1.13 (1.02, 1.25)	0; 0.344; 0
	Overall	1.12 (1.05, 1.19)	69.8; 0.036; 0.008	1.09 (1.01, 1.18)	0; 0.489; 0
Studies including both men and women	Males	0.99 (0.93, 1.07)	0; 0.744; 0	1.04 (0.93, 1.17)	0; 0.486; 0
	Females	1.11 (1.01, 1.23)	30.2; 0.238; 0.0023	1.13 (1.02, 1.25)	0; 0.344; 0
	Overall	1.05 (0.98, 1.12)	34.9; 0.175; 0.0024	1.09 (1.01, 1.18)	0; 0.489; 0
Only longitudinal studies	Males	1.02 (0.92, 1.13)	NA; NA; 0	1.04 (0.93, 1.17)	0; 0.486; 0
	Females	1.20 (1.10, 1.30)	12.5; 0.285; 0.0006	1.14 (1.03, 1.26)	0; 0.405; 0
	Overall	1.12 (1.05, 1.19)	69.8; 0.036; 0.008	1.10 (1.02, 1.18)	0; 0.473; 0
Meta-analysis using fixed-effects model	Males	1.00 (0.93, 1.07)	0; 0.744	1.04 (0.93, 1.17)	0; 0.486
	Females	1.15 (1.07, 1.23)	46.1; 0.135	1.14 (1.03, 1.26)	0; 0.405
	Overall	1.07 (1.02, 1.13)	58.4; 0.025	1.10 (1.02, 1.18)	0; 0.473

NA, not applicable. I^2 is the proportion of total variability explained by heterogeneity. Tau^2 is a measure of among-study variance.

Other forms of bias such as the healthy survivor effect should be taken into account, especially in longitudinal studies. Raaschou-Nielsen et al. (2013b) demonstrated associations between diabetes mortality and NO_x exposure; thus, diabetes patients exposed to air pollution could die and no longer participate, resulting in incorrect estimates of association if mortality was not taken into consideration.

No included study on this topic was done in developing countries. For generalizability of evidence, research should be extended to developing countries where air pollution (including indoor) is high. This could also help in understanding effects of different air pollution compositions. Indoor air pollution is also associated with diabetes as well as cardiovascular diseases (Lee et al. 2012) and is highly prevalent in developing nations (Lim et al. 2012).

Considering the ambiguity in dose–response relationship in air pollution studies (Smith and Peel 2010), future studies should assess air pollution diabetes association in a dose–response manner. This will help in identifying the point in the dose spectrum where control will yield the most benefits for health policy (Smith and Peel 2010).

Overall, the existing evidence indicates a positive association of air pollution and T2DM risk, although there is high risk of bias. High-quality longitudinal studies are needed (taking into consideration sources and composition of air pollution as well as biomarkers) to improve our understanding of this association.

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Supplemental Material

Association between Ambient Air Pollution and Diabetes Mellitus in Europe and North America: Systematic Review and Meta-Analysis

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Table S1. Association between air pollution and diabetes mellitus.

Study, exposure	Exposure contrast	Unadjusted effect estimate (95% CI)	Adjusted effect estimate (95% CI)	Confounder adjustment	Effect modification
Krämer et al. 2010[a]					
PM_{10}, monitoring stations	IQR: 10.1 $\mu g/m^3$	HR = 1.64 (1.20, 2.25)	HR = 1.16 (0.81, 1.65)	Baseline age, education, smoking, work place exposure to dust, fumes and extreme temperatures, BMI (average of baseline and follow-up).	Stronger association in women with high C3c levels: HR = 1.21 (0.70, 1.64)
NO_2, monitoring stations	IQR: 24.9 $\mu g/m^3$	HR = 1.53 (1.20, 1.95)	HR = 1.34 (1.02, 1.76)	Same as above	Stronger association in women with high C3c levels: HR = 1.29 (0.93, 1.79)
PM, traffic emission inventory	0.87 tons/year/km^2	HR = 1.23 (1.12, 1.35)	HR = 1.15 (1.04, 1.27)	Same as above	Stronger association in women with high C3c levels: HR = 1.24 (1.08, 1.41)
NO_2, traffic emission inventory	19 tons/year/km^2	HR = 1.22 (1.11, 1.34)	HR = 1.15 (1.04, 1.27)	Same as above	Stronger association in women with high C3c levels: HR = 1.24 (1.08, 1.41)
Soot, LUR	0.39 x 10^{-5}m	HR = 1.28 (1.12, 1.47)	HR = 1.27 (1.09, 1.48)	Same as above	Stronger association in women with high C3c levels: HR = 1.22 (1.02, 1.47)
NO_2, LUR	15 $\mu g/m^3$	HR = 1.47 (1.22, 1.77)	HR 1.42 (1.16, 1.73)	Same as above	Stronger association in women with high C3c levels: HR = 1.31 (1.01, 1.70)
Distance from a busy road	<100m	HR (low education) = 2.32 (1.29, 4.17)	HR (low education) = 2.54 (1.31, 4.91)	Same as above	Stronger association in women with high C3c levels HR = 3.51 (1.50, 8.23)
Distance from a busy road	<100m	HR (high education) = 0.86 (0.55, 1.36)	HR (high education) = 0.92 (0.58, 1.47)	Same as above	Same as above
Coogan et al. 2012[a]					
$PM_{2.5}$	IQR: 10 $\mu g/m^3$	NA	IRR = 1.63 (0.78, 3.44)	Time-varying age, BMI, years of education, income, number of people in a household, smoking, alcohol intake, physical activity, neighbourhood socio-economic status score, family history of diabetes.	NA
NO_x	IQR: 12.4 ppb	NA	IRR = 1.25 (1.07, 1.46)	Same as above	NA

Study, exposure	Exposure contrast	Unadjusted effect estimate (95% CI)	Adjusted effect estimate (95% CI)	Confounder adjustment	Effect modification
Andersen et al. 2012[a]					
NO_2 (35-year mean)	IQR: 4.9 μg/m^3	HR = 1.11 (1.07, 1.15)[b]	HR = 1.04 (1.00, 1.08)	Baseline age, sex, BMI, waist-to-hip ratio, smoking status, duration and intensity, ETS, educational level, physical activity and intensity, alcohol, fruit and fat consumption and calendar year.	Stronger effects among women HR = 1.07(1.01, 1.13), subjects with high waist-to-hip ratio: HR = 1.09(1.01, 1.18), non-smokers: HR = 1.12 (1.05, 1.20), subjects with <8 years of education: HR= 1.06(1.01, 1.12), subjects with COPD: HR= 1.05(1.01, 1.09) and those with asthma: HR=1.05 (1.01, 1.09)
NO_2 (15-year mean)	IQR: 5.6 μg/m^3	HR = 1.10 (1.06, 1.13)[b]	HR = 1.04 (1.01, 1.07)	Same as above	NA
NO_2 (1-year mean at baseline)	IQR: 5.6 μg/m^3	HR = 1.08 (1.05, 1.11)[b]	HR = 1.02 (0.98, 1.05)	Same as above	NA
NO_2 (1-year mean at follow-up)	IQR: 5.7 μg/m^3	HR = 1.10 (1.06, 1.13)[b]	HR = 1.04 (1.01, 1.07)	Same as above	NA
NO_x (35-year mean)	IQR: 11.4 μg/m^3	HR = 1.05 (1.03, 1.07)[b]	HR = 1.02 (1.00, 1.04)	Same as above	NA
NO_x (15-year mean)	IQR: 12.0 μg/m^3	HR = 1.05 (1.03, 1.07)[b]	HR = 1.02 (1.00, 1.04)	Same as above	NA
NO_x (1-year mean at baseline)	IQR: 10.9 μg/m^3	HR = 1.02 (1.01, 1.02)[b]	HR = 1.00 (1.00, 1.01)	Same as above	NA
NO_x (1-year mean at follow-up)	IQR: 12.0 μg/m^3	HR = 1.05 (1.03, 1.06)[b]	HR = 1.02 (1.00, 1.04)	Same as above	NA
Traffic proximity	Major road within 50m	HR = 1.20 (1.06, 1.36)[b]	HR = 1.07 (0.95, 1.21)	Same as above	NA
Traffic load	1,300 vehicles/km/day	HR = 1.05 (1.03, 1.08)[b]	HR = 1.02 (1.00, 1.04)	Same as above	NA
Puett et al. 2011[a]					
$PM_{2.5}$	IQR: 4.0 μg/m^3	HR (men) = 1.05 (0.91, 1.22)[c]	HR (men) = 1.07 (0.92, 1.24)	Age, season, calendar year, state of residence, time-varying smoking status, pack years, alcohol intake, diet and hypertension, baseline BMI and physical activity.	NA
$PM_{2.5}$	IQR: 4.0 μg/m^3	HR (women) = 1.04 (0.97, 1.12)[c]	HR (women) = 1.02 (0.94, 1.09)	Same as above	NA
$PM_{2.5}$	IQR: 4.0 μg/m^3	HR (pooled) = 1.05 (0.98, 1.12)[c]	HR (pooled) = 1.03 (0.96, 1.10)	Same as above	NA
$PM_{10\text{-}2.5}$	IQR: 4.2 μg/m^3	HR (men) = 1.05 (0.94, 1.17)[c]	HR (men) = 1.04 (0.93, 1.16)	Same as above	NA
$PM_{10\text{-}2.5}$	IQR: 4.0 μg/m^3	HR (women) = 1.07 (1.01, 1.13)[c]	HR (women) = 1.04 (0.98, 1.10)	Same as above	NA
$PM_{10\text{-}2.5}$	IQR: 4.0 μg/m^3	HR (pooled) = 1.06 (1.01, 1.12)[c]	HR (pooled) = 1.04 (0.99, 1.09)	Same as above	NA
PM_{10}	IQR: 7.2 μg/m^3	HR (men) = 1.06 (0.93, 1.20)[c]	HR (men) = 1.06 (0.94, 1.20)	Same as above	NA
PM_{10}	IQR: 7.0 μg/m^3	HR (women) = 1.06 (1.01, 1.12)[c]	HR (women) = 1.03 (0.98, 1.09)	Same as above	NA
PM_{10}	IQR: 7.0 μg/m^3	HR (pooled) = 1.06 (1.01, 1.12)[c]	HR (pooled) = 1.04 (0.99, 1.09)	Same as above	NA
Distance to road	0-49m vs. ≥200m	HR (men): 0.99 (0.82, 1.19)[c]	HR (men): 1.02 (0.85, 1.23)	Same as above	NA
Distance to road	50-99m vs. ≥200m	HR (men): 0.76 (0.51, 1.14)[c]	HR (men): 0.74 (0.49, 1.11)	Same as above	NA
Distance to road	100-199m vs. ≥200m	HR (men): 0.86 (0.66, 1.13)[c]	HR (men): 0.88 (0.67, 1.16)	Same as above	NA
Distance to road	0-49m vs. ≥200m	HR (women): 1.20 (1.08, 1.33)[c]	HR (women): 1.14 (1.03, 1.27)	Same as above	Stronger effect in women
Distance to road	50-99m vs. ≥200m	HR (women): 1.20 (1.03, 1.40)[c]	HR (women): 1.16 (0.99, 1.35)	Same as above	Same as above
Distance to road	100-199m vs. ≥200m	HR (women): 1.02 (0.92, 1.14)[c]	HR (women): 0.97 (0.88, 1.08)	Same as above	Same as above
Distance to road	0-49m vs. ≥200m	HR (pooled): 1.11 (0.92, 1.33)[c]	HR (pooled): 1.11 (1.01, 1.23)	Same as above	NA
Distance to road	50-99m vs. ≥200m	HR (pooled): 0.99 (0.64, 1.54)[c]	HR (pooled): 0.96 (0.87, 1.06)	Same as above	NA
Distance to road	100-199m vs. ≥200m	HR (pooled): 0.99 (0.86, 1.13)[c]	HR (pooled): 1.02 (0.92, 1.14)	Same as above	NA

Study, exposure	Exposure contrast	Unadjusted effect estimate (95% CI)	Adjusted effect estimate (95% CI)	Confounder adjustment	Effect modification
Brook et al. 2008[a]					
NO_2	1 ppb	NA	OR (men) = 0.99 (0.95, 1.03)	Age, sex, BMI and neighbourhood income	NA
NO_2	1 ppb	NA	OR (women) = 1.04 (1.00, 1.08)	Same as above	Stronger effect in women
NO_2	1 ppb	NA	OR (pooled) = 1.015 (0.98, 1.049)	Same as above	Same as above
Dijkema et al. 2011[a]					
NO_2	14.2-15.2 vs. 8.8-14.2 $\mu g/m^3$	OR = 0.98 (0.78, 1.23)	OR = 1.03 (0.82, 1.31)	Age, sex, BMI and average monthly income	Stronger effect in women.
NO_2	15.2-16.5 vs. 8.8-14.2 $\mu g/m^3$	OR = 1.17 (0.94, 1.45)	OR = 1.25 (0.99, 1.56)	Same as above	Same as above
NO_2	16.5-36.0 vs. 8.8-14.2 $\mu g/m^3$	OR = 0.80 (0.63, 1.01)	OR = 0.80 (0.63, 1.02)	Same as above	Same as above
Distance to nearest main road	140-220m vs. 220-1610m	OR = 1.10 (0.87, 1.39)	OR = 1.12 (0.88, 1.42)	Same as above	Same as above
Distance to nearest main road	74-140m vs. 220-1610m	OR = 1.22 (0.97, 1.53)	OR = 1.17 (0.93, 1.48)	Same as above	Same as above
Distance to nearest main road	2-74m vs. 220-1610m	OR = 0.94 (0.74-1.19)	OR = 0.88 (0.70-1.13)	Same as above	Same as above
Traffic flow at the nearest main road	5871-7306 vs. 5001-5871 vehicles/day	OR = 1.09 (0.87, 1.39)	OR = 1.02 (0.81, 1.29)	Same as above	Same as above
Traffic flow at the nearest main road	7306-9670 vs. 5001-5871 vehicles/day	OR = 0.98 (0.78, 1.23)	OR = 1.03 (0.81, 1.30)	Same as above	Same as above
Traffic flow at the nearest main road	9670-35567 vs. 5001-5871 vehicles/day	OR = 0.91 (0.72, 1.16)	OR = 0.96 (0.75, 1.22)	Same as above	Same as above
Traffic in 250m buffer	516-680 x 10^3 vs. 63-516 x 10^3 vehicles/day	OR = 1.28 (1.01, 1.61)	OR = 1.25 (0.99, 1.59)	Same as above	Same as above
Traffic in 250m buffer	680-882 x 10^3 vs. 63-516 x 10^3 vehicles/day	OR = 1.15 (0.91, 1.46)	OR = 1.13 (0.89, 1.44)	Same as above	Same as above
Traffic in 250m buffer	882-2007 x 10^3 vs. 63-516 x 10^3 vehicles/day	OR = 1.13 (0.89, 1.44)	OR = 1.09 (0.85, 1.38)	Same as above	Same as above
Chen et al. 2013[a]					
$PM_{2.5}$	10 $\mu g/m^3$	HR = 1.08 (0.99, 1.17)[d]	HR = 1.11 (1.02, 1.21)	Baseline age, sex survey year, region, marital status, education, household income, BMI, physical activity, smoking, alcohol consumption, diet, race, hypertension, urban residency, neighbourhood-level unemployment rate, education, COPD, asthma, congestive heart failure and acute myocardial infarction	Stronger effects among subjects with COPD: HR= 1.33 (1.03, 1.71), women: HR= 1.17 (1.03, 1.32), subjects aged<50 years: HR= 1.19 (1.00, 1.40) or >65 years: HR= 1.18 (1.01, 1.38) and subjects with low level of education: HR= 1.13 (1.00, 1.28).
van den Hooven et al. 2009					
Distance-weighted traffic density	158-546 vs. <158 vehicles/ day*km	OR = 0.66 (0.30, 1.48)	OR = 0.69 (0.30, 1.57)	Maternal age, education, ethnicity, BMI, parity, smoking, alcohol consumption, month and year of birth.	NA
Distance-weighted traffic density	546-1,235 vs. <158 vehicles/ day*km	OR = 1.00 (0.49, 2.05)	OR = 1.07 (0.51, 2.23)	Same as above	NA
Distance-weighted traffic density	>1,235 vs. <158 vehicles/ day*km	OR = 0.67 (0.30, 1.49)	OR = 0.79 (0.35, 1.81)	Same as above	NA
Distance to major road	150-200m vs. >200m	OR = 1.17 (0.53, 2.60)	OR = 1.07 (0.47, 2.44)	Same as above	NA
Distance to major road	100-150m vs. >200m	OR = 0.76 (0.32, 1.82)	OR = 0.77 (0.32, 1.88)	Same as above	NA

Study, exposure	Exposure contrast	Unadjusted effect estimate (95% CI)	Adjusted effect estimate (95% CI)	Confounder adjustment	Effect modification
Distance to major road	50-100m vs. >200m	OR = 1.07 (0.50, 2.31)	OR = 1.13 (0.51, 2.50)	Same as above	NA
Malmqvist et al. 2013					
NO_x	9.0-14.1 vs. 2.5-8.9 μg/m^3	OR = 1.28 (1.07, 1.54)	OR = 1.19 (0.99, 1.44)	Maternal age, parity, prepregnancy BMI, calendar year, ethnicity, T1DM	NA
NO_x	14.2-22.6 vs. 2.5-8.9 μg/m^3	OR = 1.84 (1.56, 2.18)	OR = 1.52 (1.28, 1.82)	Same as above	NA
NO_x	>22.7 vs. 2.5-8.9 μg/m^3	OR = 1.98 (1.68, 2.35)	OR = 1.69 (1.41, 2.03)	Same as above	NA
Traffic density within 200m	<2 cars/min vs. No road	OR = 0.89 (0.75, 1.06)	OR = 0.93 (0.78, 1.12)	Same as above	NA
Traffic density within 200m	2-5 cars/min vs. No road	OR = 1.04 (0.88, 1.23)	OR = 0.96 (0.81, 1.14)	Same as above	NA
Traffic density within 200m	5-10 cars/min vs. No road	OR = 1.53 (1.27, 1.84)	OR = 1.18 (0.97, 1.43)	Same as above	NA
Traffic density within 200m	>10 cars/min vs. No road	OR = 1.50 (1.24, 1.82)	OR = 1.23 (1.01, 1.51)	Same as above	NA
Hathout et al 2006					
O_3	10 ppb	OR = 2.92 (1.86, 4.58)	OR = 1.73 (1.08, 2.77)	Age at diagnosis/entry, ETS, attendance of day care, breast feeding, maternal diabetes, family history of diabetes and autoimmunity, maternal drug use, parental education.	NA
SO_4	10 μg/m^3	OR = 1.65 (1.20, 2.28)	NA	NA	NA
SO_2	1 ppb	OR = 1.42 (0.91, 2.21)	NA	NA	NA
NO_2	10 ppb	OR = 1.03 (0.71, 1.50)	NA	NA	NA
PM_{10}	10 μg/m^3	OR = 1.08 (0.87, 1.34)	NA	NA	NA
Hathout et al 2002					
O_3	IQR: 10.93 ppb	OR = 4.22 (1.96, 9.10)	OR = 4.22 (1.96, 9.10)	Age	NA
SO_4	IQR: 1.025 μg/m^3	OR = 0.56 (0.37, 0.87)	OR = 0.55 (0.35, 0.85)	Same as above	NA
SO_2	IQR: 1.235 ppb	OR = 0.54 (0.33, 0.89)	OR = 0.52 (0.31, 0.88)	Same as above	NA
NO_2	IQR: 11.175 ppb	OR = 0.57 (0.31, 1.02)	OR = 0.56 (0.30, 1.03)	Same as above	NA
PM_{10}	IQR: 22.65 μg/m^3	OR = 2.37 (1.11, 5.03)	OR = 2.37 (1.11, 5.03)	Same as above	NA
Fleisch et al. 2014					
Central-site $PM_{2.5}$	IQR: 1.7 μg/m^3	NA	OR = 0.81 (0.62, 1.08)	Age, prepregnancy BMI, pregnancy weight gain, education, race/ethnicity, family history of diabetes, prior GDM and season of last menstrual period.	NA
Central-site $PM_{2.5}$	10.0-10.7 vs. 8.3-10.0 μg/m^3	NA	OR = 0.91 (0.50, 1.65)	Same as above	NA
Central-site $PM_{2.5}$	10.7-11.7 vs. 8.3-10.0 μg/m^3	NA	OR = 0.52 (0.27, 1.00)	Same as above	NA
Central-site $PM_{2.5}$	11.7-17.2 vs. 8.3-10.0 μg/m^3	NA	OR = 0.69 (0.38, 1.27)	Same as above	NA
Spatiotemporal $PM_{2.5}$	IQR: 2.0 μg/m^3	NA	OR = 0.94 (0.67, 1.34)	Same as above	NA
Spatiotemporal $PM_{2.5}$	10.8-11.8 vs. 8.5-10.8 μg/m^3	NA	OR = 0.62 (0.30, 1.28)	Same as above	NA
Spatiotemporal $PM_{2.5}$	11.8-12.8 vs. 8.5-10.8 μg/m^3	NA	OR = 0.93 (0.48, 1.78)	Same as above	NA
Spatiotemporal $PM_{2.5}$	12.8-15.9 vs. 8.5-10.8 μg/m^3	NA	OR = 0.71 (0.35, 1.42)	Same as above	NA
Central-site black carbon	IQR: 0.16 μg/m^3	NA	OR = 0.69 (0.42, 1.13)	Same as above	NA
Central-site black carbon	0.78-0.87 vs. 0.60-0.78 μg/m^3	NA	OR = 0.75 (0.39, 1.45)	Same as above	NA

Study, exposure	Exposure contrast	Unadjusted effect estimate (95% CI)	Adjusted effect estimate (95% CI)	Confounder adjustment	Effect modification
Central-site black carbon	0.87-0.94 vs. 0.60-0.78 $\mu g/m^3$	NA	OR = 0.59 (0.25, 1.35)	Same as above	NA
Central-site black carbon	0.94-1.10 vs. 0.60-0.78 $\mu g/m^3$	NA	OR = 0.60 (0.23, 1.53)	Same as above	NA
Spatiotemporal black carbon	IQR: 0.34 $\mu g/m^3$	NA	OR = 1.02 (0.73, 1.41)	Same as above	NA
Spatiotemporal black carbon	0.55-0.70 vs. 0.14-0.55 $\mu g/m^3$	NA	OR = 1.01 (0.54, 1.87)	Same as above	NA
Spatiotemporal black carbon	0.70-0.89 vs. 0.14-0.55 $\mu g/m^3$	NA	OR = 1.12 (0.59, 2.09)	Same as above	NA
Spatiotemporal black carbon	0.89-1.69 vs. 0.14-0.55 $\mu g/m^3$	NA	OR = 0.90 (0.45, 1.79)	Same as above	NA
Neighbourhood traffic density within 100m	IQR: 1,533 vehicles/day*km	NA	OR = 1.02 (0.87, 1.18)	Same as above	NA
Neighbourhood traffic density within 100m	4,062-9,680 vs. 0-4,061 vehicles/day*km	NA	OR = 1.18 (0.66, 2.11)	Same as above	NA
Neighbourhood traffic density within 100m	9,680-19,371vs. 0-4,061 vehicles/day*km	NA	OR = 0.94 (0.51, 1.72)	Same as above	NA
Neighbourhood traffic density within 100m	19,383-30,860 vs. 0-4,061 vehicles/day*km	NA	OR = 0.74 (0.39, 1.42)	Same as above	NA
Home roadway proximity	≤200m vs. >200m	NA	OR = 0.99 (0.52, 1.88)	Same as above	NA
Pearson et al. 2010					
$PM_{2.5}$ (36km model, 2004)	10 $\mu g/m^3$	OR = 6.69 (5.53, 7.77)	OR = 3.16 (2.77, 3.74)	County-level median age, per capita income, percentage of men, per capita income, percentage of the population aged >25 years with a high school or general equivalency degree, percentage of ethnicities, prevalence of obesity, physical activity, population density and latitude (from census 2000)	NA
$PM_{2.5}$ (36km model, 2004)	10 $\mu g/m^3$	OR = 6.69 (5.53, 7.77)	OR = 2.18 (1.48, 3.49)	Same as above (from ACS 1-year)	NA
$PM_{2.5}$ (36km model, 2005)	10 $\mu g/m^3$	OR = 6.69 (5.42, 7.92)	OR = 2.51 (2.12, 3.10)	Same as above (from census 2000)	NA
$PM_{2.5}$ (36km model, 2005)	10 $\mu g/m^3$	OR = 6.69 (5.42, 7.92)	OR = 2.25 (1.62, 2.91)	Same as above (from ACS 1-year)	NA

PM: particulate matter; PM_{10}: particulate matter <10µm in diameter; $PM_{10-2.5}$: particulate matter between 2.5 and 10µm in diameter; $PM_{2.5}$: particulate matter <2.5µm in diameter; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; O_3: ozone; SO_2: sulphur dioxide; SO_4: sulphate; T1DM: type 1 diabetes mellitus; GDM: gestational diabetes mellitus; LUR: land-use regression; IQR: interquartile range; C3c: complement protein 3c; ETS: environmental tobacco smoking; BMI: body mass index; COPD: chronic obstructive pulmonary disease; NA: not available; ACS: American Community Survey.

[a]Included in meta-analysis. [b]Adjusted for only age. [c]Adjusted for age, season and year. [d]Adjusted for age, sex, year and region.

Table S2. Risk of bias assessment for included studies.

Source	Adjustment for basic DM risk factors[a] at baseline	Exposure assessment before DM diagnosis	Exposure modelled at participants' residence	Attempts to identify undiagnosed DM	Consideration of healthy survivor bias	Adjustment for noise as an environmental risk factor	Consideration of time-dependent confounding
Krämer et al. 2010[b]	Yes[c]	Yes	Yes	No	No	No	No
Andersen et al. 2012[b]	Yes[c]	Yes	Yes	No	No	No	Yes
Puett et al. 2011[b]	Yes[c]	Yes	Yes	No	No	No	Yes
Coogan et al. 2012[b]	Yes[d]	No	Yes	No	No	No	Yes
Chen et al. 2013[b]	Yes[b]	Yes	Yes	No	No	No	No
Brook et al. 2008[b]	Yes[e]	No	Yes	No	NA	No	NA
Dijkema et al. 2011[b]	Yes[f]	No	Yes	Yes	NA	No	NA
Pearson et al. 2010	Yes[g]	NA	NA	NA	NA	No	NA
Malmqvist et al. 2013	Yes[c]	No	Yes	No	NA	No	NA
Van den Hooven et al. 2009	Yes[c]	Yes	Yes	No	NA	No	NA
Hathout et al. 2002	Yes[h]	Yes	Yes	No	NA	No	NA
Hathout et al. 2006	Yes	Yes	Yes	Yes	NA	No	NA
Fleisch et al. 2014	Yes[d]	No	Yes	Yes	NA	No	NA

[a]Include age, BMI, socio-economic status, smoking, family history and physical activity. [b]Included in meta-analysis. [c]Except family history. [d]Except physical activity. [e]Except family history, physical activity; [f]except family history, physical activity, smoking. [g]On ecologic scale. [h]Only age. NA: not applicable.

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Air Pollution and Diabetes Risk

Assessing the Evidence to Date

Many studies have reported associations between ambient air pollution and cardiovascular disease, asthma, and cancer.[1] Diabetes mellitus also is a risk factor for vascular and respiratory diseases, and development of these outcomes in people with diabetes may be exacerbated by exposure to air pollution.[2] In this issue of *EHP*, a team of European scientists conducted a systematic review to evaluate whether air pollution exposure is also associated with developing diabetes itself.[3]

The researchers systematically searched databases for English-language articles addressing diabetes and outdoor air pollution in human subjects. They screened 636 studies and identified 13 that addressed the research question of interest. Eight pertained to type 2 diabetes, two pertained to type 1 diabetes, and three pertained to gestational diabetes. Seven of the studies on type 2 diabetes—selected because they reported air particle concentrations the same way—were pooled in a meta-analysis.

Based on three available longitudinal studies on exposure to fine particulate matter ($PM_{2.5}$), the authors estimated a 10% increased risk of type 2 diabetes per 10-mg/m^3 increase in exposure. For nitrogen dioxide (NO_2), there were two longitudinal and two cross-sectional studies available, which suggested an 8% increase in type 2 diabetes per 10-mg/m^3 increase in exposure.[3]

For both NO_2 and $PM_{2.5}$, estimated effects were more pronounced in females than males.[3] "This was one of the surprising findings of our study, considering that men are usually at higher risk for type 2 diabetes," says coauthor Ikenna Eze, a PhD candidate at the Swiss Tropical and Public Health Institute. "There could also be some unexplained sex-based physiologic differences which could account for this." Alternatively, women generally tend to stay around the home more than men,[4] hence residence-based exposure estimates may have better captured their actual exposures.

Positive associations reported in the epidemiologic literature give credence to the hypothesis that air pollution exposure may increase the risk of developing diabetes, says Patricia Coogan, an epidemiology research professor at Boston University and coauthor of one of the studies reviewed.[5] "Even more convincing, I think, are the animal and clinical studies indicating that air pollution can affect insulin sensitivity and other biologic pathways relevant to diabetes," Coogan says.

Ursula Krämer, a professor at the IUF-Leibniz Research Institute for Environmental Medicine whose study was included in the meta-analysis,[6] believes the association between air pollution exposure and development of diabetes is plausible. "Subclinical inflammation is a major driving force for the incidence of diabetes, and particle pollution can cause subclinical inflammation," she says. "I fully agree with the main conclusion of the authors: Research should be expanded to developing countries, where a steep increase in diabetes type 2 was observed in the last decade and where outdoor and indoor pollution is much higher than in Europe and North America."

Wendee Nicole was awarded the inaugural Mongabay Prize for Environmental Reporting in 2013. She writes for *Discover*, *Scientific American*, *National Wildlife*, and other magazines.

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Subclinical inflammation, which can be caused by exposure to particulate matter, is "a major driving force" behind diabetes.
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Long-term air pollution exposure and diabetes in a population-based Swiss cohort

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ABSTRACT

Air pollution is an important risk factor for global burden of disease. There has been recent interest in its possible role in the etiology of diabetes mellitus. Experimental evidence is suggestive, but epidemiological evidence is limited and mixed. We therefore explored the association between air pollution and prevalent diabetes, in a population-based Swiss cohort.

We did cross-sectional analyses of 6392 participants of the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults [SAPALDIA], aged between 29 and 73 years. We used estimates of average individual home outdoor PM_{10} [particulate matter <10 μm in diameter] and NO_2 [nitrogen dioxide] exposure over the 10 years preceding the survey. Their association with diabetes was modeled using mixed logistic regression models, including participants' study area as random effect, with incremental adjustment for confounders.

There were 315 cases of diabetes (prevalence: 5.5% [95% confidence interval (CI): 2.8, 7.2%]). Both PM_{10} and NO_2 were associated with prevalent diabetes with respective odds ratios of 1.40 [95% CI: 1.17, 1.67] and 1.19 [95% CI: 1.03, 1.38] per 10 $\mu g/m^3$ increase in the average home outdoor level. Associations with PM_{10} were generally stronger than with NO_2, even in the two-pollutant model. There was some indication that beta blockers mitigated the effect of PM_{10}. The associations remained stable across different sensitivity analyses.

Our study adds to the evidence that long term air pollution exposure is associated with diabetes mellitus. PM_{10} appears to be a useful marker of aspects of air pollution relevant for diabetes. This association can be observed at concentrations below air quality guidelines.

1. Introduction

Ambient air pollution, indoor air pollution and hyperglycemia constitute major risks for the global burden of disease (Lim et al., 2012). Air pollution is associated with cardiovascular diseases (Auchincloss et al., 2008; Hoffmann et al., 2007), and chronic respiratory diseases (Künzli et al., 2009; Schikowski et al., 2010) and has been shown to contribute to hospitalizations and deaths among cardiac disease patients (Goldberg et al., 2013), and diabetic patients (Goldberg et al., 2013; O'Neill et al., 2005; Zanobetti and Schwartz, 2001). Type 2 diabetes is increasing globally and is already one of the major causes of death (Lim et al., 2012). Type 2 diabetes and cardiovascular diseases share similar risk factors. Air pollution could be involved in the etiology of type 2 diabetes mellitus. Postulated mechanisms of action include oxidative stress and low grade inflammation, endothelial dysfunction, visceral adipose tissue inflammation, endoplasmic reticulum stress and mitochondrial dysfunction (Liu et al., 2013; Rajagopalan and Brook, 2012) with resulting impairment in insulin signaling (Xu et al., 2013).

Animal and human biomarker studies, including sparse epidemiological studies contribute to this evidence. Animal studies suggest a contribution of fine particles to insulin resistance, especially in association with a high fat diet (Sun et al., 2009; Xu et al., 2011; Yan et al., 2011). Chuang et al. (2007) demonstrated an alteration in glycosylated hemoglobin C, blood lipids and blood pressure in young adults in Taipei, after exposure to particulate matter and ozone.

Epidemiological evidence is sparse and findings are mixed. Longitudinal studies in European and North American populations (Andersen

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et al., 2012; Chen et al., 2013; Coogan et al., 2012; Krämer et al., 2010; Puett et al., 2011), found inconsistent associations between incident diabetes mellitus and PM_{10} [particulate matter < 10 μm in diameter], NO_2 [nitrogen dioxide], NO_x [nitrogen oxides], $PM_{2.5}$ [particulate matter < 2.5 μm in diameter], $PM_{10-2.5}$ [particulate matter with diameter between 2.5 and 10 μm] and residential proximity to traffic. Although the previous studies taken together with experimental evidence support the evidence for an association between inhaled pollutants and diabetes, several aspects may contribute to uncertainties and inconsistencies. Limiting factors toward more conclusive evidence include differences in (a) exposure metrics and assessment; (b) diabetes definition; (c) population characteristics and (d) covariates considered (Papazafiropoulou et al., 2011; Rajagopalan and Brook, 2012). Two epidemiological studies have investigated the association between air pollution and prevalent type 2 diabetes, with contradictory results on NO_2 effects (Brook et al., 2008; Dijkema et al., 2011). Noise can positively correlate with air pollution (Foraster, 2013; Kim et al., 2012) and has been implicated in cardiovascular diseases (Dratva et al., 2012; Sorensen et al., 2011), as well as more recently with diabetes (Sorensen et al., 2013). The quality and quantity of sleep have been shown to be significant predictors of the risk of type 2 diabetes (Cappuccio et al., 2010). Thus, noise can be considered a potential confounder in air pollution epidemiology studies.

To add to the epidemiologic evidence base on the newly uncovered, potentially causal relationship between air pollution and diabetes, we investigated the association between ambient/traffic-related air pollution and prevalent diabetes mellitus in the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults [SAPALDIA], taking noise exposure, individual and area-level socio-economic index into consideration.

2. Materials and methods

2.1. Study population and health examinations

At baseline [SAPALDIA 1; 1991], the study population of SAPALDIA included 9561 randomly selected participants aged 18–65 years. These participants were selected from eight different areas in Switzerland, representing a wide range of environmental conditions in Switzerland. Subjects had extensive health examinations which involved computer-assisted interviews, lung function and allergy testing. At the first follow-up [SAPALDIA 2; 2002], the health assessments were repeated in 8047 participants, with more detailed interviews, including information on diabetes and other chronic non-communicable diseases, blood testing for biomarkers and genotyping. This is described in detail elsewhere (Ackermann-Liebrich et al., 2005). For the purpose of the present analysis, we had a sample of 6392 follow-up participants, aged 29–73 years, who had complete information on all the variables of interest, for assessing the association between air pollution and diabetes mellitus.

2.2. Definition of diabetes mellitus

At SAPALDIA 2, participants were asked “do you have diabetes mellitus?” and “was it diagnosed by a physician?” Participants' non-fasting blood samples were taken to measure blood markers, including non-fasting blood glucose, glycosylated hemoglobin C [HbA1c] and blood lipids. Based on the available information, we defined diabetes as present if at least one of the following conditions was met i) intake of any anti-diabetic medication; ii) self-reported, physician-diagnosed diabetes mellitus; iii) non-fasting blood glucose of >11.1 mmol/L or iv) HbA1c of >6.5% or 48 mmol/mol. Since this is an adult population [minimum age of 29 years] and >90% of diabetes in adults is of type 2, we assumed the majority of diabetic cases in this population to be type 2 diabetes mellitus.

2.3. Individual assignment of exposures

We considered markers of ambient air pollution [PM_{10}] and traffic-related air pollution [NO_2] as our air pollution exposure indicators. Estimates of mean ambient levels of these pollutants were available for the residential addresses of the participants in the years 1990 and 2000, the years before health assessments (Liu et al., 2007). They were obtained from validated dispersion models, with different emission inventories for both years. They have a spatial resolution of 200 × 200 m (Liu et al., 2007). Annual trends at fixed monitoring sites and participants' residential histories were used to estimate average ambient residential levels of the two pollutants over periods of 1 to 10 years prior to the first follow-up assessment in 2002. The dispersion model for PM_{10} provided good predictions both at background and traffic sites, whereas the model for NO_2 provided better predictions at traffic sites while underestimating levels at background sites (Liu et al., 2007). For this reason, the dispersion model for NO_2 was extended to a hybrid model involving land-use regression components (Liu et al., 2012). For this analysis, we primarily used the modeled average ambient levels of PM_{10} and NO_2 at participants' residential addresses over the 10 years preceding the first follow-up survey.

We obtained estimates of road traffic and railway noise from sonBASE, the Swiss national noise database (FOEN, 2009a,b). This database, developed by the Swiss Federal Office of Environment, provides average railway and road traffic noise estimates for day [0600 h–2200 h] and night [2200 h–0600 h]. Noise propagation was estimated with 10 × 10 meter grids and for individual buildings using the StL86 + emission model for road traffic noise and SEMIBEL [Swiss emission model for the estimation of railway noise] for railway noise (FOEN, 2009b). These estimates were then assigned to participants' residential addresses. From the day and night estimates, we estimated the average day–night [L_{dn}] noise exposure level by applying a penalty of 10 dB on the night noise estimates for both road traffic and railway noise. The L_{dn} value at the participant's address of the first follow-up survey was used as measure of individual noise exposure in the regression analysis.

2.4. Potential confounding variables

From the computer-assisted interviews at SAPALDIA 2, we extracted information on potential confounders. These included participants' age, sex [male, female], height and weight to compute the body mass index [BMI; kg/m^2], and educational attainment [low corresponding to primary education; intermediate corresponding to secondary, middle, or vocational school; and high corresponding to technical college or university]. Neighborhood-level socio-economic index was obtained for participants' residential areas. This index was defined using neighborhood characterization based on median rent, occupation and education of heads of households and crowding of households, combined in a principal component analysis (Panczak et al., 2012). We also extracted information on physical activity [≤0.5 h per week, 0.5–2 h per week and >2 h per week of vigorous activity], smoking [never, former, current and pack years smoked], environmental tobacco smoking in the past 12 months [never smoker, and former smoker] and alcohol consumption [never, ≤once a day, and >once a day], and occupational exposure to gases, dusts and fumes [yes/no]. In addition, we extracted information on consumption of raw vegetables [never, ≤3 days per week, and >3 days per week], consumption of citrus fruits [never, ≤3 days per week, and >3 days per week] and consumption of other fruits [never, ≤3 days per week, and >3 days per week]. We also extracted information on some existing co-morbidities including hypertension [yes/no], and chronic obstructive pulmonary disease [COPD; defined by GOLD standard: forced expiratory volume in 1 s (FEV_1) ÷ forced vital capacity (FVC) < 0.7; yes/no].

Since the parameter of air pollution exposure was the mean ambient residential level over the ten years preceding the first follow-up survey, we also considered some baseline exposure characteristics, as potential

confounders. We extracted information on baseline smoking history [never, former, current and pack years smoked], environmental tobacco smoking in the past 12 months [never smoker, former smoker] and occupational exposure to gases, dusts or fumes [yes/no].

2.5. Statistical analysis

We included 6392 participants with complete information on the variables of interest in this analysis. First, we estimated the prevalence of diabetes mellitus among the study sample. We then evaluated the distribution of various characteristics among participants, stratified by diabetes status.

Second, we assessed the association between air pollution and prevalent type 2 diabetes mellitus using mixed logistic regression models, with a random intercept for the different study areas. We selected potential confounders based on literature review and plausibility and added them to the model in an incremental manner. Our fully-adjusted model included age, sex, BMI, educational status, neighborhood socio-economic index, smoking status, pack years of cigarettes smoked, environmental tobacco smoking status, occupational exposure to gases, dusts or fumes, consumption of alcohol, raw vegetables, citrus fruits and other fruits, and average railway and road traffic noise exposure. In some exploratory analyses, we additionally adjusted for self-reported hypertension, inflammatory markers including high sensitivity C-reactive protein and dyslipidemia. We assessed this association singly for each pollutant [single pollutant model] and in combination [two-pollutant model].

Third, we assessed potential effect modifiers. The pre-selected candidates included age group [≤50 years, and >50 years], sex, obesity [BMI > 30 kg/m^2], educational level [low, intermediate, and high], physical activity [low, medium, and high], COPD [yes/no], hypertension [yes/no] and intake of beta-blockers [yes/no]. Beta-blockers have been shown to be protective on the cardiac effects of

Table 1
Background characteristics of participants by diabetes status.

Characteristic (%)	Diabetes mellitus N = 315	No diabetes mellitus N = 6077	p-Value [chi-square]
Females	34.6	52.2	<0.001
Smoking status [yes/no]			<0.001
Never	35.6	43.9	
Former	42.5	31.0	
Current	21.9	25.1	
ETS [yes/no]			0.768
Never smoker	7.0	6.6	
Former smoker	6.0	6.5	
Physical activity [yes/no]			<0.001
<0.5 h/week	58.1	37.7	
0.5–2 h/week	23.8	34.2	
>2 h/week	18.1	28.1	
Educational level [yes/no]			<0.001
Low	11.4	5.9	
Intermediate	65.4	65.6	
High	23.2	28.5	
Work exposure to gas/dusts/fumes [yes/no]	25.7	27.6	0.474
Alcohol consumption [yes/no]			<0.001
Never	14.6	8.9	
≤Once/day	71.4	82.3	
>Once/day	14.0	8.8	
Raw vegetable consumption [yes/no]			0.288
Never	0.3	0.6	
≤3 days/week	20.3	18.8	
>3 days/week	79.4	80.6	
Citrus fruits consumption [yes/no]			0.045
Never	12.7	8.2	
≤3 days/week	54.0	56.2	
>3 days/week	33.4	35.7	
Other fruits consumption [yes/no]			0.053
Never	1.9	1.8	
≤3 days/week	25.7	33.6	
>3 days/week	72.4	64.6	
Duration of residence < 10 years	30.8	42.4	<0.001
Duration of residence ≥ 10 years	69.1	57.6	
Self-reported hypertension [yes/no]	52.4	17.7	<0.001
COPD (FEV_1/FVC < 0.7) [yes/no]	22.5	19.7	0.209
Dyslipidemia [yes/no]	73.7	46.6	<0.001
High hs-CRP [yes/no]	72.4	48.8	<0.001
Mean (SD)			T-test
Age [years]	60.8 (8.1)	51.7 (11.4)	<0.001
BMI [kg/m^2]	30.3 (5.1)	25.6 (4.3)	<0.001
Pack-years of smoking	16.4 (25.1)	10.5 (17.9)	<0.001
Neighborhood socio-economic index	63.6 (10.1)	62.1 (10.4)	0.005
10-year mean PM_{10} [μg/m^3]	24.4 (7.2)	22.2 (7.4)	<0.001
10-year mean NO_2 [μg/m^3]	29.2 (10.5)	26.7 (11)	<0.001
Mean railway noise [dB]	11.6 (13.5)	10.3 (13.0)	0.076
Mean street noise [dB]	49.9 (9.0)	49.4 (8.8)	0.365

ETS: Environmental tobacco smoking; COPD: chronic obstructive pulmonary disease. FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity. hs-CRP: high sensitivity C-reactive protein. High hs-CRP is defines as hs-CRP ≥ 1.0 mmol/L, the median hs-CRP. Dyslipidemia defined as triglyceride ≥ 1.7 mmol/L and/or high density lipoprotein ≤ 1.03 mmol/L in men or ≤1.29 mmol/L in women. Low education corresponds to primary school level, intermediate corresponds to secondary, middle, or vocational school, and high education corresponds to technical college or university. IQR: Inter-quartile range.

$PM_{2.5}$ (Folino et al., 2009; Lotti, 2011). Non-selective beta blockers have also been shown to improve insulin sensitivity among cardiac and diabetic patients (Hara et al., 2003; Kveiborg et al., 2006), possibly through their anti-atherogenic, anti-inflammatory and oxygen perfusion improvement properties (Bell, 2004). We generated interaction terms between each of the potential effect modifiers and the variables of PM_{10} and NO_2 exposure, and added these interaction terms to the fully-adjusted model one by one. We estimated separate effects of the respective air pollutant variable for the groups compared, from the same model. Heterogeneity of these separate estimates was assessed using the likelihood ratio test and the p-values were noted. Finally, we did some sensitivity analyses, using the fully-adjusted single-pollutant model, to check the robustness of the estimated association of air pollution on the prevalence rate of diabetes mellitus. In the first sensitivity analysis, we restricted the analysis to those who had lived in the same residence between SAPALDIA 1 and SAPALDIA 2, since our noise data was from a single measurement during follow-up. Next, we excluded participants with any heart disease from the model. In another sensitivity analysis, we excluded cases that reportedly started anti-diabetic medication before or at baseline. We also restricted the diabetes definition to each of the diagnostic criterion used to identify diabetes cases, excluding the diabetes cases not matching the criterion, from the controls. We also adjusted for participation bias using the inverse probability weighting (ignoring area as a random effect). We did this by deriving a model for the probability of participation based on informative predictor variables assessed at baseline, i.e., age, sex, BMI, nationality, educational status, chronic disease status and lifestyle characteristics. We then weighted each participant based on their probabilities and added it to the fully adjusted model. Lastly, we tested linearity of association by introducing quadratic terms of the exposure variables to the model.

In most analyses, participants' study area was treated as a random effect [except in some sensitivity analyses]. This is to account for the gradient between health outcomes and exposure levels across study areas, and not exclusively focusing only on within-area gradients, which leads to loss of some statistical power.

We used STATA statistical software version 12 (StataCorporation, 2011) for all statistical analyses and defined statistical significance at the 5% level.

3. Results

3.1. Characteristics of study population

The prevalence of diabetes mellitus in the study sample was 5.5% [95% confidence interval (CI): 2.8, 7.2%]. The mean age of the participants was 52 years and about 50% of them were females (Table 1). Males constituted 65% of the diabetics, were more overweight/obese [64% vs. 43%], smoked twice the pack-years of females [14 vs. 7.7] and were more often current smokers [27% vs. 22%], but mean age was the same for males and females. The mean PM_{10} exposure in the study population was 22.3 $\mu g/m^3$ [WHO air quality guideline: 20 $\mu g/m^3$ (WHO, 2006)] whereas mean NO_2 exposure was 26.8 $\mu g/m^3$ [WHO: 40 $\mu g/m^3$ (WHO, 2006)]. The mean railway noise exposure was 10.4 dB whereas the mean road traffic noise was 49.5 dB. Participants with diabetes were older, had higher body mass index, smoked more and were more likely exposed to environmental tobacco smoke. Furthermore, diabetic subjects were less educated, and consumed less fruits but more alcohol. In addition, diabetic subjects had higher exposures to PM_{10} and NO_2 and were more likely to remain in the same residential area over the course of follow-up. Diabetic subjects were also more likely to be hypertensive and have COPD [Table 1]. Table A1 summarizes excluded subjects vs. included subjects based on the background characteristics and shows no substantial differences between these groups. Fig. A1 shows the identification of diabetes cases for this study.

3.2. Association between air pollution and diabetes mellitus

For every 10 $\mu g/m^3$ increase in home outdoor PM_{10} or NO_2, the fully adjusted odds ratio for prevalent diabetes mellitus was 1.40 [95% CI: 1.17, 1.67] and 1.19 [95% CI: 1.03, 1.38], respectively. The unadjusted odds ratio for prevalent diabetes mellitus was 1.46 [95% CI: 1.20, 1.77] and 1.20 [95% CI: 1.03, 1.39] respectively [Table 2]. Additional adjustment for neither age and sex nor educational level and neighborhood-level socio-economic index appreciably changed the estimates. Additional adjustment for lifestyle characteristics such as physical activity, diet, smoking and alcohol consumption, reduced the home outdoor PM_{10} estimate by 11% [OR: 1.35 (95% CI: 1.12, 1.63)]; and NO_2 estimate by 3% [OR: 1.17 (95% CI: 1.02, 1.36)]. The effect estimate for home outdoor NO_2 and PM_{10} increased by 4% [OR: 1.21 (95% CI: 1.05, 1.39)] and 9% [OR: 1.40 (1.21, 1.71)] respectively, upon additional adjustment for body mass index. Additional adjustment for noise further reduced the effect estimates, but these estimates remained stable all through the adjustments, including hypertension, high sensitivity C-reactive protein (hs-CRP) and dyslipidemia [Table 2].

For the multi-pollutant model, the unadjusted odds ratio for home outdoor PM_{10} and NO_2 was 1.37 [95% CI: 1.02, 1.84] and 1.02 [95% CI: 0.84, 1.25] respectively. These estimates remained fairly stable following additional adjustments [Table 3].

A multivariate comparison across study areas, showed a consistent association between adjusted diabetes prevalence rates and the community air pollution levels ($r = 0.88$ and 0.70 for PM_{10} and NO_2 respectively). Areas with higher air pollution levels tended to have higher rates of diabetes [Figs. A2 and A3]. Also, the effect estimates did not substantially change when we removed one area at a time in our model [result not shown].

Table 2
Association between home outdoor air pollution and diabetes mellitus [single pollutant models].

	NO_2 OR [95% CI]	PM_{10} OR [95% CI]
Unadjusted	1.20 [1.03, 1.39]	1.46 [1.20, 1.77]
Adjusted for age and gender	1.23 [1.06, 1.43]	1.43 [1.18, 1.74]
+ adjusted for educational level and neighborhood SEI	1.22 [1.05, 1.41]	1.45 [1.23, 1.72]
+ adjusted for lifestyle characteristics[a]	1.17 [1.02, 1.36]	1.35 [1.12, 1.63]
+ adjusted for body mass index	1.21 [1.05, 1.39]	1.44 [1.21, 1.71]
+ adjusted for noise	1.19 [1.03, 1.38]	1.40 [1.17, 1.67]
+ adjusted for hypertension	1.17 [1.01, 1.36]	1.37 [1.14, 1.65]
+ adjusted for high hs-CRP and dyslipidemia	1.21 [1.04, 1.40]	1.41 [1.17, 1.69]

SEI: socio-economic index. OR: odds ratio. OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2. CI: confidence interval. hs-CRP: high sensitivity C-reactive protein. High hs-CRP defined as CRP level > sample median (1.0 mmol/L). Dyslipidemia defined as triglyceride ≥ 1.7 mmol/L and/or high density lipoprotein ≤ 1.03 mmol/L in men or ≤1.29 mmol/L in women. Area was treated as a random effect in all models. + indicates additional adjustment. N = 6392 at all levels of adjustment except for hs-CRP and dyslipidemia where N = 6111.

[a] Include alcohol consumption, smoking, passive smoking, work exposure to dust gas and fumes, consumption of alcohol, fruits and raw vegetables and physical activity.

Table 3
Association between home outdoor air pollution and diabetes mellitus [two-pollutant models].

	NO_2 OR [95% CI]	PM_{10} OR [95% CI]
Unadjusted	1.03 [0.83, 1.27]	1.41 [1.02, 1.96]
Adjusted for age and gender	1.10 [0.87, 1.37]	1.28 [0.90, 1.82]
+ adjusted for educational level and neighborhood SEI	1.03 [0.84, 1.28]	1.40 [1.03, 1.90]
+ adjusted for lifestyle characteristics[a]	1.03 [0.83, 1.28]	1.31 [0.95, 1.79]
+ adjusted for body mass index	1.04 [0.86, 1.27]	1.37 [1.02, 1.85]
+ adjusted for noise	1.02 [0.84, 1.25]	1.37 [1.02, 1.84]
+ adjusted for hypertension	1.02 [0.82, 1.26]	1.35 [0.99, 1.84]
+ adjusted for high hs-CRP and dyslipidemia	1.04 [0.84, 1.29]	1.35 [0.99, 1.84]

SEI: socio-economic index. OR: odds ratio. OR values represent % increase in diabetes prevalence per 10 μg/m^3 increase in PM_{10} or NO_2. CI: confidence interval. hs-CRP: high sensitivity C-reactive protein. High hs-CRP defined as CRP level > sample median (1.0 mmol/L). Dyslipidemia defined as triglyceride ≥ 1.7 mmol/L and/or high density lipoprotein ≤ 1.03 mmol/L in men or ≤1.29 mmol/L in women. Area was treated as a random effect in all models. + indicates additional adjustment. N = 6392 at all levels of adjustment except for hs-CRP and dyslipidemia where N = 6111.

[a] Include alcohol consumption, smoking, passive smoking, work exposure to dust gas and fumes, consumption of alcohol, fruits and raw vegetables and physical activity.

3.3. Effect modification

We did not find any statistically significant interaction term with the selected potential modifiers [Table 4]. Intake of beta-blocker may be protective for PM_{10} with OR 0.23 [95% CI: 0.02, 3.32] vs. 1.41 [95% CI: 1.18, 1.69] for those not taking the medication.

3.4. Sensitivity analyses

Restricting the analysis to subjects who did not change their residential address between baseline and follow-up assessments and to persons without self-reported heart disease did not substantially alter the association between air pollution and diabetes [Table 5]. Excluding participants who reported diabetes medication intake before baseline assessment increased the estimates of association by 2% for both pollutants [Table 5]. Associations remained positive and significant when we narrowed diabetes definition to each criterion used for case identification except for narrowing the definition of diabetes to reported intake of anti-diabetic medication where associations remained positive but statistically insignificant [Table A2]. This may imply under-reporting of diabetic medication intake among those we identified as diabetic. The mean probability of participation in this study (from baseline) was 66.2%; therefore, we adjusted for participation bias (IPW) which gave adjusted odds ratios of 1.39 [95% CI: 1.15, 1.67] and 1.18 [95% CI: 1.05, 1.34] per 10 μg/m^3 increase in PM_{10} and NO_2 respectively [Table 5]. There was evidence that associations might be slightly non-linear. The coefficients of the quadratic terms of NO_2 and PM_{10} were negative (−0.00152 and −0.00144), with respective p-values of 0.004 and 0.164. This might imply attenuation of effects at higher levels of exposure. When treating area as a fixed effect, no significant association between air pollution exposure and diabetes mellitus could be seen anymore and the 95% CI of these effect estimates got wide [Table A3]. We did not observe strong heterogeneity in area-specific effects of PM_{10} and NO_2.

4. Discussion

In this analysis, we found that long-term exposure to PM_{10} and NO_2 were positively associated with prevalent diabetes mellitus in the SAPALDIA cohort, at concentrations below the air quality guidelines. As mentioned earlier, we assume the diabetes cases to be predominantly type 2, since >90% of adult diabetes is type 2 diabetes. The associations were independent of traffic-related noise exposure, individual and area-level socioeconomic status. They were in fact remarkably insensitive to adjustment for potential confounders. Based on evidence and physical properties, $PM_{2.5}$ could be a better predictor of health effects of air pollution than PM_{10}, but the associations would essentially be the same due to the high spatial and temporal correlations in the SAPALDIA study areas (measured at a later point), given their ratio of ~0.80.

In contrast to the results for NO_2, those for PM_{10} were very different when area was controlled as a fixed instead of a random effect. The absence of significant associations within areas, shown by the fixed effect estimates, could have several reasons. First, power to detect within-area associations is clearly lower for PM_{10} due to its low spatial variation within these rather small geographic areas. NO_2 has instead larger contrasts within areas as it picks up the local contrasts of traffic related pollution. Second, PM_{10} is known to have different compositions across areas. Depending on the PM_{10} composition, the diabetogenic toxicity may vary, thus, adding to the heterogeneity in the within-area effects. Instead, NO_2 is generally an indicator of local traffic-related pollution, which is a comparable source all across Switzerland. Lastly, the prevalence of possible susceptibility factors varied across areas, for instance the proportion of high physical activity and alcohol intake > once/day varied from 8.1 to 42% and 1.7 to 21.9% respectively. However, effect estimates for PM_{10} and NO_2 remained quite stable when we additionally considered interaction terms between these factors and the exposure variables or study area. Thus, variation in susceptibility factors is unlikely to explain the observed difference in the associations within and across study areas. Since associations between air pollution and diabetes across areas might be confounded by lifestyle characteristics at the area level, we conducted additional sensitivity analyses including area means of socio-demographic and lifestyle characteristic. Again, effect estimates of PM_{10} and NO_2 remained remarkably stable.

This study adds to the growing, but still inconsistent evidence on the cross-sectional and longitudinal association between air pollution and possible type 2 diabetes. Brook et al. (2008) found a positive association between NO_2 and prevalent diabetes mellitus in women, but not men, who attended respiratory clinics in Hamilton and Toronto, Canada [OR: 1.04; 95% CI: 1.00, 1.08 for every 1 ppb increase in NO_2]. Dijkema et al. did not find any association of diabetes with NO_2 and traffic proximity estimates (Dijkema et al., 2011). In a purely ecologic comparison, Pearson et al. (2010) found a positive association between $PM_{2.5}$ and diabetes prevalence at the county level.

The longitudinal studies on incident diabetes were a bit more consistent. Kraemer et al. found the hazard of diabetes to be increased by 15–42% per interquartile range (IQR) of PM or traffic-related exposure measured as NO_2 in a German cohort of 1775 adult females (Krämer et al., 2010). Chen et al. reported a hazard ratio of 1.11 [95% CI: 1.02, 1.21], for diabetes as recorded in the Ontario diabetes database, per 10 μg/m^3 increase in 6-year average $PM_{2.5}$ in a Canadian cohort of 62,012 adults (Chen et al., 2013). In a Danish registry-based study involving 51,818 participants (Andersen et al., 2012), NO_2 was also associated with confirmed diabetes cases [HR = 1.04; 95% CI: 1.00, 1.08 per 2.6 ppb interquartile range of NO_2]. Coogan et al. studied 3992 African–American women in Los Angeles and reported an incidence risk ratio of 1.25 [95% CI: 1.07, 1.46] per 12.4 ppb IQR of NO_x and 1.63 [95% CI: 0.78, 3.44] per 10 μg/m^3 increase in $PM_{2.5}$ (Coogan et al.,

Table 4
Modification of the association between air pollution and diabetes mellitus.

Variable	Categories	NO_2 OR [95% CI]	PM_{10} OR [95% CI]
Age	≤50 years	1.22 [0.87, 1.71]	1.34 [0.81, 2.20]
	>50 years	1.18 [1.01, 1.38]	1.42 [1.18, 1.71]
	Interaction (p-value)	0.925	0.813
Sex	Males	1.25 [1.06, 1.48]	1.53 [1.29, 1.90]
	Females	1.11 [0.91, 1.36]	1.18 [0.89, 1.58]
	Interaction (p-value)	0.300	0.146
Obesity (BMI > 30 kg/m^2)	No	1.19 [1.00, 1.40]	1.37 [1.10, 1.67]
	Yes	1.13 [0.93, 1.34]	1.28 [0.99, 1.70]
	Interaction (p-value)	0.639	0.702
Hypertension	No	1.14 [0.90, 1.43]	1.34 [0.98, 1.84]
	Yes	1.19 [1.01, 1.41]	1.38 [1.12, 1.71]
	Interaction (p-value)	0.687	0.881
COPD ($FEV_1/FVC < 0.7$)	No	1.15 [0.98, 1.35]	1.32 [1.08, 1.61]
	Yes	1.31 [1.02, 1.67]	1.61 [1.11, 2.34]
	Interaction (p-value)	0.326	0.331
Educational level	Low	1.13 [0.83, 1.55]	1.32 [0.78, 2.48]
	Medium	1.22 [1.03, 1.44]	1.46 [1.18, 1.79]
	High	1.17 [0.91, 1.51]	1.23 [0.84, 1.79]
	Interaction (p-value)	0.885	0.698
Physical activity	Low	1.14 [0.96, 1.35]	1.37 [1.11, 1.70]
	Medium	1.23 [0.94, 1.60]	1.31 [0.90, 1.90]
	High	1.38 [1.03, 1.86]	1.73 [1.07, 2.80]
	Interaction (p-value)	0.456	0.618
Intake of Beta-blockers	No	1.19 [1.03, 1.38]	1.41 [1.18, 1.69]
	Yes	1.83 [0.52, 6.39]	0.23 [0.02, 3.32]
	Interaction (p-value)	0.388	0.185

OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2; OR: odds ratio, CI: confidence interval; kg/m^2: kilogram per meter squared; COPD: chronic obstructive pulmonary disease, FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity. OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2 in each category. Area was treated as a random effect in all models. The odds ratio of each category represents a stratified analysis for the category. Odds ratio in each category represents the effect in that group whereas the p-value of interaction term represents the p-value of the likelihood ratio test. All models were adjusted for age, sex, educational status, neighborhood socio-economic index, smoking status, pack-years of smoking, environmental tobacco smoking, occupational exposure to dusts, gases and fumes, consumption of alcohol, raw vegetables and fruits, physical activity, body mass index and noise. Low education corresponds to primary school level, intermediate corresponds to secondary, middle, or vocational school, and high education corresponds to technical college or university.

2012) whereas Puett et al., in the Health Professionals Follow-up Study and Nurses' Health Study, found an association only among female nurses living <50 m from a roadway [HR: 1.14, 95% CI: 1.03, 1.27] (Puett et al., 2011).

In our two-pollutant model, there was an attenuation of the effect of NO_2 from 1.19 [95% CI: 1.03, 1.38] to 1.02 [0.84, 1.25]. The Black Women Health Study reported a similar pattern with the attenuation of NO_x coefficient in the model that included $PM_{2.5}$ (Coogan et al., 2012). Unlike some studies that found a stronger effect in women (Brook et al., 2008; Chen et al., 2013), we did not find any substantial gender differences in our analysis. One of the reasons for observing a gender difference, apart from chance or hormonal differences, is potential exposure misclassification, because exposure estimates were based on residential addresses and women are believed to stay more around the home than men (Brook et al., 2008). Our exposure estimates were also based on the residential addresses and we found a slightly weaker effect in women, which might be a chance finding. Similar to the Canadian cohort (Chen et al., 2013) and the Danish cohort (Andersen et al., 2012), we did not find any significant interactions with any co-morbidities. Unlike the Danish study (Andersen et al., 2012), we did not find any interaction with physical activity, even though we observed stronger effects among the physically active for both pollutants. Whereas Kraemer et al. found a higher effect of living near a busy road in women of low education [HR: 2.54; 95% CI: 1.31–4.91, p = 0.006], we did not find any interaction with educational level.

4.1. Biological mechanisms linking air pollution to development of diabetes mellitus

Air pollution causes subclinical inflammation and appears to mediate components of the metabolic syndrome including impaired vascular endothelial function, and alterations in the central autonomic tone, visceral and brown adipose tissue, with mitochondrial and hepatic insulin receptor dysfunction (Liu et al., 2013; Rajagopalan and Brook, 2012). Apart from the experimental studies on mouse models which showed insulin resistance among rats, regardless of the type of diet given (Sun et al., 2009; Xu et al., 2011), human epidemiological studies have also demonstrated insulin resistance after air pollution exposure. Thiering et al. found a positive association between long-term exposure to NO_2 and PM_{10}, and homeostatic model assessment (HOMA) of insulin resistance among 10-year old children in Germany. Insulin resistance increased by 17% [95% CI: 5.0, 30.3] and 18.7% [95% CI: 2.9, 36.9] for every 2SD increase in NO_2 and PM_{10} respectively (Thiering et al., 2013). Similarly, Kelishadi and colleagues found positive associations between exposure to PM_{10}, and NO_2 [and other markers of air pollution], and insulin resistance [and other markers of inflammation and oxidative stress] among children in Iran (Kelishadi et al., 2009).

4.2. Strengths and limitations of this study

This study draws from the extensive database of the SAPALDIA study. This is the first cross-sectional study assessing this association with detailed confounding adjustment, including several lifestyle characteristics, health status as well as noise exposure. Our air pollution estimates were derived annually, over the 10 years preceding the first follow-up. This provided reliable estimates for cumulative exposure of the participants in this study. To limit outcome misclassification, we tried to identify undiagnosed cases through tests for non-fasting blood glucose and HbA1c, the gold standard for diagnosis of diabetes mellitus.

One major limitation of this study was the inclusion of all cases of self-reported, physician-diagnosed diabetes in the analysis irrespective of time of diagnosis. We did not have this information for all diabetes cases. However, we had information on some who reported

Table 5
Sensitivity analyses.

	N [cases/controls]	NO_2 OR [95% CI]	PM_{10} OR [95% CI]
Subjects living in same residence over 10-year follow-up period	3719 [218/3719]	1.17 [1.00, 1.37]	1.36 [1.09, 1.70]
Subjects without self-reported heart disease	5951 [259/5692]	1.21 [1.04, 1.41]	1.41 [1.16, 1.71]
Exclusion of diabetes reported at or before baseline assessment	6373 [296/6077]	1.21 [1.04, 1.41]	1.42 [1.17, 1.72]
Adjustment for participation bias (Inverse probability weighting)	6392 [315/6077]	1.18 [1.05, 1.34]	1.39 [1.15, 1.67]

OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2. OR: odds ratio, CI: confidence interval. Area was treated as a random effect in all models. All models were adjusted for age, sex, educational status, neighborhood socio-economic index, smoking status, pack-years of smoking, environmental tobacco smoking, occupational exposure to dusts, gases and fumes, consumption of alcohol, raw vegetables and fruits, physical activity, body mass index and noise. All sensitivity analyses were done using the fully-adjusted single-pollutant models.

starting anti-diabetic medication, 90% of whom started taking the medication after the baseline examination. We also performed a sensitivity analysis, excluding those who reported taking diabetes medication before baseline. Another limitation was that air pollution was modeled at participants' residences. We did not have estimates for exposure at work and at other places where outdoor activities may take place. We expect this misclassification to be mostly non-systematic, thus, leading to bias toward the null. Fortunately, attenuation due to ignoring exposure at work is expected to be small (some 10%), because people spend more of their time at home. Nevertheless, we adjusted for occupational exposure to vapor, dust and fumes, which is unlikely to confound our main findings because it is not really correlated with our exposure of interest.

To the best of our knowledge, this is the first epidemiological study to consider noise exposure as a potential confounder of the association between diabetes and ambient air pollution exposure. Experimental evidence associating noise with diabetes mellitus (Spiegel et al., 2005; Tasali et al., 2009) postulates mechanisms through sleep deprivation, imbalance of the autonomic nervous system with a relative increase in sympathetic tone, release of stress hormones and consequent increase in blood pressure, blood lipids, glucose level, clotting and viscosity. Our consideration of noise could be a strength and a limitation. As discussed above, one may hypothesize interrelated pathways where both noise and air pollution may be relevant, thus, as in the case of cardiovascular outcomes, taking noise into account in air pollution–diabetes research is a strength (Tetreault et al., 2013). On the other side, we had only outdoor noise estimates available. As discussed by Foraster, outdoor noise estimates may not be a good proxy for personal exposure to noise, thus, it is not clear to what degree our models were able to properly control for independent effects of noise (Foraster, 2013). Finally, we had only one noise exposure estimate, at participants' residences for the entire follow-up period. To address this limitation, we also did a sensitivity analysis restricting the analysis to participants having lived in the same residence between baseline and follow-up.

The potential bias due to differential non-participation deserves further investigation. Analyses involving IPW help to correct at least some of the bias, but some bias may persist. All longitudinal studies on diabetes determinants face this challenge. Diabetic individuals with more advanced disease and disease-related handicaps are more likely to die or no longer participate. Air pollution is thought to contribute to the progression of diabetes and to susceptibility for cardiovascular events (Rajagopalan and Brook, 2012). Also, our finding of effect attenuation at higher rather than lower levels of exposure (opposing the usual threshold thinking), deserves further investigation. This calls for extension of air pollution research to areas with higher pollution levels and larger contrasts as observed in many developing countries.

In conclusion, this study adds to the evidence for a moderate and independent association between air pollution and diabetes. The results point to the need of future studies to consider the composition of PM. The observed association at concentrations below air quality standards parallels associations with mortality and points to continuous needs in air quality regulation.

Conflict of interest

All authors declare no actual or potential conflict of financial or other interests.

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All authors contributed equally to the development of this manuscript.

Appendix A

Table A1
Characteristics of participants included/excluded from the study.

Variables (%)	Baseline participants excluded only (N = 1604)	Follow-up participants excluded (N = 1655)	Participants Included (N = 6392)	N of baseline participants excluded/N of follow-up participants excluded/N included in the analysis
Females	45.3	54.5	51.3	1604/1655/6392
Smoking status at baseline				
Never smoker	35.6	40.1	46.9	1602/1648/6392
Ex-smoker	22.4	20.6	23.2	
Current smoker	42.0	39.3	29.9	
Smoking status at follow-up				0/1642/6392
Never smoker		37.5	43.5	
Ex-smoker		30.6	31.6	
Current smoker		31.9	24.9	
ETS in never smokers				
Baseline	12.4	13.2	13.3	1602/1648/6392
Follow-up		5.3	6.7	0/1261/6392
ETS in ex-smokers				
Baseline	8.2	7.5	7.2	1602/1648/6392
Follow-up		6.3	6.4	0/1261/6392
Occupational exposure to dust/gases/fumes				
Baseline	36.6	31.3	30.6	1600/1628/6392
Follow-up		26.3	27.5	0/171/6392
Physical activity[a]				0/141/6392
<0.5 h/week		45.4	38.7	
0.5–2 h/week		31.2	33.7	
>2 h/week		23.4	27.6	
Educational level at baseline				1594/1643/6390
Low	27.4	19.0	13.7	
Intermediate	57.6	65.7	68.9	
High	15.0	15.3	17.5	
Educational level at follow-up				0/1649/6392
Low		18.5	6.2	
Intermediate		64.5	65.6	
High		17.0	28.2	
Alcohol consumption[a]				0/170/6392
Never		10.6	9.2	
≤Once a day		75.9	81.8	
>Once a day		13.5	9.0	
Raw vegetable consumption[a]				0/172/6392
Never		0	0.6	
≤3 days/week		16.9	18.8	
>3 days/week		83.1	80.6	
Citrus fruits consumption[a]				0/172/6392
Never		7.1	8.4	
≤3 days/week		52.9	56.1	
>3 days/week		40.0	35.5	
Other fruits consumption[a]				0/168/6392
Never		1.2	1.8	
≤3 days/week		31.6	33.2	
>3 days/week		67.3	65.0	
Areas[a]: Basel		15.1	12.9	0/195/824
Wald		17.9	18.1	0/231/1154
Davos		7.6	8.0	0/98/512
Lugano		16.1	14.5	0/208/928
Montana		5.8	9.2	0/75/589
Payerne		18.5	13.8	0/238/885
Aarau		3.4	15.1	0/44/968
Geneva		15.5	8.3	0/199/532
Diabetes cases[a]		4.9	4.9	0/1045/6392
COPD (FEV1/FVC < 0.7) cases[a]		22.7	19.8	0/185/6392
Hypertension cases[a]		15.6	19.3	0/1025/6392
Dyslipidemias[a]		48.5	47.9	0/206/6111
High hs-CRP[a]		57.8	53.4	0/206/6111
Mean (SD)				
Age at baseline (years)	40.7 (12.0)	40.7 (12.1)	41.3 (11.4)	1604/1655/6392
Age at follow-up (years)		51.8 (12.1)	52.2 (11.4)	0/1655/6392
BMI at baseline (kg/m^2)	24.4 (4.3)	24.1 (4.1)	23.8 (3.6)	1571/1618/6363
BMI at follow-up (kg/m^2)		26.6 (5.4)	25.9 (4.4)	0/206/6392
Neighborhood SEI[a]		62.7 (10.7)	63.5 (10.1)	0/1598/6392
10-year mean PM_{10} (μg/m^3)[a]		22.7 (7)	22.3 (7.4)	0/1532/6392

Table A1 (*continued*)

Variables (%)	Baseline participants excluded only (N = 1604)	Follow-up participants excluded (N = 1655)	Participants Included (N = 6392)	N of baseline participants excluded/N of follow-up participants excluded/N included in the analysis
10-year mean NO_2 ($\mu g/m^3$)[a]		28.3 (11.4)	26.8 (11)	0/1532/6392
Mean smoking pack-years		12.4 (19.9)	10.8 (18.4)	0/1493/6392
Mean railway noise (dB)[a]		10.5 (13.1)	10.4 (13.1)	0/1595/6392
Mean street noise (dB)[a]		49.7 (9.4)	49.5 (8.8)	0/1595/6392

ETS: Environmental tobacco smoking; COPD: chronic obstructive pulmonary disease. FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity. hs-CRP: high sensitivity C-reactive protein. High hs-CRP is defines as hs-CRP ≥ 1.0 mmol/L, the median hs-CRP. Dyslipidemia defined as triglyceride ≥ 1.7 mmol/L and/or high density lipoprotein ≤ 1.03 mmol/L in men or ≤1.29 mmol/L in women. Low education corresponds to primary school level, intermediate corresponds to secondary, middle, or vocational school, and high education corresponds to technical college or university. SEI: socio-economic index. IQR: inter-quartile range.

[a] Measured only at follow-up.

Table A2
Association between air pollution and diabetes mellitus, stratified by case definition criteria.

	Self-reported, physician-diagnosed diabetes		Non-fasting blood glucose ≥ 11.1 mmol/L or HbA1c ≥ 0.065.		Self-reported diabetes medication	
	NO_2	PM_{10}	NO_2	PM_{10}	NO_2	PM_{10}
OR [95% CI]	1.18 [0.99,1.40] N = 6306; Cases = 229	1.31 [1.02, 1.67]	1.26 [1.09, 1.45] N = 6298; Cases = 221	1.48 [1.19, 1.82]	1.19 [0.96, 1.48] N = 6224; Cases = 147	1.12 [0.84, 1.51]

OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2. OR: odds ratio, CI: confidence interval. Area was treated as a random effect in all models. All models were adjusted for age, sex, educational status, area socio-economic index, smoking status, pack-years of smoking, environmental tobacco smoking, occupational exposure to dusts, gases and fumes, consumption of alcohol, raw vegetables and fruits, physical activity, body mass index and noise. Diabetes cases not matching the criterion were excluded from the controls.

Table A3
Association between air pollution and diabetes with fixed effect models.

	NO2 OR [95% CI]	PM10 OR [95% CI]
Fully adjusted model treating study area as a fixed effect	1.11 [0.87, 1.40]	0.86 [0.47, 1.60]
Fully adjusted model ignoring study area	1.21 [1.07,1.36]	1.40 [1.17, 1.68]

OR values represent % increase in diabetes prevalence per 10 $\mu g/m^3$ increase in PM_{10} or NO_2. OR: odds ratio, CI: confidence interval. Area was treated as a fixed effect in all models. All models were adjusted for age, sex, educational status, area socio-economic index, smoking status, pack-years of smoking, environmental tobacco smoking, occupational exposure to dusts, gases and fumes, consumption of alcohol, raw vegetables and fruits, physical activity, body mass index and noise. N = 6392 at all levels of adjustment.

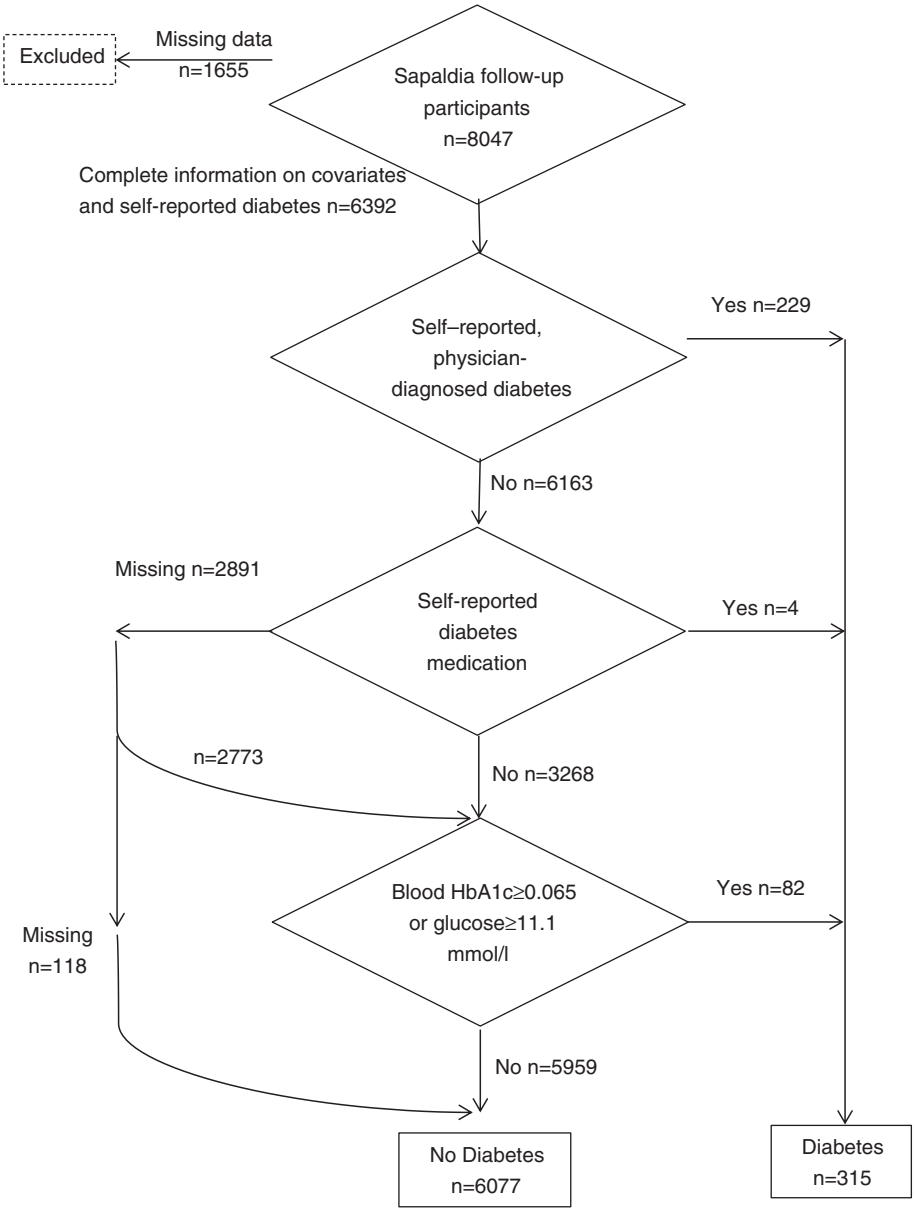

Fig. A1 Diabetes case identification flow chart.

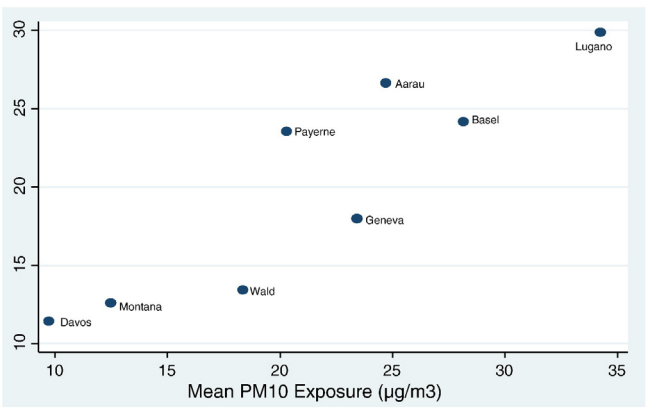

Fig. A2 Correlation between adjusted diabetes prevalence and mean PM_{10} by area.

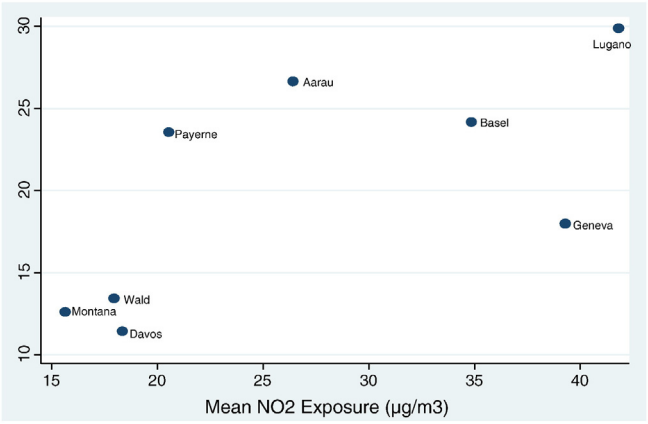

Fig. A3 Correlation between adjusted diabetes prevalence and mean NO_2 by area.

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PART IV UNDERSTANDING THE MECHANISMS INVOLVED IN AMBIENT AIR POLLUTION AND TYPE 2 DIABETES RELATIONSHIPS

6. Article: Long-term exposure to air pollution exposure and metabolic syndrome in adults.

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RESEARCH ARTICLE

Long-Term Exposure to Ambient Air Pollution and Metabolic Syndrome in Adults

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Abstract

Air pollutants (AP) play a role in subclinical inflammation, and are associated with cardiovascular morbidity and mortality. Metabolic syndrome (MetS) is inflammatory and precedes cardiovascular morbidity and type 2 diabetes. Thus, a positive association between AP and MetS may be hypothesized. We explored this association, (taking into account, pathway-specific MetS definitions), and its potential modifiers in Swiss adults. We studied 3769 participants of the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults, reporting at least four-hour fasting time before venepuncture. AP exposures were 10-year mean residential PM_{10} (particulate matter <10μm in diameter) and NO_2 (nitrogen dioxide). Outcomes included MetS defined by World Health Organization (MetS-W), International Diabetes Federation (MetS-I) and Adult Treatment Panel-III (MetS-A) using four- and eight-hour fasting time limits. We also explored associations with individual components of MetS. We applied mixed logistic regression models to explore these associations. The prevalence of MetS-W, MetS-I and MetS-A were 10%, 22% and 18% respectively. Odds of MetS-W, MetS-I and MetS-A increased by 72% (51-102%), 31% (11-54%) and 18% (4-34%) per 10μg/m^3 increase in 10-year mean PM_{10}. We observed weaker associations with NO_2. Associations were stronger among physically-active, ever-smokers and non-diabetic participants especially with PM_{10} ($p<0.05$). Associations remained robust across various sensitivity analyses including ten imputations of missing observations and exclusion of diabetes cases. The observed associations between AP exposure and MetS were sensitive to MetS definitions. Regarding the MetS components, we observed strongest associations with impaired fasting glycemia, and positive but weaker associations with hypertension and waist-circumference-based obesity. Cardio-metabolic effects of AP may be majorly driven by impairment of glucose homeostasis, and to a less-strong extent, visceral adiposity. Well-designed prospective studies are needed to confirm these findings.

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Introduction

Metabolic syndrome (MetS) represents a group of symptoms including central obesity, hypertension, atherogenic dyslipidaemias and insulin resistance. World Health Organization (WHO) defines MetS (MetS-W) as diagnosis of impaired fasting glycaemia (IFG; or treatment for type 2 diabetes) and of any two out of central obesity, hypertension, hypertriglyceridemia (HTG) and low high-density lipoproteins (HDL) (or treatment for specific dyslipidaemia), and urinary albumin excretion ratio $\geq$20μg/min [1]. International Diabetes Federation (IDF) defines MetS (MetS-I) as central obesity and any two out of IFG, hypertension, HTG and low HDL [2], whereas Adult Treatment Panel (ATP) III defines MetS (MetS-A) as diagnosis of any three of five major components [3, 4]. MetS greatly contributes to global disease burden, occurring in about 25% of adults [2]. It predisposes to cardiovascular events and type 2 diabetes. Similarly, air pollutants (AP) are common, top risk factors for disease burden [5] and have been associated with cardiovascular [6–8]-and diabetes-related events [9–11]. Controlling disease burden from cardiovascular morbidity and diabetes implies that prevention of MetS and excessive AP exposure are crucial. Identifying modifiable risk factors to MetS will improve attribution of the burden and support public health control strategies.

MetS enhanced susceptibility to adverse effects of short-term AP exposure. Experimental exposure to diesel exhaust resulted in more haemoconcentration and thrombocytosis in MetS subjects compared to healthy ones [12]. MetS subjects also developed cardiovascular symptoms when exposed to ultrafine particles [13]. Susceptibility to low grade systemic inflammation on exposure to long- term particulate matter <10μm (PM_{10}) was enhanced by MetS [14]. Thus, a link between AP exposure and MetS is plausible but has not been studied. Previous MetS-related studies have focused on PM effects. Unlike PM, which is a marker of general pollution and particle exposure, Nitrogen dioxide (NO_2) is more specific for traffic-related pollution. Studying NO_2 will reveal if traffic exposure contributes to the association, or whether the observed association solely reflects a particle effect (pointing towards an innate immunity activation pathway) or a contribution of different sources. Studying the various definitions of MetS will not only assess the sensitivity of associations to definition, but will also aid the understanding of pathways most likely driving the cardio-metabolic effects of AP on a population level. We therefore explored associations between long-term AP exposure and MetS in adults from a general population sample.

Materials and Methods

Ethics Statement

Ethical clearance for the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA) was obtained from the Swiss Academy of Medical Sciences, the National Ethics Committee for Clinical Research (UREK, Project Approval Number 123/00) and the Cantonal Ethics Committees of the eight health examination areas (Aargau, Basel, Geneva, Grisons, Ticino, Valais, Vaud and Zurich). Participants were required to give written consent prior to the conduct of any health examination.

We used data from 3769 follow-up participants of the SAPALDIA study aged 29–73 years. Details of this study are explained elsewhere [15] but briefly, SAPALDIA began in 1991 with 9651 participants randomly drawn from eight Swiss communities representing a wide range of environmental conditions in Switzerland. 8047 individuals participated in the follow-up study in 2001/2002. Participants completed computer-assisted interviews on health and lifestyle, and had physical examinations including blood sampling, at follow-up, into a bio bank for biomarker and genetic assays. Inclusion in the present study required participation in the follow-

up study, complete data on outcomes and covariates and at least four-hour fasting time before the follow-up examination. The reduction in sample size for this study is primarily explained by the exclusion of non-fasting subjects. Fasting status was not required for SAPALDIA participation.

Definition of MetS

Participants reported their fasting time at first follow-up physical examination (including venepuncture). Height, weight, blood pressure (BP), plasma glucose and lipids were measured. Blood pressure was measured twice at rest, on the left arm, at least three minutes apart, in a sitting position. The mean value of both measures was computed for analyses. Participants were asked about physician diagnoses of diabetes, hypertension, dyslipidaemia and use of medication for these conditions. We defined hypertension as BP (mmHg) $\geq$ 140/90 (MetS-W) and >130/85 (MetS-I; MetS-A) or a physician diagnosis/ treatment. We defined low HDL as plasma HDL (mmol/l) <0.9 (MetS-W) and <1.03 (MetS-I; MetS-A) in males and <1.0 and <1.30 respectively in females and/or diagnosis/ treatment of dyslipidaemias. We defined HTG as plasma triglyceride (mmol/l) $\geq$1.7 and/or diagnosis/ treatment of dyslipidaemias, and impaired fasting glycaemia (IFG) as plasma glucose $\geq$6.1mmol/l (MetS-W) and $\geq$5.6mmol/l (MetS-I; MetS-A) and/or diagnosis/treatment of diabetes. Waist circumference (WC) was not measured at this visit, but was measured at the next follow-up visit. We derived a prediction model, with optimal Bayesian Information Criterion, for waist circumference measured at the next follow-up:

$$\begin{aligned}\text{Waist circumference (cm)} = {} & \beta_0 + \beta_1 * \text{sex} + \beta_2 * \text{age} + \beta_3 * \text{age}^2 + \beta_4 * \text{BMI} \\ & + \beta_5 * \text{BMI}^2 + \beta_6 * \text{age} * \text{bmi} + \beta_7 * \text{sex} * \text{age} + \beta_8 * \text{sex} * \text{age}^2 \\ & + \beta_9 * \text{sex} * \text{BMI} + \beta_{10} * \text{sex} * \text{BMI}^2 + \beta 11 * \text{sex} * \text{age} * \text{BMI} \\ & + \beta_{12} * \text{alcohol} + \beta_{13} * \text{physical activity} + \beta_{14} * \text{ex-smoker} \\ & + \beta_{15} * \text{current smoker}.\end{aligned}$$

We applied this model, using the covariate values of the second survey and added the residuals from the third survey, to back-predict waist circumference for present analyses. We used cross-validation to assess our imputation model, randomly splitting the follow-up sample into a training and a validation sample. The mean imputation error was not significantly different from zero, and the correlations of the imputation errors and the independent variables were also not significantly different from zero. The adjusted R^2 of the imputation model was 0.79 and the squared correlation between the imputed and the actual values was of the same size.

We thus defined central obesity (MetS-I) for a European population as WC $\geq$94cm and $\geq$80cm for males and females respectively. We also defined central obesity (MetS-A) as WC $\geq$102cm and $\geq$88cm for males and females respectively. Central obesity can be assumed if BMI>30 kg/m^2 [2]. Finally, we defined MetS-W, MetS-I and MetS-A based on the above criteria.

Assignment of exposures

We considered estimates of residential exposure to PM_{10} and NO_2. Annual means of AP for 1990 and 2000 were estimated from dispersion models using various emission inventories including road and rail traffic, residential, agricultural, heavy equipment and industrial emissions [16] on a 200x200m grid, and linked to participants' addresses.[17] Estimates of NO_2 exposure were obtained from a hybrid model incorporating land-use regression, since the dispersion model alone did not optimally predict NO_2 near traffic sites.[18] Annual data of AP at

monitoring sites and participants' residential histories were used to estimate annual means of residential exposure levels during the follow-up period and to assign estimates of average residential exposure over the 12 month and 10 year period, respectively, preceding the follow-up examination.[17]

Potential confounders

Consistent with our previous report on diabetes [10], we considered the following characteristics, measured at follow-up, as potential confounders: age, sex, educational attainment ($\leq$9, >9 years), smoking status (never, former, current) and pack-years, passive smoke exposure (yes/no), occupational exposure to vapours, gases, dusts or fumes (VGDF; yes/no), alcohol consumption (including beers, wines, liquors and spirits) (never, $\leq$ once a day, > once a day), consumption of raw vegetables (including salads, juices), citrus fruits (including juices) and other fruits (including juices) (never, $\leq$ 3 days per week, >3 days per week respectively), and self-reported vigorous physical activity defined as participation in activities making one sweat or breathless (<0.5 and $\geq$0.5 hours/week). We also considered neighbourhood-level socio-economic index (SEI) of participants, derived from a principal component analysis using median rent, number of residents of households, educational level and occupation of household heads [19].

Statistical Analyses

We summarized participants' characteristics by different MetS definitions and also by inclusion/exclusion status. We estimated the prevalence of MetS-W, MetS-I and MetS-A, and their associations with 10-year-means of exposure metrics, using mixed logistic models with a random intercept for study area. Since metabolic syndrome is common [2] and given the prevalence in our study sample, we applied mixed Poisson models to estimate incidence rate ratios and used a heuristic approach to obtain robust confidence intervals [20]. Our fully-adjusted model included participants' age, sex, educational attainment, neighbourhood SEI, smoking status and pack-years, passive smoke and VGDF exposure, consumption of alcohol, vegetables, citrus fruits and other fruits, and physical activity and BMI. We adjusted for continuous BMI to capture its variation within obesity and non-obesity groups. Using this fully-adjusted model, we also explored independent associations of PM_{10} and NO_2 with MetS in two- pollutant models. We also explored associations between AP and components of MetS. All these models additionally included BMI except for the AP- obesity model. We repeated these analyses among participants reporting at least eight-hour fasting time (N = 367).

We assessed potential effect modification by age ($\leq$50, >50 years), sex, and physical activity, diabetes and smoking status by stratification and interaction, given previously reports on their role as potential modifiers of AP and diabetes association [21]. Sensitivity analyses included: imputation of 75 observations (10 imputations) with missing data using chained equations; excluding participants who had IFG or obesity but not identified as MetS; treating study area as fixed factor; omitting study area from the models. We applied inverse probability weighting (IPW) to explore non-participation bias. We defined alternative MetS including MetS-I with BMI-based central obesity and MetS-I with North American cut-offs for waist circumference. We performed all analyses with STATA version 13 (Stata Corporation, Texas).

Results

Characteristics of participants

Table 1 shows the characteristics of included participants by MetS status. The distribution of established risk factors with MetS generally followed expectations (e.g. male sex, smoking,

Table 1. Background Characteristics of participants.

Characteristic (%)	MetS-W[a]	MetS-I[b]	MetS-A[c]	No MetS[d]
N	382	771	663	2617
Females	40.1	46.0	40.8	58.0
Education >9 years	85.1	88.9	88.5	93.6
Never smokers	37.2	43.3	44.6	45.0
ETS exposure	49.5	46.3	46.4	46.7
Occupational exposure to VGDF	45.0	45.2	45.1	42.4
Alcohol intake: None	13.1	9.9	9.9	9.9
≤ once/day	72.2	76.4	75.3	81.7
> once/day	14.7	13.7	14.8	8.4
Citrus fruits intake: None	12.8	9.5	8.7	7.6
≤3days/week	54.2	54.5	55.7	56.8
>3days/week	33.0	36.0	35.6	35.6
Fruit intake: None	2.1	2.1	2.1	2.1
≤3days/week	26.4	30.2	30.8	33.7
>3days/week	71.5	67.7	67.1	64.2
Raw vegetables intake: None	0	1.0	0.7	0.7
≤3days/week	20.7	18.0	18.6	18.5
>3days/week	79.3	81.0	80.7	80.8
Vigorous physical activity ≥0.5hours/week	42.7	53.0	52.8	60.1
Impaired fasting glycaemia (IFG)[e]	100	56.3	67.8	7.9/20.7[h]
Low high-density lipoproteins (HDL)[f]	41.6	51.1	65.6	6.9/14.7[h]
High triglycerides	91.6	83.4	89.4	34.3
Obesity (BMI>30kg/m^2)	49.0	36.4	34.0	9.3
Hypertension[g]	81.9	82.4	82.0	25.5/36.3[h]
Area:				
Basel	13.4	11.3	10.0	10.6
Wald	14.6	13.7	16.5	16.1
Davos	2.6	8.6	8.2	9.1
Lugano	25.1	17.6	19.8	17.3
Montana	5.2	10.1	10.5	11.6
Payerne	14.7	15.2	12.3	11.9
Aarau	16.0	14.5	13.6	13.6
Geneva	8.4	8.9	9.1	9.9
Mean (SD)				
Age (years)	61.4(7.3)	58.1 (9.1)	57.9 (9.2)	51.2 (11.5)
BMI (kg/m^2)	30.3(4.9)	29.1(3.9)	28.7 (4.0)	24.8 (3.9)
Predicted waist circumference (cm)	100.7 (11.9)	100.3 (10.6)	98.8 (11.7)	83.5 (11.4)
Neighborhood SEI	61.7(10.3)	62.5(9.9)	62.9 (9.5)	63.2 (10.0)
Pack-years of cigarettes smoked	15.9(24.7)	13.4(22.4)	13.6 (22.2)	9.8 (16.6)
10-year PM_{10} (μg/m^3)	25.0(7.4)	22.7(7.9)	22.8 (8.1)	22.2 (7.8)

(*Continued*)

Table 1. (*Continued*)

Characteristic (%)	MetS-W[a]	MetS-I[b]	MetS-A[c]	No MetS[d]
10-year NO_2 (μg/m^3)	29.9(11.4)	27.6(11.6)	27.5 (11.8)	27.2 (11.3)

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. MetS-A: Adult Treatment Panel III-defined metabolic syndrome. ETS: environmental tobacco smoke. VGDF: vapours, gases, dusts or fumes. SEI: socio-economic index expressed as a percentage. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide.

[a]defined as IFG and any two of central obesity, hypertension, low HDL and high triglycerides.

[b]defined as central obesity and any two of IFG, hypertension, low HDL and high triglycerides.

[c]defined as any three of IFG, central obesity, hypertension, low HDL and high triglycerides.

[d] defined as not having a, b and c.

[e]defined by WHO as fasting blood glucose≥6.1mmol/L and/or diagnosis of type2diabetes; and by IDF and ATP-III as fasting blood glucose≥5.6mmol/L and/or diagnosis of type2diabetes. High triglycerides defined as fasting triglycerides≥1.7mmol/L or treatment for this condition.

[f] defined by WHO as ≤ 0.9 mmol/L (males), ≤ 1.0 mmol/L (females); and by IDF and ATP-III as < 1.03 mmol/L (males), < 1.29 mmol/L (females), or treatment for this condition.

[g]defined by WHO as ≥140/90, or treatment of previously diagnosed hypertension; and by IDF and ATP-III as blood pressure >130/85 mm Hg or previously diagnosed hypertension.

[h]proportion in controls according to MetS-W/ MetS-I or MetS-A criteria respectively.

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physical inactivity were more prevalent in MetS). The MetS cases also had higher exposures to AP than the controls (Table 1).

MetS-W had a weakly positive correlation with MetS-I (kappa = 0.25), but both correlated better with the MetS-A (kappa = 0.40 and 0.67 respectively). Differences between included and excluded participants are shown in S1 Table. Included participants tended to be older, more educated, never-smokers, more exposed to occupational dusts and less physically active (S1 Table).

Table 2. Association between air pollutants and metabolic syndrome (4-hour fasting time).

	Model	10-year mean PM_{10} OR (95%CI)	P-Value	10-year mean NO_2 OR (95%CI)	P-value
MetS-W	Model 1	1.64 (1.35, 1.98)	<0.001	1.20 (1.02, 1.41)	0.025[c]
Cases = 382	Model 2	1.58 (1.29, 1.95)	<0.001	1.21 (1.02, 1.43)	0.026[c]
	Model 3	1.72 (1.46, 2.02)	<0.001	1.22 (1.02, 1.46)	0.033[c]
MetS-I[a]	Model 1	1.23 (1.05, 1.45)	0.009	1.10 (1.00, 1.22)	0.056
Cases = 771	Model 2	1.21 (0.99, 1.49)	0.058	1.10 (0.97, 1.24)	0.154
	Model 3	1.31 (1.11, 1.54)	0.002	1.17 (1.04, 1.31)	0.011
MetS-A[b]	Model 1	1.12 (1.00, 1.24)	0.047[c]	1.03 (0.95, 1.10)	0.505
Cases = 663	Model 2	1.10 (0.98, 1.24)	0.117	1.01 (0.93, 1.09)	0.899
	Model 3	1.18 (1.04, 1.34)	0.011	1.05 (0.95, 1.17)	0.339

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighborhood socio-economic index, occupational exposure to vapors, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10μg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models.

[a] MetS-I defined using predicted waist circumference and European cut-off for central obesity (≥94cm for men and ≥80cm for women).

[b] MetS-A defined using predicted waist circumference and North-American cut-off for central obesity (≥102cm for men and ≥88cm for women).

[C] Lost statistical significance following Bonferroni correction at P<0.016 (0.05/3). PM_{10} and NO_2 are not testing independent hypothesis.

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Associations between AP and MetS

The odds of MetS-W, MetS-I and MetS-A increased by 72% (46–102%), 31% (11–54%) and 18% (4–34%) per 10μg/m^3 increase in 10-year mean home outdoor PM_{10} (Table 2). We also observed positive but less strong associations per 10μg/m^3 increase in 10-year mean home outdoor NO_2 (Table 2).

Translated into incidence rate ratios, the risk of MetS-W, MetS-I and MetS-A increased by 52% (35–70%), 12% (4–19%), and 9% (0–19%) per 10μg/m^3 increase in 10-year mean PM_{10}, and weaker associations were also observed with NO_2 (S2 Table). Among the outcomes, we observed strongest associations with MetS-W, and associations were stronger with PM_{10} than NO_2 (Table 2). Restriction of analyses to subjects reporting eight-hour fasting time provided similar results albeit with limited statistical power. While odds ratios for MetS-W slightly decreased, those for MetS-I and MetS-A increased, and no association was observed between NO_2 and MetS-A (S3 Table). In multi-pollutant MetS models, associations with PM_{10} persisted across outcomes, while those with NO_2 were strongly decreased or lost (S4 Table).

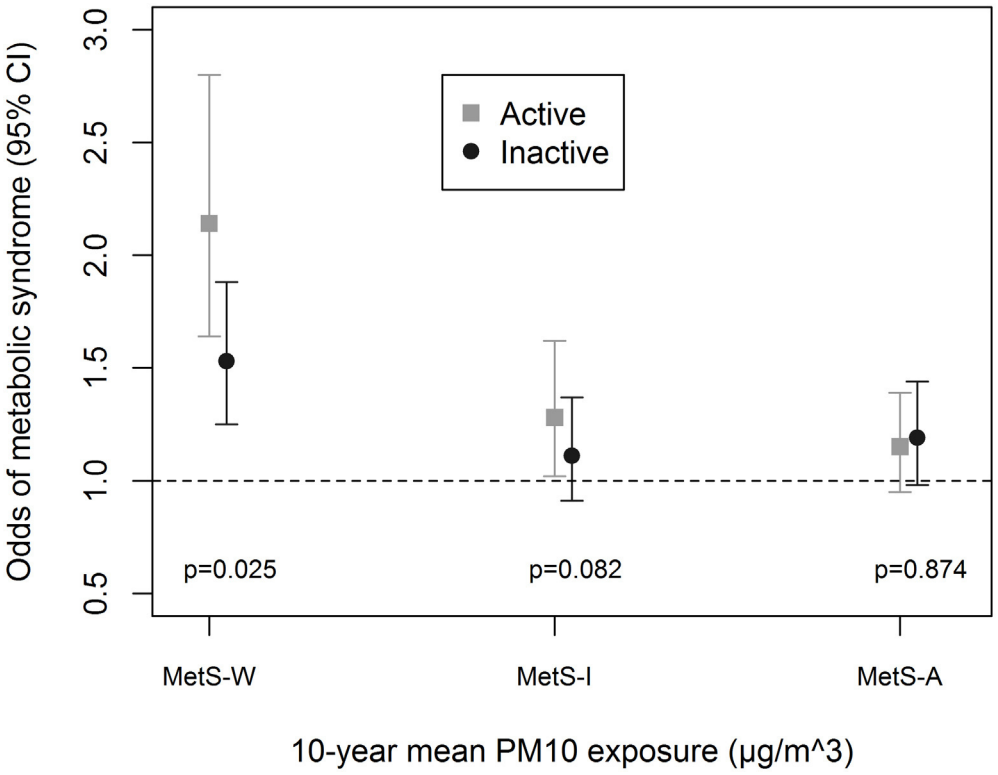

Fig 1. Effect modification by vigorous physical activity. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel-III criteria. Active defined as vigorous physical activity ≥30 minutes per week. Inactive defined as vigorous physical activity <30minutes per week. Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. Odds ratio values refer to increments of 10μg/m^3 in PM_{10} exposure. Total N = 3684; N(physically-active) = 2115.

doi:10.1371/journal.pone.0130337.g001

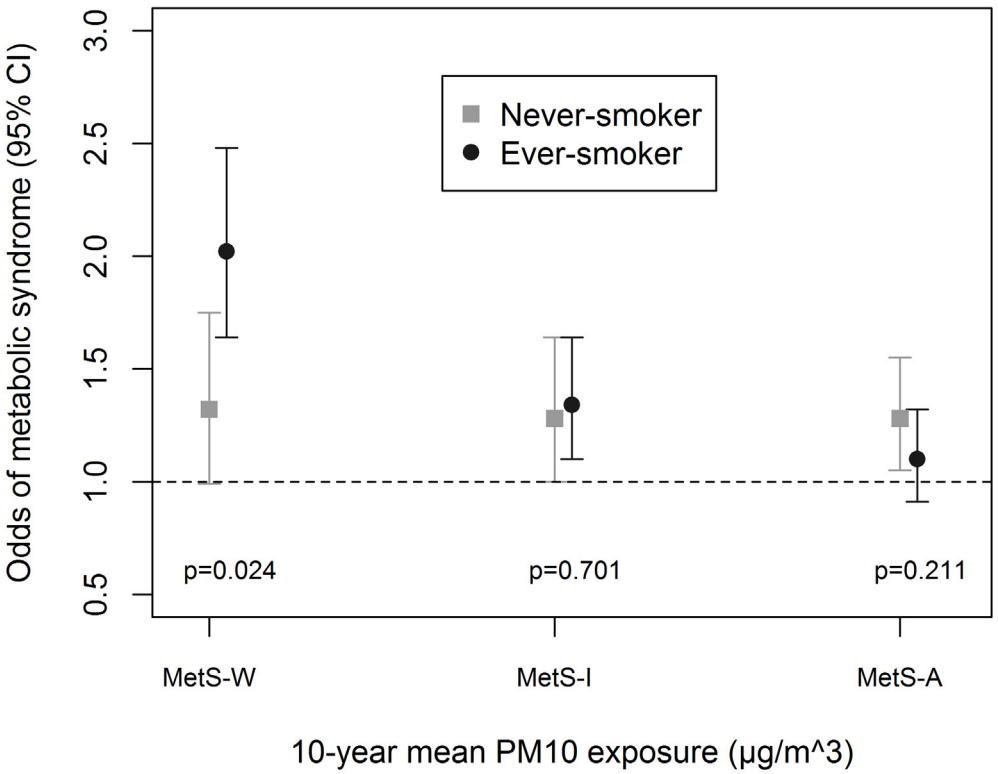

Fig 2. Effect modification by smoking status. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel-III criteria. Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. Odds ratio values refer to increments of 10µg/m^3 in PM_{10} exposure. Total N = 3684; N(never-smoker) = 1623.

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Modification of AP and MetS association

Associations were enhanced by being physically-active (Fig 1), an ever-smoker (Fig 2) and non-diabetic (Fig 3). We observed significant interaction between these variables and PM_{10} in association with MetS-W ($P_{interaction}$ = 0.025, 0.024 and 0.020 respectively). Similar trends were observed with MetS-I, and associations with NO_2 even though interaction terms were non-significant (S5 Table). We observed no significant gender (Fig 4 and S5 Table) and age-group (Fig 5 and S5 Table) differences in the AP-MetS association, even though there was indication for a stronger association among males and participants >50 years (Figs 4 and 5, S5 Table). With MetS-A, there was a significant modification of NO_2 effect by age ($P_{interaction}$ = 0.021; S5 Table). Other interactions were largely non-significant (S5 Table).

Sensitivity Analyses

Estimates of associations were remarkably robust across sensitivity analyses. Multiple imputations of 75 observations marginally improved effect estimates. IPW adjustment for participation bias and exclusion of diabetes cases did not appreciably change these estimates (Table 3). Ignoring study area gave very similar results as the fully-adjusted random-effects model whereas area-specific slopes reduced the effect estimates especially for PM_{10} (Table 3).

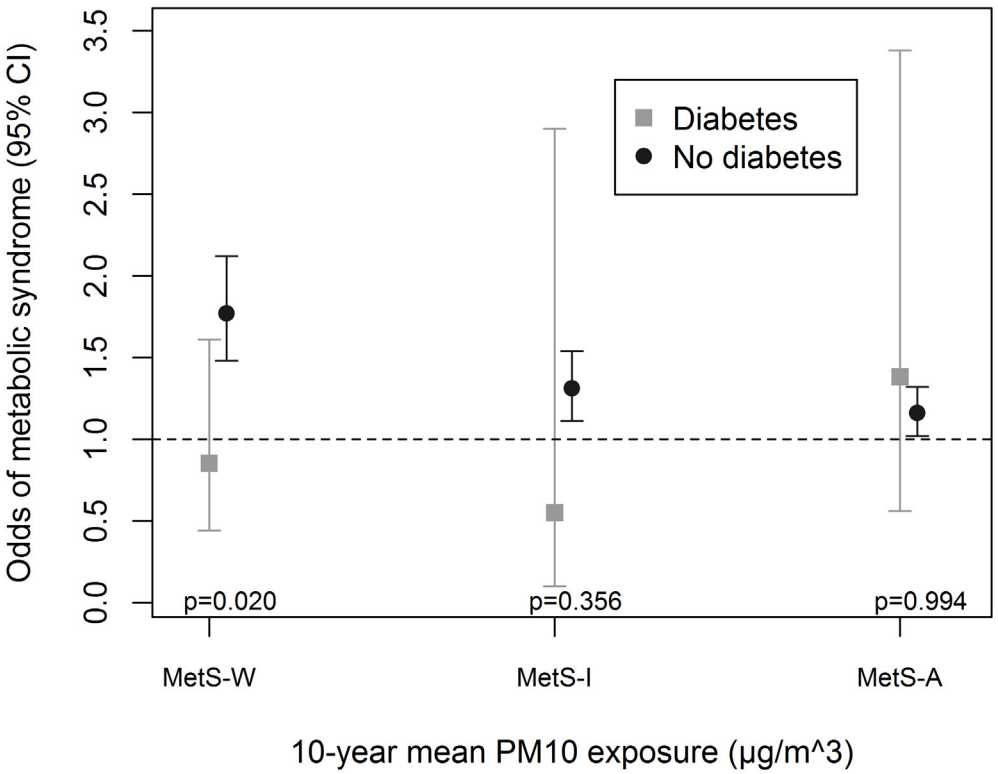

Fig 3. Effect modification by diabetes status. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel-III criteria. Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. Odds ratio values refer to increments of 10µg/m^3 in PM_{10} exposure. Total N = 3684; N(diabetes) = 144.

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We observed weaker associations with MetS-I based on BMI-defined central obesity, and MetS-I based on North-American cut-offs for central obesity in a European population (S6 Table).

Association between AP and MetS components

There were positive associations between AP and IFG (Table 4). Associations were consistent across exposure metrics. We also observed positive associations with hypertension, which were strongest with NO_2. We also found stronger associations with central obesity defined by waist circumference compared to central obesity defined by BMI. We found no appreciable associations with other components, although eight-hour MetS estimates appeared to be stronger than four-hour MetS estimates (Table 4).

Discussion

We found positive associations between markers of long-term AP exposure and MetS, which were sensitive to definition in this sample of Swiss adults. Associations were most pronounced with MetS-W, which reflects a glucose metabolism-dependent pathway, and weaker with

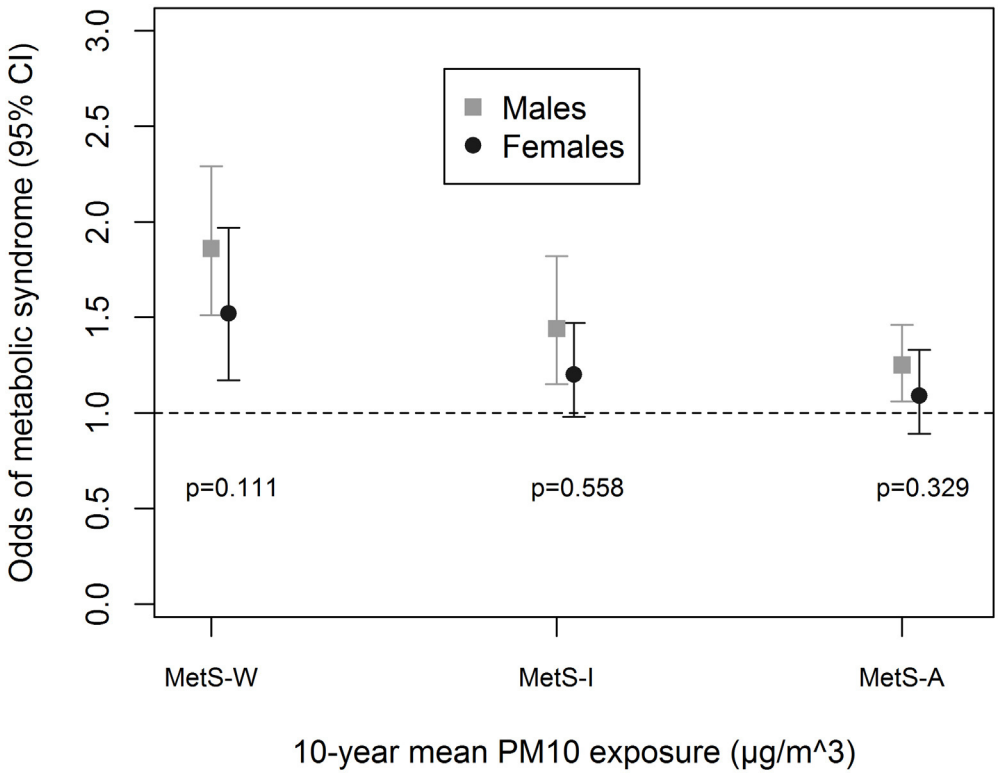

Fig 4. Effect modification by sex. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel-III criteria. Fully adjusted models include age, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. Odds ratio values refer to increments of 10µg/m^3 in PM_{10} exposure. Total N = 3684; N(males) = 1746.

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MetS-I which is based on visceral adiposity, and MetS-A which does not depend on a particular pathway. Our results therefore suggest that AP seems to impact particularly on insulin resistance part of MetS—aligned with impact on adipose tissue inflammation observed in animal models [22–24] and homeostatic model of insulin resistance observed in humans [25, 26]. Given the cross-sectional nature of the analysis and the sub-group findings, one cannot derive etiologic conclusions. But the plausibility of underlying mechanisms warrants further longitudinal investigations of these highly relevant results.

Potential mechanisms of action

MetS reflects a status of low grade systemic inflammation, and exposure to PM has been associated with blood markers of inflammation [27]. Exposure to PM_{10} increased the expression of inflammatory and MetS genes in mice [28]. MetS may predispose to the expression of inflammatory markers [14] and autonomic dysfunction [22, 23, 29] associated with chronic AP exposure. The components of MetS have also been positively linked to AP. Exposure to AP has been linked to hypertension [30, 31], alterations in blood lipids [32, 33], insulin resistance [22, 23] and obesity [34, 35]. Exposure to passive smoke, a contributor to PM also induces inflammatory responses and lipid changes, and has been positively associated with MetS-I [36]. In

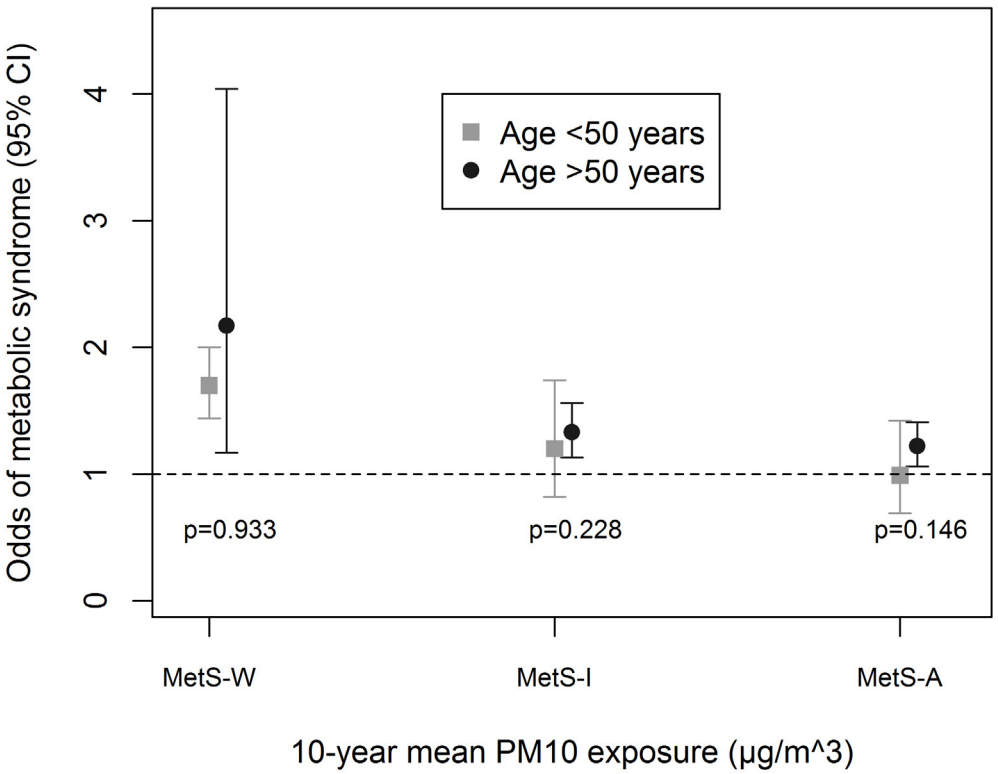

Fig 5. Effect modification by age group. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel-III criteria. Fully adjusted models include sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. Odds ratio values refer to increments of 10µg/m^3 in PM_{10} exposure. Total N = 3684; N(age≤50) = 1393.

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addition, sub-acute exposures to low levels of $PM_{2.5}$ induced insulin resistance in healthy young adults [26], whereas exposure to ambient levels of PM_{10} and NO_2 induced insulin resistance in children [25]. Based on the evidence from human insulin resistance studies, our finding of strongest association with MetS-W and the results of the individual MetS components, the insulin resistance pathway may be the strongest pathway through which AP exert their cardio-metabolic effects. This is also supported by the finding of slightly stronger association with waist circumference-based central obesity as opposed to BMI-based central obesity, with the former being a better indicator for insulin resistance.

Changes in inflammatory markers and blood lipids were non-significant in young adults when exposed to AP [37]. Conversely, significant changes were observed in middle-aged/older subjects, reversible with omega-3-fatty acid [38]. This supports our finding of stronger associations among older people. Smoking is a known risk factor for cardio-metabolic diseases [39], hence our finding of stronger effect among ever-smokers may be additive effect on the already existing effect of smoking exposure. Stronger effects among ever-smokers was observed for MetS-W and MetS-I, but not for MetS-A. This may be explained by the facts that the never-smokers, in our study population, were less physically-active (S7 Table) and had higher predicted waist circumference (90 vs. 88cm) compared to ever-smokers. Whereas the findings for

Table 3. Sensitivity Analyses.

	10-year mean PM_{10}			10-year mean NO_2		
	MetS-W OR (95%CI)	MetS-I OR (95%CI)	MetS-A OR (95%CI)	MetS-W OR (95%CI)	MetS-I OR (95%CI)	MetS-A OR (95%CI)
Fully-adjusted, random-effect model	1.72 (1.46, 2.02)	1.31 (1.11, 1.54)	1.18 (1.04, 1.34)	1.22 (1.02, 1.46)	1.17 (1.04, 1.31)	1.05 (0.95, 1.17)
P-value	<0.001	0.002	0.011	0.033	0.011	0.339
Fully-adjusted random-effect model with multiple imputations	1.81 (1.52, 2.15)	1.39 (1.20, 1.62)	1.17 (1.02,1.35)	1.28 (1.15, 1.43)	1.23 (1.11, 1.12)	1.07 (0.98, 1.17)
P-value	<0.001	<0.001	0.021	<0.001	<0.001	0.156
IPW analysis for participation bias.	1.74 (1.49, 2.03)	1.29 (1.12, 1.49)	1.17 (1.02, 1.33)	1.31 (1.19, 1.46)	1.15 (1.04, 1.27)	1.05 (0.96, 1.15)
P-value	<0.001	0.001	0.023	<0.001	0.005	0.292
Model excluding diabetes cases	1.77 (1.48, 2.12)	1.31 (1.11, 1.54)	1.16 (1.02, 1.32)	1.22 (1.00, 1.50)	1.17 (1.05, 1.32)	1.04 (0.94, 1.16)
P-value	0.020	0.356	0.994	0.110	0.091	0.597
Model excluding diabetes cases reporting medication	1.80 (1.51, 2.14)	1.30 (1.10, 1.53)	1.17 (1.03, 1.34)	1.15 (0.92, 1.43)	1.17 (1.04, 1.32)	1.05 (0.94, 1.16)
P-value	<0.001	0.002	0.015	0.226	0.009	0.421
Model, ignoring study area	1.72 (1.46, 2.02)	1.30 (1.13, 1.50)	1.18 (1.04, 1.34)	1.31 (1.18, 1.46)	1.16 (1.06, 1.28)	1.06 (0.98, 1.16)
P-value	<0.001	<0.001	0.011	<0.001	0.002	0.159
Model, including study area as fixed effect	1.10 (0.63, 2.09)	1.35 (0.86, 2.11)	1.19 (0.74, 1.91)	1.09 (0.88, 1.36)	1.21 (0.99, 1.48)	0.96 (0.79, 1.14)
P-value	0.733	0.194	0.474	0.419	0.058	0.576

Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. MI: multiple imputations. IPW: inverse probability weighting. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR refer to increments of 10μg/m^3 in PM_{10}, and NO_2 exposure respectively. All analyses were done with four-hour fasting participants.

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MetS-W and MetS-I appear to contradict a previous finding of stronger AP effects (on diabetes) among never-smokers [21], our finding with MetS-A supports it. We did not observe any associations among the diabetes cases. This may be because of their use of medication for blood glucose control. It may also be due their very small number which limits the statistical power to see any associations.

We observed stronger associations among the physically active. This observation was independent of MetS definition and persisted in the sub-sample with eight-hour fasting time. Stronger AP associations among the physically active (with diabetes) were shown elsewhere [10, 21]. This may be expected if the physically-active spend more time outdoors, thus, their outdoor concentrations may better capture their actual exposure. Also, due to their deeper inhalation while active, the physically-active have higher exposure of their lung tissues to AP for the same ambient concentration. Physical activity improves lung function [40] and has been shown to enhance response to volatile organic compounds [41].

As shown (S7 Table), the physically-active lived in less polluted areas. Being physically inactive was also associated with areas of high outdoor $PM_{2.5}$ concentrations in normal-weight people in previous studies [42]. One may conjecture that the observed interaction with physical activity may be partly due to some other non-considered covariates. The inactive subjects were exposed to other risk factors for MetS at a higher level than the active subjects (S7 Table), thus,

Table 4. Association between air pollutants and components of metabolic syndrome.

	Fasting time (hours)	10-year mean PM_{10} OR (95%CI)	P-value	10-year mean NO_2 OR (95%CI)	P-value
Impaired fasting Glycaemia (IFG;WHO)	4	1.82 (1.60, 2.08)	<0.001	1.15 (0.98, 1.34)	0.080
	8	2.27 (1.43, 3.62)	0.001	1.33 (0.98, 1.79)	0.063
Impaired fasting Glycaemia (IFG; IDF/ATP-III)	4	1.45 (1.19, 1.78)	<0.001	1.06 (0.93, 1.21)	0.388
	8	1.84 (1.30, 2.60)	0.001	1.36 (1.08, 1.72)	0.008
Low high-density lipoproteins (WHO)	4	0.95 (0.76, 1.19)	0.657	0.88 (0.76, 1.01)	0.071
	8	0.89 (0.47, 1.70)	0.735	0.76 (0.49, 1.19)	0.229
Low high-density lipoproteins (IDF/ATP-III)	4	0.99 (0.87, 1.12)	0.847	0.95 (0.87, 1.05)	0.303
	8	0.99 (0.63, 1.56)	0.982	0.86 (0.66, 1.13)	0.287
High triglycerides	4	0.90 (0.77, 1.05)	0.169	0.94 (0.85, 1.03)	0.194
	8	1.14 (0.78, 1.67)	0.494	0.94 (0.73, 1.21)	0.630
Hypertension (WHO)	4	1.12 (0.97, 1.29)	0.130	1.11 (1.01, 1.20)	0.022
Hypertension (IDF/ATP-III)	4	1.11 (0.95, 1.30)	0.172	1.12 (1.03, 1.23)	0.011
Central obesity (BMI>30kg/m2)	4	1.00 (0.83, 1.21)	0.971	1.01 (0.89, 1.14)	0.898
Central obesity[a]	4	1.19 (0.90, 1.58)	0.218	1.06 (0.90, 1.26)	0.465

Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index (BMI). Model for obesity excludes BMI. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. Traffic PM_{10} refers to dispersion models including only traffic-related emissions. OR: odds ratio. CI: confidence interval. OR values represent fold increase in odds of outcomes per 10μg/m^3 of PM_{10}, NO_2, and 1μg/m^3 of traffic PM_{10} exposure. IFG defined as fasting blood glucose≥6.1mmol/L and/or diagnosis of type2diabetes. High triglycerides defined as fasting triglycerides≥1.7mmol/L or treatment for this condition. Low HDL defined by IDF and ATP-III as < 1.03 mmol/L (males), < 1.29 mmol/L (females), or treatment for this condition, and by WHO as ≤ 0.9 mmol/L (males), ≤ 1.0 mmol/L (females). Hypertension defined by IDF and ATP-III as blood pressure >130/85 mm Hg and by WHO as ≥140/90, or treatment of previously diagnosed hypertension. Participants' study area was treated as a random effect in all models. N (4 hours fasting time) = 3684. N (8 hours fasting time) = 367.

[a]Central obesity defined using the predicted waist circumference and European cut-offs (≥94cm for males and ≥80cm for females)

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the relative role of AP in MetS development may be less crucial in them. Use of more objective measures of visceral adiposity should improve the definition of MetS.

Strengths and Limitations

This study derives from the large SAPALDIA database, with detailed information on health, socio-demographic and lifestyle characteristics. This allowed us to have a clean case definition and detailed confounder adjustment. We had validated annual estimates of residential AP exposures from which long-term exposure estimates were derived. To the best of our knowledge, this is the first study to assess direct associations between AP and MetS. Its results may help in understanding the pathways involved in the effects of AP on cardiovascular disease and diabetes.

A major limitation is the cross-sectional design which precludes etiologic inferences. We did not measure waist circumference at this visit but had a validated prediction model based on trends at the next follow-up visit. As we do not have urinary albumin excretion ratio for our participants, we may have misclassified some MetS-W cases. We used four-hour fasting time to define MetS, instead of conventional eight hours in our main analysis. This was due to the small sample of participants who reported a fasting time of at least eight hours, limiting our statistical power. However, associations were also positive in the subjects who fasted for eight hours. Four-hour fasting blood samples can be used for patient diagnosis in ambulatory settings [43]. Also, non-fasting triglycerides were shown to be a predictor of cardiac events in

women [44]. $PM_{2.5}$ was not modelled in this study, thus we relied on PM_{10}. While one may argue $PM_{2.5}$ to be more relevant for systemic effects, the lack thereof, is unlikely to bias this analysis. In Switzerland, $PM_{2.5}$ contributes 70–80% to the PM_{10} fraction and both are highly correlated within and across SAPALDIA areas (R~0.8).

We used two markers of ambient pollution with partly different characteristics. Our results indicate possible larger effects of PM_{10} compared to NO_2. This may largely be because PM_{10} represents a mixture of different particles, unlike NO_2 which measures a specific gas. Particulate matter has been shown to be stronger activators of innate immunity in comparison with gaseous pollutants [22, 23].

We did not have estimates of indoor or occupational AP for our participants, but any misclassification that could be caused by this is expected to be non-systematic, leading to a null bias. We considered occupational exposure to VGDF, which partly adjusts for indoor occupational exposure. Only 46% of follow-up and 38% of baseline participants was studied. A substantial percentage of non-inclusion was due to subjects who had venepuncture in less than four-hour fasting time. Despite this low participation, all study areas and other characteristics were well represented in this study sample. Sensitivity analyses using IPW suggested that participation bias was non-substantial. Despite this finding, some bias may still persist. The weaker precision from the fixed effect model, especially for PM_{10}, could be due to poor within-area spatial contrasts exhibited by PM_{10} compared to the traffic-related exposures [10, 45].

It is unclear if the associations with PM_{10} are due to the inflammation elicited by physical effects of particles and/or the innate immunity response elicited by its biological components. These and other questions deserve further investigation by future well-designed longitudinal studies. The studies should consider measured waist circumference as a component of MetS, and explore associations with PM components. Also, physical activity must be more objectively measured. Our findings, if confirmed, are of great public health relevance, as they may call for physical activity promotion to be adapted to various environmental contrasts.

Supporting Information

S1 Table. Characteristics of participants included and excluded in the study. ETS: environmental tobacco smoke. VGDF: vapours, gases, dusts and fumes. MVPA: moderate to vigorous physical activity. Hypertension defined as blood pressure >130/85 mm Hg or treatment of previously diagnosed hypertension. SEI: socio-economic index expressed as a percentage. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide.
(DOCX)

S2 Table. Incidence rate ratios of metabolic syndrome in association with air pollutants. MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of $10\mu g/m^3$ in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N = 3684
(DOCX)

S3 Table. Association between air pollutants and metabolic syndrome (8 hours fasting time). MetS-W: World Health Organization-defined metabolic syndrome. MetS-I:

International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10μg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N = 367
(DOCX)

S4 Table. Association between air pollutants and metabolic syndrome (two-pollutant models). MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10μg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N = 3684
(DOCX)

S5 Table. Effect modification of NO_2 and metabolic syndrome association. Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. NO_2: nitrogen dioxide. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. OR: Odds ratios OR values refer to increments of 10μg/m^3 in NO_2 exposure. Total N = 3684; N(age≤50) = ; N(males) = 1746; N(physically-active) = 2115; N(never-smoker) = 1623; N(diabetes) = 144.
(DOCX)

S6 Table. Association between air pollutants and alternative definitions of metabolic syndrome. MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. MetS-ATP-III: Adult treatment panel III criteria- defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10μg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N (Four-hour fasting time) = 3684; N (Eight-hour fasting time) = 367.
(DOCX)

S7 Table. Participants' characteristics by self-reported physical activity. MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel III criteria. ETS: environmental tobacco smoke. VGDF: vapours, gases, dusts and fumes. IFG defined as fasting blood glucose≥6.1mmol/L and/or diagnosis of type2diabetes. High triglycerides defined as fasting triglycerides≥1.7mmol/L or treatment for this condition. Low

HDL defined by IDF as < 1.03 mmol/L (males), < 1.29 mmol/L (females), or treatment for this condition, and by WHO as ≤ 0.9 mmol/L (males), ≤ 1.0 mmol/L (females). Hypertension defined by IDF and ATP-III as blood pressure >130/85 mm Hg and by WHO as ≥140/90, or treatment of previously diagnosed hypertension. SEI: socio-economic index. PM_{10}: particulate matter <10μm in diameter from all sources. NO_2: nitrogen dioxide.
(DOCX)

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Author Contributions

Conceived and designed the experiments: NPH. Performed the experiments: NPH ICE ES MF MI AVE T. Rochat T. Rothe MWG NK CS. Analyzed the data: NPH ICE ES CS. Contributed reagents/materials/analysis tools: NPH. Wrote the paper: NPH ICE ES MF MI AVE T. Rochat T. Rothe MWG NK CS.

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SUPPLEMENTARY TABLES

Long-term exposure to ambient air pollution and metabolic syndrome in adults.

Ikenna C. Eze, Emmanuel Schaffner, Maria Foraster, Medea Imboden, Arnold von Eckardstein, Margaret Gerbase, Thomas Rothe, Thierry Rochat, Nino Künzli, Christian Schindler, Nicole Probst-Hensch.

S1 Table: Characteristics of participants included and excluded in the study

Characteristic (%)	Included (N=3684)	Excluded (N=5967)	P-value (Chi^2)
Females	52.6	51.1	0.223
Education >9 years	92.0	61.0	<0.001
Smoking status: Never	44.0	40.6	<0.001
Former	32.0	31.2	
Current	24.0	28.2	
ETS exposure	46.8	48.0	0.256
Occupational exposure to VGDF	43.0	19.5	<0.001
Alcohol intake: None	10.1	8.0	<0.001
≤ once/day	79.9	83.9	
> once/day	9.9	8.1	
Citrus fruits intake: None	8.4	8.3	0.989
≤3days/week	56.0	56.1	
>3days/week	35.6	35.6	
Fruits intake: None	2.1	1.4	0.089
≤3days/week	32.5	33.9	
>3days/week	65.4	64.7	
Raw vegetables intake: None	0.8	0.4	0.099
≤3days/week	18.6	19.0	
>3days/week	80.6	80.6	
Vigorous physical activity ≥0.5hours/week	57.6	65.6	<0.001
Central obesity (BMI>30kg/m^2)	17.0	14.8	0.015
Hypertension	38.5	38.3	0.966
Area: Basel	10.9	15.5	<0.001
Wald	15.4	20.4	<0.001
Davos	8.7	7.2	0.016
Lugano	17.7	12.1	<0.001
Montana	10.8	6.6	<0.001

Payerne	12.8	16.3	<0.001
Aarau	13.9	12.5	0.063
Geneva	9.7	9.4	0.653
Mean (SD)			T-test
Age (years)	53.3(11.4)	50.9(11.5)	<0.001
Body mass index (kg/m^2)	26.0(4.6)	25.7(4.4)	0.005
Blood glucose (mmol/L)	5.6(1.6)	5.6(1.5)	0.996
Triglycerides (mmol/L)	1.9(1.3)	1.8(1.2)	<0.001
High-density lipoproteins (mmol/L)	1.5(0.5)	1.5(0.4)	0.7
Neighbourhood SEI	63.1(10.0)	63.6(10.4)	0.030
Pack-years of smoking	11.0(18.7)	11.1(18.6)	0.753
10-year PM_{10} ($\mu g/m^3$)	22.5(7.9)	22.3(6.9)	0.482
10-year NO_2 ($\mu g/m^3$)	27.4(11.4)	26.8(10.8)	0.018

ETS: environmental tobacco smoke. VGDF: vapours, gases, dusts and fumes. MVPA: moderate to vigorous physical activity. Hypertension defined as blood pressure >130/85 mm Hg or treatment of previously diagnosed hypertension. SEI: socio-economic index expressed as a percentage. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide.

S2 Table: Incidence rate ratio (IRR) of metabolic syndrome in association with air pollutants.

	Model	10-year mean PM_{10} IRR (95%CI)	10-year mean NO_2 IRR (95%CI)
MetS-W; Cases=382	Model 1	1.55 (1.31, 1.83)	1.18 (1.02, 1.37)
	Model 2	1.45 (1.25, 1.69)	1.17 (1.02, 1.34)
	Model 3	1.52 (1.35, 1.70)	1.19 (1.02, 1.40)
MetS-I [a]; Cases=771	Model 1	1.14 (1.03, 1.26)	1.07 (1.00, 1.14)
	Model 2	1.08 (1.00, 1.18)	1.03 (0.99, 1.07)
	Model 3	1.12 (1.04, 1.19)	1.06 (1.01, 1.11)
MetS-A[b]; Cases=663	Model 1	1.11 (1.02, 1.20)	1.04 (0.96, 1.11)
	Model 2	1.06 (0.98, 1.15)	1.00 (0.98, 1.02)
	Model 3	1.09 (1.00, 1.19)	1.02 (0.96, 1.08)

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10µg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N=3684

[a] MetS-I defined using predicted waist circumference and European cut-off for central obesity (≥94cm for men and ≥80cm for women).

[b] MetS-A defined using predicted waist circumference and North-American cut-off for central obesity (≥102cm for men and ≥88cm for women).

S3 Table: Association between air pollutants and metabolic syndrome (8 hours fasting time).

	Model	10-year mean PM_{10} OR (95%CI)	10-year mean NO_2 OR (95%CI)
MetS-W Cases=34	Model 1	1.41 (0.76, 2.61)	1.17 (0.80, 1.72)
	Model 2	1.54 (0.86, 2.76)	1.11 (0.75, 1.64)
	Model 3	1.62 (0.81, 3.26)	1.16 (0.73, 1.84)
MetS-I[a] Cases=62	Model 1	1.82 (0.91, 3.63)	1.13 (0.77, 1.64)
	Model 2	2.26 (0.93, 5.52)	1.23 (0.74, 2.05)
	Model 3	2.23 (1.04, 4.79)	1.30 (0.78, 2.19)
MetS-A[b] Cases=56	Model 1	1.35 (0.85, 2.15)	0.94 (0.71, 1.26)
	Model 2	1.51 (0.77, 2.97)	0.85 (0.57, 1.26)
	Model 3	1.56 (0.82, 2.98)	0.87 (0.78, 1.26)

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10µg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N=367

[a] MetS-I defined using predicted waist circumference and European cut-off for central obesity (≥94cm for men and ≥80cm for women).

[b] MetS-A defined using predicted waist circumference and North-American cut-off for central obesity (≥94cm for men and ≥80cm for women).

S4 Table: Association between air pollutants and metabolic syndrome (two-pollutant models).

	Model	10-year meanPM_{10} OR (95%CI)	10-year mean NO_2 OR (95%CI)
MetS-W; Cases=382	Model 1	1.79 (1.40, 2.30)	0.90 (0.72, 1.13)
	Model 2	1.65 (1.21, 2.25)	0.96 (0.76, 1.21)
	Model 3	1.84 (1.40, 2.42)	0.94 (0.78, 1.13)
MetS-I [a] ; Cases=771	Model 1	1.24 (1.00, 1.54)	0.99 (0.86, 1.54)
	Model 2	1.21 (0.94, 1.57)	1.00 (0.84, 1.19)
	Model 3	1.25 (0.97, 1.61)	1.04 (0.88, 1.23)
MetS-A[b] ; Cases=663	Model 1	1.25 (1.04, 1.50)	0.91 (0.80, 1.03)
	Model 2	1.26 (1.04, 1.53)	0.89 (0.77, 1.01)
	Model 3	1.28 (1.04, 1.58)	0.93 (0.81, 1.08)

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10µg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N=3684

[a] MetS-I defined using predicted waist circumference and European cut-off for central obesity (≥94cm for men and ≥80cm for women).

[b] MetS-A defined using predicted waist circumference and North-American cut-off for central obesity (≥102cm for men and ≥88cm for women).

S5 Table: Effect modification of NO_2 and metabolic syndrome association.

	MetS-W OR (95%CI)	MetS-I OR (95%CI)	MetS-A OR (95%CI)
Age: Age≤50	1.24 (1.04, 1.49)	1.05 (0.81, 1.36)	0.83 (0.65, 1.08)
Age>50	1.34 (0.91, 1.97)	1.20 (1.07, 1.34)	1.12 (1.01, 1.23)
P-value	0.664	0.091	0.021
Sex: Males	1.27 (1.01, 1.61)	1.27 (1.08, 1.48)	1.10 (0.96, 1.25)
Females	1.22 (1.01, 1.48)	1.08 (0.94, 1.25)	1.02 (0.89, 1.16)
P-value	0.258	0.407	0.434
Vigorous physical activity ≥0.5 hrs/wk: Yes	1.27 (1.08, 1.49)	1.26 (1.06, 1.48)	1.08 (0.95, 1.23)
No	1.22 (0.93, 1.62)	1.04 (0.90, 1.20)	1.02 (0.87, 1.18)
P-value	0.299	0.077	0.593
Never-smoker: Yes	1.17 (0.94, 1.46)	1.19 (0.98, 1.46)	1.11 (0.98, 1.27)
No	1.26 (0.97, 1.62)	1.20 (1.03, 1.36)	1.00 (0.85, 1.16)
P-value	0.130	0.804	0.252
Diabetes: Yes	0.84 (0.58, 1.23)	0.49 (0.12, 2.03)	1.56 (0.76, 3.22)
No	1.22 (1.00, 1.50)	1.17 (1.05, 1.32)	1.04 (0.94, 1.16)
P-value	0.110	0.091	0.597

Fully adjusted models include age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts and fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, physical activity and body mass index. NO_2: nitrogen dioxide. All analyses were done with four-hour fasting participants. Participants' study area was treated as a random effect in all models. OR: Odds ratios OR values refer to increments of $10\mu g/m^3$ in NO_2 exposure. Total N=3684; N(age≤50)= ; N(males)=1746 ; N(physically-active)=2115 ; N(never-smoker) =1623 ; N(diabetes)=144.

S6 Table: Association between air pollution and alternative definitions of metabolic syndrome

	Model	10-year mean PM_{10} OR (95%CI)	10-year mean NO_2 OR (95%CI)
MetS-I [a] ; Cases = 492	Model 1	1.07 (0.90, 1.26)	1.04 (0.92, 1.18)
	Model 2	1.05 (0.88, 1.25)	1.04 (0.91,1.18)
	Model 3	1.19 (0.97, 1.46)	1.15 (1.00, 1.33)
MetS-I [b] Cases = 479	Model 1	1.15 (0.90, 1.47)	1.08 (0.93, 1.23)
	Model 2	1.11 (0.84, 1.48)	1.03 (0.88, 1.22)
	Model 3	1.12 (0.84, 1.48)	1.05 (0.88, 1.24)
MetS-I [a, c] ; Cases=50	Model 1	1.61 (0.84, 3.07)	1.37 (0.93, 2.03)
	Model 2	1.63 (0.87, 3.04)	1.30 (0.88, 1.91)
	Model 3	2.24 (1.01, 4.98)	1.73 (1.05, 2.85)
MetS-I [b, c] Cases=39	Model 1	1.51 (0.67, 3.38)	0.93 (0.59, 1.47)
	Model 2	1.78 (0.67, 4.73)	0.94 (0.54, 1.64)
	Model 3	1.98 (0.77, 5.08)	1.02 (0.57, 1.82)

MetS-W: World Health Organization-defined metabolic syndrome. MetS-I: International Diabetes Federation-defined metabolic syndrome. MetS-ATP-III: Adult treatment panel III criteria- defined metabolic syndrome.. Model 1: Crude; Model 2: Model 1+ age, sex, educational attainment, neighbourhood socio-economic index, occupational exposure to vapours, gases, dusts or fumes, smoking status, smoked pack-years, exposure to passive smoke, consumption of fruits and raw vegetables, and physical activity; Model 3: Model 2+ body mass index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide. OR: odds ratio. CI: confidence interval. OR values refer to increments of 10µg/m^3 in PM_{10} and NO_2 exposure respectively. Participants' study area was treated as a random effect in all models. N (Four-hour fasting time)=3684; N (Eight-hour fasting time)=367.

[a] MetS-I defined using BMI>30kg/m^2 to define central obesity.

[b] MetS-I defined using predicted waist circumference and North-American cut-off for central obesity (≥102cm for men and ≥88cm for women).

[c] Eight-hour fasting time

S7 Table: Participants' characteristics by self-reported physical activity

Characteristic (%)	Physical activity <0.5 hours/week (N=1569)	Physical activity ≥0.5 hours/week (N=2115)	P-value (Chi^2)
Females	59.6	47.4	<0.001
Education >9 years	87.8	95.7	<0.001
Never-smokers	41.7	45.8	0.015
ETS exposure	51.9	43.1	<0.001
Occupational exposure to VGDF	41.4	44.0	0.116
Alcohol intake: None	14.3	7.2	<0.001
≤ once/day	73.2	84.8	
> once/day	12.5	8.0	
Citrus fruits intake: None	10.0	7.2	0.002
≤3days/week	56.3	55.9	
>3days/week	33.7	36.9	
Fruits intake: None	2.4	1.8	0.187
≤3days/week	31.8	33.2	
>3days/week	65.8	65.0	
Raw vegetables intake: None	1.0	0.6	0.318
≤3days/week	20.6	17.1	
>3days/week	78.4	82.3	
Low HDL (WHO)	16.6	13.9	0.081
Low HDL (IDF)	30.4	22.7	<0.001
High triglycerides (≥1.7 mmol/L)	51.7	45.0	<0.001
Impaired fasting glycaemia (IFG; WHO)	16.8	12.6	<0.001
Impaired fasting glycaemia (IFG; IDF)	38.2	30.1	<0.001
Hypertension (WHO)	41.9	35.3	<0.001
Hypertension (IDF)	53.2	47.9	0.002
MetS-I [a]	38.0	26.5	<0.001
MetS-W [b]	13.9	7.7	<0.001
MetS-A[c]	24.9	18.9	<0.001
Area: Basel	9.0	12.2	0.002

Wald	12.3	17.9	<0.001
Davos	6.5	10.3	<0.001
Lugano	32.0	7.0	<0.001
Montana	7.9	13.0	<0.001
Payerne	12.3	13.1	0.510
Aarau	9.4	17.3	0.003
Geneva	10.4	9.2	0.236
Mean (SD)			T-test
Age (years)	55.6 (11.1)	51.7 (11.3)	<0.001
Body mass index (kg/m^2)	26.3 (4.8)	25.6 (4.2)	0.004
Predicted waist circumference (cm)	89.6 (13.5)	88.7 (12.9)	0.149
Neighborhood SEI	61.9 (10.3)	63.4 (9.7)	<0.001
Davos	6.5	10.3	<0.001
Lugano	32.0	7.0	<0.001
Montana	7.9	13.0	<0.001

MetS-W: Metabolic syndrome according to World Health Organization. MetS-I: Metabolic syndrome according to International Diabetes Federation. MetS-A: Metabolic syndrome according to Adult Treatment Panel III criteria. ETS: environmental tobacco smoke. VGDF: vapours, gases, dusts and fumes. IFG defined as fasting blood glucose≥6.1mmol/L and/or diagnosis of type2diabetes. High triglycerides defined as fasting triglycerides≥1.7mmol/L or treatment for this condition. Low HDL defined by IDF as < 1.03 mmol/L (males), < 1.29 mmol/L (females), or treatment for this condition, and by WHO as ≤ 0.9 mmol/L (males), ≤ 1.0 mmol/L (females). Hypertension defined by IDF and ATP-III as blood pressure >130/85 mm Hg and by WHO as ≥140/90, or treatment of previously diagnosed hypertension. SEI: socio-economic index. PM_{10}: particulate matter <10µm in diameter from all sources. NO_2: nitrogen dioxide.

[a]defined as central obesity and any two of IFG, hypertension, low HDL and high triglycerides.

[b]defined as IFG and any two of central obesity, hypertension, low HDL and high triglycerides.

[c]defined as any three of five components.

7. Article: Air pollution and diabetes association: Modification by type 2 diabetes genetic risk score

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Air pollution and diabetes association: Modification by type 2 diabetes genetic risk score

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ABSTRACT

Exposure to ambient air pollution (AP) exposure has been linked to type 2 diabetes (T2D) risk. Evidence on the impact of T2D genetic variants on AP susceptibility is lacking. Compared to single variants, joint genetic variants contribute substantially to disease risk. We investigated the modification of AP and diabetes association by a genetic risk score (GRS) covering 63 T2D genes in 1524 first follow-up participants of the Swiss cohort study on air pollution and lung and heart diseases in adults. Genome-wide data and covariates were available from a nested asthma case-control study design. AP was estimated as 10-year mean residential particulate matter <10 μm (PM_{10}). We computed count-GRS and weighted-GRS, and applied PM_{10} interaction terms in mixed logistic regressions, on odds of diabetes. Analyses were stratified by pathways of diabetes pathology and by asthma status. Diabetes prevalence was 4.6% and mean exposure to PM_{10} was 22 μg/m^3. Odds of diabetes increased by 8% (95% confidence interval: 2, 14%) per T2D risk allele and by 35% (−8, 97%) per 10 μg/m^3 exposure to PM_{10}. We observed a positive interaction between PM_{10} and count-GRS on diabetes [$OR_{interaction}$ = 1.10 (1.01, 1.20)], associations being strongest among participants at the highest quartile of count-GRS [OR: 1.97 (1.00, 3.87)]. Stronger interactions were observed with variants of the GRS involved in insulin resistance [($OR_{interaction}$ = 1.22 (1.00, 1.50)] than with variants related to beta-cell function. Interactions with count-GRS were stronger among asthma cases. We observed similar results with weighted-GRS. Five single variants near *GRB14*, *UBE2E2*, *PTPRD*, *VPS26A* and *KCNQ1* showed nominally significant interactions with PM_{10} ($P < 0.05$). Our results suggest that genetic risk for T2D may modify susceptibility to air pollution through alterations in insulin sensitivity. These results need confirmation in diabetes cohort consortia.

1. Introduction

Epidemiologic evidence shows a positive association between air pollution and type 2 diabetes (T2D) risk (Eze et al., 2014a, 2015a; Park et al., 2015). The underlying mechanisms and susceptibilities are still subject to active research. Effects of inhaled pollutants that are supported by experimental and epidemiological evidence include the contribution to systemic inflammation, autonomic imbalance, weight gain, and to insulin resistance, thought to be in part the result of inhalants stimulating an innate immune response, influencing endoplasmic reticulum, glucose and lipid metabolism, and activating the central nervous system (Rao et al., 2015).

Abbreviations: BCF, beta cell function; BMI, body mass index; CI, confidence interval; CNG, Centre National de Génotypage; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; GEI, gene-environment interaction; GWAS, genome-wide association studies; GRS, genetic risk score; GRS_{IR}, genetic risk score of variants in the insulin resistance pathway; GRS_{BCF}, genetic risk score of variants in the beta-cell function pathway; HbA1c, glycosylated haemoglobin; HWE, Hardy-Weinberg equilibrium; IPW, inverse probability weighting; IR, insulin resistance; MAF, minor allele frequency; OR, odds ratio; $PM_{2.5}$, particulate matter with diameter < 2.5 μm; PM_{10}, particulate matter with diameter < 10 μm; RAF, risk allele frequency; SAPALDIA, Swiss cohort study on air pollution and lung and heart diseases in adults; SNP, single nucleotide polymorphism; T1D, type 1 diabetes; T2D, type 2 diabetes.

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Gene-environment interaction (GEI) can inform on biological pathways by which air pollution affects diabetes, an aspect of relevance to air quality regulation. So far, GEI studies in areas of air pollution have focused on candidate genes in the domains of oxidative stress and inflammation on cardio-respiratory and metabolic outcomes (Curjuric et al., 2012; Eze et al., 2016; Minelli et al., 2011; Zanobetti et al., 2011). The degree of reduction in markers of heart rate variability, in relation to air pollutants, was associated with deletions in *GSTM1* (Chahine et al., 2007), long GT repeats of *HMOX-1* (Schwartz et al., 2005), wild-type *HFE* (Park et al., 2006), and *IL6*-572GC (Adam et al., 2014). A stronger effect of ozone on lung function was reported among carriers of combined *NQOI* wild-type/*GSTM1* null genotype, *GSTP1* and long GT repeats on *HMOX-1* (Alexeeff et al., 2008; Chen et al., 2007). A variant in *CDH13* showed the strongest signal in a genome-wide interaction study between PM_{10} and lung function decline (Imboden et al., 2015). Particle number significantly increased fibrinogen concentrations in individuals with high genetic risk score (GRS) of genes in the oxidative stress pathway, and increased C-reactive proteins and intracellular adhesion molecule-1 concentrations in individuals with higher genetic scores of metal-processing gene variants (Bind et al., 2014).

Over the years, T2D susceptibility loci have been increasingly identified through meta-analyses of agnostic genome-wide analyses. So far, >60 T2D genetic risk variants have been identified (Morris et al., 2012). By selecting diabetes gene risk variants identified in genome-wide association studies (GWAS) for interaction with air pollution, a novel mechanistic understanding may evolve. This approach has been applied to factors other than air pollution, and to single diabetes gene risk variants (Cornelis and Hu, 2012).

Physical activity and variants near the *FTO* gene are one of the most studied GEI in T2D (Kilpelainen and Franks, 2014), demonstrating an attenuation of the effect of an *FTO* variant on BMI among the physically active compared to the inactive (Kilpelainen et al., 2011). Variants near *HNF1B* (Brito et al., 2009) and *CDKN2A* also interacted with physical activity on T2D incidence (Moore et al., 2008). The Pro12Ala variant of *PPARG* was shown to modify the association between physical activity and glucose regulation in people with (Adamo et al., 2005) and without diabetes (Kahara et al., 2003). Evidence from GEI studies on nutrition and T2D also demonstrated that the carriers of this *PPARG* variant are more responsive to the beneficial effects of unsaturated fat and less susceptible to the adverse effects of saturated fat on glucose regulation and/or body mass index (Lamri et al., 2012). Carriers of a *TCF7L2* risk variant had a lower T2D risk when they were on low glycemic diet (Cornelis et al., 2009a). An *SLC30A8* variant modified the negative relationship between zinc intake and glucose homeostasis (Kanoni et al., 2011).

Compared to single genetic variants, a combination of genetic variants may contribute more substantially to disease risk and might thus be useful to better characterize high-risk populations (Talmud et al., 2015; Vassy et al., 2014). Few studies have explored the impact of the T2D genetic risk score on its associated phenotypes such as coronary artery disease (Hamad et al., 2015), or explored its modifying effect on the diabetes association with basic risk factors including age, sex, physical activity (Langenberg et al., 2014), weight gain (Andersson et al., 2013), obesity and family history (Cornelis et al., 2009b; Langenberg et al., 2014). No study explored the interaction of the T2D genetic risk score with air pollution.

Several studies on the effects of T2D risk variants on quantitative traits of glucose metabolism have identified pathways through which some of these variants impact on T2D. Pathways through which the risk variants impact directly on T2D include the impairment of beta-cell function (BCF) and insulin resistance (IR) (Dimas et al., 2014; Harder et al., 2013; Manning et al., 2012; Perry and Frayling, 2008; Scott et al., 2012) or other pathways may confer insulin resistance indirectly through obesity risk increasing genetic variants (near *FTO* and *M4CR*) (Perry and Frayling, 2008; Scott et al., 2014).

We generated GWAS-derived polygenic risk scores and explored modification of our previously reported association between air pollutants and diabetes (Eze et al., 2014a) among participants of the Swiss cohort study on air pollution and lung and heart diseases in adults (SAPALDIA), in general and in pathway-analyses approach. Genome-wide data and detailed covariate information were available from a previous nested asthma case-control study design.

2. Materials and methods

2.1. Study population and sample selection

The SAPALDIA study has been described elsewhere (Martin et al., 1997) but in brief, the participants include 9651 population-representative adults, aged 18 to 60 years when they were recruited in 1991, from eight Swiss communities (Aarau, Basel, Davos, Geneva, Lugano, Montana, Payerne, and Wald) which represent the diverse geographic characteristics of Switzerland. At baseline (SAPALDIA1) and first follow-up in 2002 (SAPALDIA2), 8047 participants had computer-assisted interviews on health and lifestyle characteristics. Venipuncture for biomarker and genetic assays was also done at follow-up. Details of follow-up participation rates can be found elsewhere (Ackermann-Liebrich et al., 2005). Participants gave prior written informed consent (including to genetic testing). The study protocols were approved by the Swiss National Ethics Committee and the Regional Ethics Committees of the eight study centers. As part of the European asthma consortium, GABRIEL, a nested asthma case-control study was designed using the SAPALDIA2 samples and data involving 1612 participants (Moffatt et al., 2010). Participants were identified as having asthma if they responded "yes" to the question: "have you ever been diagnosed of asthma"? Corresponding controls were selected from participants who responded "no" to this question. Eligible participants in the GABRIEL study comprised 654 asthma cases and 958 randomly selected asthma controls (Moffatt et al., 2010) and underwent genome-wide typing. The present cross-sectional analyses include 1524 (615 asthma cases and 913 controls) SAPALDIA2 participants who had genome-wide data and data on other relevant variables for current research question.

2.2. Case identification

We identified participants with diabetes as having at least one of the following at follow-up: a self-report of physician-diagnosed diabetes; use of diabetes medication in the past month; non-fasting blood glucose >11.1 mmol/L or HbA1c > 0.065. HbA1c was measured only in participants with non-fasting glucose > 6.1 mmol/L (Eze et al., 2014b). We did not have information on diabetes status at baseline, thus precluding the study of incident diabetes.

2.3. Air pollution exposure assignment

Consistent with our previous publication (Eze et al., 2014a), we considered 10-year mean residential exposure to particulate matter <10 μm (PM_{10}) as our air pollution exposure measure of interest. We did not consider nitrogen dioxide (NO_2) in this study because PM_{10} showed a sustained effect on diabetes and metabolic syndrome independent of NO_2 (in adjusted two-pollutant models) in the SAPALDIA cohort (Eze et al., 2014a, 2015b). PM_{10} was assigned to participants' residential addresses in 1990 and 2000 using validated dispersion models, at a resolution of 200 m × 200 m, based on various emission inventories including road and rail traffic, agriculture and industries (Liu et al., 2007). Annual estimates of ambient residential PM_{10} levels of up to ten years of follow-up were computed using annual trends at fixed monitoring stations closest to the residential addresses, and participants' residential histories. We computed 10-year means as a marker of long-term exposure to PM_{10} (Eze et al., 2014a).

2.4. Genotyping, imputation and selection of T2D risk variants

Genomic DNA was extracted using PUREGENE™ DNA Purification Kit (GENTRA Systems, Minneapolis, USA), from EDTA-buffered whole blood (Ackermann-Liebrich et al., 2005). Whole genome genotyping was done at the Centre National de Génotypage (CNG, Evry, France) within the nested asthma case-control study (N = 1612), using Illumina Human610K Quad BeadChip (Illumina, San Diego, CA, USA) covering 567′589 autosomal single nucleotide polymorphisms (SNPs) (Moffatt et al., 2010). Following quality control, 35 participants were excluded for having low genotyping call rate (<97%), leaving 1577 participants with high quality genome-wide data for analyses. Successfully genotyped SNPs were imputed to 2.5 million SNPs using MaCH v1.0 (Li et al., 2010; Soler Artigas et al., 2015).

T2D risk variants were selected if they were identified or confirmed as achieving genome-wide significance ($P < 5 \times 10^{-8}$) irrespective of population ancestry. A recent meta-analysis identified 65 T2D variants reaching genome-wide significance (Morris et al., 2012). We included 63 T2D in our GRS. Genotype data on two variants (rs6819243 near gene *MAEA* and rs4458523 near *WFS1*) on chromosome 4 (including proxies with $R^2 \geq 0.8$) were not captured on the Illumina 610 K Quad BeadChip, thus, the genetic risk scores computed for this study were based on 63 T2D SNPs each representing the top GWAS-identified variant of one T2D associated locus.

2.5. Genetic risk scores

We computed two polygenic risk scores, "count-GRS" and "weighted-GRS", based on the 63 selected SNPs. We calculated the count-GRS by summing up the number of risk alleles across the 63 SNPs, giving a minimum of 52 risk alleles and a maximum of 82 risk alleles. Count-GRS assumes that alleles contribute to disease risk in an additive manner, i.e., with a value of 0 for non-risk and 1 for each risk allele (Cornelis et al., 2009b). The additive model is more plausible when the genetic model is unknown (Balding, 2006). We calculated the weighted-GRS by first, weighting by size of the beta-coefficients derived from the largest genome-wide meta-analysis on T2D (Morris et al., 2012). We weighted the SNPs by multiplying the number of risk alleles of each SNP (i.e., 0, 1, and 2) by the reported beta-coefficient associated with the SNP. Next, we summed up the products across the 63 SNPs. To facilitate interpretation of effect size per risk allele, and enable comparison with count-GRS, we standardized the weighted-GRS by dividing it by 5.34 (the sum of the beta-coefficients) and multiplying by 63 (the possible maximal number of risk variants) (Cornelis et al., 2009b). The minimum and maximum weighted-GRS were 49.9 and 84.6 risk alleles respectively.

We also computed count- and weighted-GRS, using the same procedure, for the major pathway markers of T2D pathology including insulin resistance (count-GRS_{IR}; weighted-GRS_{IR}; involving variants near *GCKR*, *GRB14*, *IRS1*, *PPARG*, *ANKRD55*, *KLF14*, *HMGA2*, *FTO*, *M4CR* and *PEPD*) and beta-cell function (count-GRS_{BCF}; weighted-GRS_{BCF}; involving variants near *PROX1*, *THADA*, *UBE2E2*, *ADCY5*, *IGF2BP2*, *CDKAL1*, *DGKB*, *GCK*, *ANK1*, *SLC30A8*, *GLIS3*, *CDKN2A/B*, *CDC123*, *HHEX/IDE*, *TCF7L2*, *KCNQ1*, *KCNJ11*, *ARAP1*, *MTNR1B*, *C2CD4A* and *BCAR1*) or of related traits likely to mediate insulin resistance based on two T2D GWAS variants, one near *FTO* and one in the *M4CR* gene.

2.6. Potential confounders

Similar to our previous publication on air pollution and diabetes (Eze et al., 2014a), we considered the following potential confounders: age (years; continuous), sex, body mass index (kg/m^2; continuous), years of formal education (≤9; >9), neighborhood socio-economic index (expressed as a percentage; developed from a principal component analysis involving occupation and educational level of household head, median rent and number of persons in a household (Panczak et al., 2012)). Additionally we considered active smoking history (never, former, current; and pack-years), exposure to passive smoke (yes/no) and occupational vapors, gases, dusts and fumes (yes/no), as well as nutritional habits like alcohol consumption (including beers, wines, spirits and liquors: never; ≤1 glass/day; >1 glass/day); consumption of at least one portion of fruits and raw vegetables respectively (never; ≤3 days/week; >3 days/week) and moderate physical activity (defined as at least 150 min/week of participation in activities that make one out of breath). All models were adjusted for genome-wide population stratification.

2.7. Statistical analyses

We summarized characteristics at follow-up of participants with and without diabetes and contrasted them to follow-up participants not included in the current analysis. We assessed risk allele frequency (RAF) and Hardy-Weinberg equilibrium of the selected risk variants. We explored associations of diabetes with GRS and with ambient air pollution in this sample.

We first assessed interactions between PM_{10} and each of the 63 T2D genetic risk variants on diabetes. Then we fitted interaction terms between the GRS and PM_{10}, on a continuous scale to assess potential risk dependent effect modification. We also explored associations between PM_{10} and diabetes across quartiles of GRS. For the pathway-related genetic risk, we fitted interaction terms between count- and weighted-GRS, and PM_{10} for insulin resistance, obesity-mediated insulin resistance and beta cell function pathway separately (count-GRS_{IR} and count-GRS_{BCF}) to explore their specific interaction with PM_{10} on diabetes. In sensitivity analyses, we repeated all analyses with weighted-GRS by i) stratifying our analyses by asthma status, ii) omitting BMI from covariates, iii) assessing impact of selection bias by applying inverse probability weighting (IPW) to the models and iv) performing models with study center as fixed effect. All analyses were performed with STATA version 14 [STATA Corporation, Texas, USA] and involved mixed logistic regression models, with random intercepts by study area.

3. Results

Characteristics at the first follow-up (SAPALDIA2) of participants and non-participants in the presented analyses and comparison of the included participants with and without diabetes are presented in Table 1.

Overall, the participant characteristics were similarly distributed between the included (no diabetes) and excluded participants, despite a low inclusion rate of ~20% (Table 1). Among included participants, diabetes prevalence was 4.6%, and highly comparable to the diabetes prevalence in the non-participants (4.7%); and mean PM_{10} exposure was 22.1 μg/m^3 in participants and 22.5 μg/m^3 in non-participants. Compared to participants without diabetes, diabetes cases were more likely to be male, of lower social status, obese, smokers and consumed more alcohol. Moreover, they were exposed to higher PM_{10} concentrations. Prevalent asthma and mean count- and weighted GRS were also significantly higher among participants with diabetes (Table 1). Mean (SD) count-GRS was 67 (4.8) risk alleles whereas mean (SD) weighted-GRS was 66.5 (5.3) risk alleles. Both GRS were normally distributed in the study population (0.4 ≤ P-value of Shapiro-Wilk test ≤0.8). Table 2 describes all included SNPs, indicating chromosomal location, nearby gene, risk allele and its frequency. All SNPs were in Hardy-Weinberg Equilibrium ($P > 0.01$), with risk allele frequency (RAF) ≥3%.

The previously published positive association between PM_{10} and diabetes (Eze et al., 2014a) persisted in this smaller sample in both crude (odds ratio (OR): 1.23 (0.88, 1.73) per 10μg/m^3 exposure to PM_{10}) and adjusted models (OR: 1.35 (0.92, 1.97)). Additional adjustment for count-GRS (while adjusting for BMI), and removal of BMI (while adjusting for count-GRS) only increased the odds of diabetes by 2% and 6% respectively.

Table 1
Characteristics of participants at first follow-up of SAPALDIA study, included and excluded from present study.

Characteristics (% or mean (SD))	Diabetes N = 70	No diabetes N = 1454	Excluded % or mean (SD); N
Age (years)	60.7 (8.4)	51.5 (11.3)[a]	52.1 (11.6); 6156
Females	33.8	51.7[a]	52.1; 6156
Body mass index (kg/m^2)	30.6 (5.2)	25.7 (4.3)[a]	25.9 (4.5); 5074
Formal education ≤9 years	15.5	6.1[a]	8.7; 6145[b]
Neighborhood socio-economic index (%)	60.9 (9.8)	63.6 (10.2)[a]	63.3 (10.3); 6466
Ever-smokers	70.4	56.1[a]	58.1; 6523
Pack-years smoked	16.6 (27.4)	9.9 (17.6)[a]	11.3 (18.7); 5972
Exposure to passive smoke	54.9	47.5	47.6; 6523
Occupational exposure to vapors, gases, dusts and fumes	48.6	43.0	31.0; 6523[b]
Alcohol consumption >1 glass/day	11.4	8.2	9.4; 5038
Consumption of fruits - never/seldom	7.0	8.9	8.9; 5036
Consumption of raw vegetables - Never/seldom	4.2	2.0	2.0; 5040
150 min of moderate physical activity /week	51.4	46.8	49.9; 5019
Asthma cases	52.1	39.9[a]	43.4; 53
Diabetes cases	100	0[a]	4.7
10-year mean PM_{10} (μg/m^3)	23.1 (7.0)	22.0 (7.0)	22.5 (7.5); 6052
Total count-GRS	68.5 (4.8)	66.9 (4.7)[a]	67.3 (3.9); 53
Total weighted-GRS	68.2 (5.2)	66.4 (5.3)[a]	66.6 (4.4); 53
Insulin resistance count-GRS	10.8 (2.1)	10.4 (1.9)	10.4 (1.8); 53
Insulin resistance weighted-GRS	10.7 (2.2)	10.4 (2.0)	10.4 (2.0); 53
Beta-cell function count-GRS	24.1 (3.1)	23.5 (3.2)	20.7 (2.7); 53
Beta-cell function weighted-GRS	20.7 (2.7)	19.9 (3.1)[a]	2.2 (0.3); 53
Insulin resistance (obesity variants) count-GRS	1.6 (0.9)	1.4 (0.9)	1.6 (1.0); 53
Insulin resistance (obesity variants) weighted-GRS	1.7 (1.0)	1.4 (1.0)[a]	1.6 (1.0); 53

GRS: genetic risk score; PM_{10}: particulate matter <10 μm in diameter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults.
[a] Significant difference in proportion or mean between diabetes cases and participants without diabetes ($P < 0.05$).
[b] Significant difference in proportions or means between participants and non-participants of the presented analyses ($P < 0.05$).

The direction of association of 41 alleles agrees with that of published risk alleles on T2D, despite perfect agreement in the RAFs (Supplementary Table 1). We observed positive associations between count- and weighted-GRS and diabetes in our sample. In the crude model, odds of diabetes was increased by 7% (2, 12%) and 6% (2, 11%) per unit of count- and weighted-GRS respectively. Adjusted models, which did not depend on adjustment for BMI or PM_{10}, showed similar results (Table 2).

Table 2 also shows the results of the single SNP interactions with PM_{10} on the odds of diabetes. Interaction with only five variants (near *GRB14*, *UBE2E2*, *PTPRD*, *VPS26A* and *KCNQ1*) showed nominal significance ($P < 0.05$). Although nominally non-significant, we observed strong interaction signals with variants near *THADA*, *PPARG*, *KLF14*, *ZMIZ1*, *DUSP8*, *ARAP1*, *PRC1* and *FTO* (Table 2). No single variant interaction remained significant following Bonferroni correction at $P < 0.0016$ (0.1/63), false discovery rate $P < 0.0016$ ($0.1 * 1/63$) or family-wise error rate $P < 0.0016$ ($1-(1-0.1)^{1/63}$).

Looking at the combination of T2D variants, we observed a significant positive interaction between 10-year mean PM_{10} and 63-loci GRS (Table 2). The association between PM_{10} and diabetes increased across quartiles of count-GRS, being strongest among those in the highest quartile of genetic risk (Table 3). Compared to those at lowest genetic risk (Q1), odds of diabetes (per 10 μg/m^3 exposure to PM_{10}) increased by 106% among those at highest risk (Q4). Interactions between PM_{10} and weighted-GRS on odds of diabetes were similar, and sometimes stronger, compared to those observed with count-GRS (Table 3).

Fig. 1 shows interaction odds ratios for PM_{10} and pathway-specific GRS. Odds of diabetes (per 10μg/m^3 exposure to PM_{10}) increased by 22% (95% CI: 0, 49%) per T2D risk allele of insulin resistance GRS (count-GRS_{IR}).

We observed a positive and weaker interaction with beta cell function GRS (count-GRS_{BCF}), the odds of diabetes (per 10 μg/m^3 exposure to PM_{10}) increased by 6% (−8, 22%) per T2D risk allele of count-GRS_{BCF} (Fig. 1). Interactions with weighted-GRS were almost identical to those observed with count-GRS for both pathways (Supplementary Table 2), and were insensitive to BMI in the interaction model.

Interactions with 63-loci GRS were comparable between asthma cases and controls, but pathway-specific GRS_{IR} showed stronger significant interactions with PM_{10} among asthma cases (Fig. 2).

When considering only obesity-dependent variants in the count-GRS_{IR}, asthma cases had a more than twofold increased odds of diabetes (per 10 μg/m^3 exposure to PM_{10} and per T2D risk variant) (Fig. 2). These observations were also very consistent with weighted-GRS (Supplementary Table 2) and were insensitive to BMI. When comparing participants by asthma status, significant differences were only observed for age, BMI, alcohol consumption and diabetes status (Supplementary Table 3).

Sensitivity analyses proved robust results. In particular, interactions were not sensitive to body mass index. Adjusting the analyses for selection bias or treating study area as fixed effect also did not change the results of PM_{10}-GRS interactions (Table 4).

4. Discussion

This is the first study to show a positive interaction between T2D polygenic risk and particulate matter, on prevalent diabetes. Individuals at higher genetic risk for diabetes were more susceptible to PM_{10}. This was especially true for genetic variants functionally related to T2D through alteration of insulin sensitivity. Our findings, which remained robust across sensitivity analyses, also indicate that stronger associations may be observed in pathway-based analyses, providing a promising handle to disentangle the complex disease etiology by assessing gene-environment interactions.

Similar to our finding of a positive relationship between T2D polygenic risk and diabetes and its modifiability by air pollution, in the Health Professionals Follow-up and Nurses' Health Study, a ten-SNP score-associated risk of T2D was higher among the obese and persons with family history of diabetes (Cornelis et al., 2009b). Another study of a GRS of 49 SNPs also showed the positive association with incident T2D to be modified by age and obesity (Langenberg et al., 2014). A study by Andersson et al (Andersson et al., 2013) showed a polygenic risk score of 46 SNPs to predict T2D especially among weight gainers (Andersson et al., 2013). A 65-loci GRS was associated with prevalent

Table 2
Interactions of PM_{10} with candidate SNPs and genetic risk scores on the odds of diabetes in the SAPALDIA study.

RS number	CHR	Gene$_{(pathway)}$[a]	Risk/other allele	Risk allele frequency	Association with diabetes[b]	Increase in odds of diabetes per 10 μg/m^3 increase in PM_{10}[b]
					OR (95% CI)	OR (95% CI)
rs10923931	1	*NOTCH2*	T/G	0.09	0.89 (0.46, 1.71)	0.73 (0.29, 1.87)
rs2075423	1	*PROX1*$_{(BCF)}$	G/T	0.64	0.71 (0.49, 1.04)	1.15 (0.66, 1.99)
rs780094	2	*GCKR*$_{(IR)}$	C/T	0.54	1.07 (0.74, 1.55)	0.77 (0.46, 1.27)
rs10203174	2	*THADA*$_{(BCF)}$	C/T	0.89	2.20 (1.02, 4.71)[c]	0.35 (0.11, 1.13)
rs243088	2	*BCL11A*	T/A	0.48	0.95 (0.66, 1.37)	0.71 (0.43, 1.19)
rs7569522	2	*RBMS1*	A/G	0.47	1.16 (0.78, 1.73)	0.76 (0.43, 1.32)
rs13389219	2	*GRB14*$_{(IR)}$	C/T	0.62	0.85 (0.58, 1.24)	2.19 (1.26, 3.80)[c]
rs2943640	2	*IRS1*$_{(IR)}$	C/A	0.66	1.19 (0.80, 1.17)	1.33 (0.74, 2.38)
rs1801282	3	*PPARG*$_{(IR)}$	C/G	0.89	0.75 (0.42, 1.33)	0.55 (0.23, 1.28)
rs1496653	3	*UBE2E2*$_{(BCF)}$	A/G	0.82	0.80 (0.49, 1.31)	1.98 (1.01, 3.90)[c]
rs12497268	3	*PSMD6*	G/C	0.84	0.95 (0.56, 1.61)	1.28 (0.55, 2.96)
rs6795735	3	*ADAMTS9*	C/T	0.56	1.27 (0.85, 1.88)	0.69 (0.40, 1.21)
rs11717195	3	*ADCY5*$_{(BCF)}$	T/C	0.80	1.35 (0.83, 2.20)	0.65 (0.31, 1.37)
rs4402960	3	*IGF2BP2*$_{(BCF)}$	T/G	0.31	1.25 (0.84, 1.86)	1.17 (0.65, 2.12)
rs17301514	3	*ST64GAL1*	A/G	0.10	1.51 (0.86, 2.63)	0.97 (0.42, 2.26)
rs459193	5	*ANKRD55*$_{(IR)}$	G/A	0.74	0.99 (0.63, 1.57)	1.11 (0.60, 2.07)
rs6878122	5	*ZBED3*	G/A	0.30	1.00 (0.67, 1.49)	1.00 (0.56, 1.79)
rs7756992	6	*CDKAL1*$_{(BCF)}$	G/A	0.28	1.24 (0.82, 1.88)	1.20 (0.65, 2.21)
rs4299828	6	*ZFAND3*	A/G	0.72	0.96 (0.61, 1.50)	1.06 (0.55, 2.06)
rs3734621	6	*KCNK16*	C/A	0.03	1.03 (0.37, 2.88)	1.20 (0.34, 4.24)
rs17168486	7	*DGKB*$_{(BCF)}$	T/C	0.15	0.98 (0.58, 1.66)	1.85 (0.83, 4.15)
rs849135	7	*JAZF1*	G/A	0.50	0.87 (0.60, 1.25)	1.27 (0.76, 2.14)
rs10278336	7	*GCK*$_{(BCF)}$	A/G	0.60	1.01 (0.68, 1.49)	1.13 (0.64, 1.99)
rs17867832	7	*GCC1*	T/G	0.92	0.89 (0.44, 1.79)	1.01 (0.36, 2.81)
rs13233731	7	*KLF14*$_{(IR)}$	G/A	0.54	1.46 (0.99, 2.15)	1.61 (0.91, 2.83)
rs516946	8	*ANK1*$_{(BCF)}$	C/T	0.73	1.05 (0.69, 1.60)	0.75 (0.41, 1.39)
rs7845219	8	*TP53INP1*	T/C	0.50	1.06 (0.74, 1.52)	0.90 (0.53, 1.53)
rs3802177	8	*SLC30A8*$_{(BCF)}$	G/A	0.73	0.88 (0.59, 1.31)	1.38 (0.75, 2.53)
rs10758593	9	*GLIS3*$_{(BCF)}$	A/G	0.42	1.07 (0.74, 1.55)	1.00 (0.58, 1.74)
rs16927668	9	*PTPRD*	T/C	0.24	1.26 (0.83, 1.91)	0.50 (0.28, 0.92)[c]
rs10811661	9	*CDKN2A/B*$_{(BCF)}$	T/C	0.80	1.54 (0.92, 2.58)	0.86 (0.42, 1.79)
rs17791513	9	*TLE4*	A/G	0.94	1.57 (0.63, 3.94)	0.95 (0.20, 4.41)
rs2796441	9	*TLE1*	G/A	0.61	1.08 (0.73, 1.59)	1.01 (0.58, 1.73)
rs11257655	10	*CDC123*$_{(BCF)}$	T/C	0.20	0.86 (0.54, 1.37)	1.23 (0.62, 2.43)
rs12242953	10	*VPS26A*	G/A	0.93	0.79 (0.41, 1.53)	2.96 (1.04, 8.41)[c]
rs12571751	10	*ZMIZ1*	A/G	0.54	1.01 (0.69, 1.48)	1.52 (0.90, 2.57)
rs1111875	10	*HHEX/IDE*$_{(BCF)}$	C/T	0.61	1.03 (0.71, 1.51)	1.30 (0.77, 2.21)
rs7903146	10	*TCF7L2*$_{(BCF)}$	T/C	0.33	1.33 (0.91, 1.94)	0.81 (0.47, 1.40)
rs2334499	11	*DUSP8*	T/C	0.40	0.80 (0.55, 1.15)	1.59 (0.92, 2.75)
rs163184	11	*KCNQ1*$_{(BCF)}$	G/T	0.47	1.16 (0.80, 1.69)	1.87 (1.09, 3.20)[c]
rs5215	11	*KCNJ11*$_{(BCF)}$	C/T	0.37	0.87 (0.59, 1.28)	1.00 (0.56, 1.77)
rs1552224	11	*ARAP1*$_{(BCF)}$	A/C	0.86	1.79 (0.90, 3.55)	0.50 (0.18, 1.37)
rs10830963	11	*MTNR1B*$_{(BCF)}$	G/C	0.27	1.28 (0.79, 2.07)	0.68 (0.34, 1.37)
rs11063069	12	*CCND2*	G/A	0.18	1.93 (1.12, 3.33)[a]	1.40 (0.64, 3.08)
rs10842994	12	*KLHDC5*	C/T	0.82	1.26 (0.75, 2.12)	1.10 (0.53, 2.30)
rs2261181	12	*HMGA2*$_{(IR)}$	T/C	0.12	0.81 (0.43, 1.51)	1.47 (0.59, 3.66)
rs7955901	12	*TSPAN8*	C/T	0.47	1.24 (0.85, 1.81)	1.25 (0.73, 2.13)
rs12427353	12	*HNF1A* (*TCF1*)	G/C	0.80	1.39 (0.81, 2.37)	1.34 (0.63, 2.88)
rs1359790	13	*SPRY2*	G/A	0.73	0.96 (0.63, 1.44)	1.16 (0.66, 2.05)
rs4502156	15	*C2CD4A*$_{(BCF)}$	T/C	0.57	1.12 (0.77, 1.64)	0.99 (0.58, 1.72)
rs7177055	15	*HMG20A*	A/G	0.70	1.02 (0.68, 1.53)	0.90 (0.51, 1.60)
rs11634397	15	*ZFAND6*	G/A	0.65	1.24 (0.82, 1.86)	1.41 (0.77, 2.59)
rs2007084	15	*AP3S2*	G/A	0.93	1.26 (0.58, 2.74)	0.65 (0.19, 2.20)
rs12899811	15	*PRC1*	G/A	0.31	1.00 (0.67, 1.50)	1.64 (0.94, 2.86)
rs9936385	16	*FTO*$_{(IR)}$	C/T	0.42	1.35 (0.93, 1.96)	1.59 (0.92, 2.73)
rs7202877	16	*BCAR1*$_{(BCF)}$	T/G	0.90	2.31 (1.06, 5.03)[c]	0.52 (0.17, 1.60)
rs2447090	17	*SRR*	A/G	0.64	0.83 (0.57, 1.21)	0.82 (0.46, 1.44)
rs11651052	17	*HNF1B* (*TCF2*)	G/A	0.49	0.92 (0.63, 1.33)	1.47 (0.86, 2.53)
rs12970134	18	*MC4R*$_{(IR)}$	A/G	0.28	1.08 (0.72, 1.63)	0.95 (0.52, 1.72)
rs10401969	19	*CILP2*	C/T	0.07	0.15 (0.03, 0.87)[c]	1.07 (0.07, 15.6)
rs8182584	19	*PEPD*$_{(IR)}$	T/G	0.38	1.20 (0.81, 1.76)	0.87 (0.50, 1.51)
rs8108269	19	*GIPR*	G/T	0.32	1.02 (0.68, 1.51)	1.07 (0.59, 1.95)
rs4812829	20	*HNF4A*	A/G	0.18	1.16 (0.72, 1.89)	0.71 (0.35, 1.44)
Count genetic risk score					1.08 (1.02, 1.14)[c]	1.10 (1.01, 1.20)[c]
Weighted genetic risk score					1.09 (1.03, 1.14)[c]	1.07 (0.99, 1.16)[d]

BCF: Beta-cell function; CI: confidence intervals; IR: Insulin resistance; OR: Odds ratio; PM_{10}: particulate matter <10 μm in diameter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults; SNPs: Single nucleotide polymorphisms.

[a] SNPs were genotyped using Illumina Human610Kquad BeadChip and imputations done using MaCH v1.0 software.

[b] All models adjusted for age, sex, educational level, neighborhood socio-economic index, smoking status and pack years, passive smoke exposure, consumption of alcohol, fruits and vegetables, physical activity, body mass index and genome-wide population stratification.

[c] $P < 0.05$.

[d] $P < 0.1$.

Table 3
Associations between PM_{10} and quartiles of count-GRS on the odds of diabetes in the SAPALDIA study.

	Quartile	N	Range of risk alleles	Association with diabetes[a]	Increase in odds of diabetes per 10 μg/m^3 increase in PM_{10}[b]
Count-GRS				OR (95% CI)	OR (95% CI)
	Q_1	385	51.67–63.83	Reference	0.82 (0.41, 1.65)
	Q_2	378	63.84–67.09	0.93 (0.40, 2.14)	0.92 (0.55, 1.54)
	Q_3	381	67.10–70.22	1.66 (0.76, 3.61)	1.54 (0.95, 2.49)[d]
	Q_4	380	70.23–82.33	1.86 (0.86, 3.99)	1.97 (1.00, 3.87)[c]
	Q_4 vs. Q_1	765	51.67–82.33	2.31 (1.03, 5.19)[c]	2.06 (0.69, 6.19)
Weighted-GRS					
	Q_1	381	49.92–62.84	Reference	0.83 (0.39, 1.74)
	Q_2	382	62.85–66.49	1.28 (0.55, 2.99)	1.04 (0.62, 1.73)
	Q_3	380	66.50–70.13	1.40 (0.60, 3.28)	1.21 (0.73, 1.99)
	Q_4	381	70.14–84.62	3.28 (1.48, 7.27)[c]	2.01 (1.04, 3.88)[c]
	Q_4 vs. Q_1	762	49.92–84.62	3.61 (1.55, 8.42)[c]	2.53 (0.82, 7.76)

Abbreviations: CI, confidence intervals; GRS, genetic risk score; OR, Odds ratio; PM_{10}, particulate matter < 10 μm in diameter; SAPALDIA, Swiss cohort study on air pollution and lung and heart diseases in adults.
[a] ORs and 95%CIs represent increase in odds of diabetes per risk allele.
[b] ORs and 95%CIs represent increase in odds of diabetes per 10 μg/m^3 increase in exposure to PM_{10}.
[c] $P < 0.05$.
[d] $P < 0.1$.

T2D among people with European ancestry (Talmud et al., 2015) whereas a 62-loci GRS equally predicted T2D in both blacks and whites (Vassy et al., 2014).

Experimental and epidemiologic evidence have demonstrated the contribution of fine particulate matter to insulin resistance. $PM_{2.5}$ was shown to enhance insulin resistance in a mouse model of diet-induced obesity (Sun et al., 2009). Kelishadi and colleagues found $PM_{2.5}$ to be associated with markers of insulin resistance among Iranian children (Kelishadi et al., 2009). On the other hand, NO_2 was also associated with insulin resistance among two cohorts of German children (Thiering et al., 2013). In a study of 25 healthy adults, Brook and colleagues found an association between a sub-acute exposure to $PM_{2.5}$ and insulin resistance (Brook et al., 2013). Postulated mechanisms for the observed association include systemic inflammation, alteration of insulin signaling following oxidative stress, endothelial vasoconstriction, hypothalamic-adrenal stress response and augmentation of sympathetic activity (Liu et al., 2013; Rajagopalan and Brook, 2012).

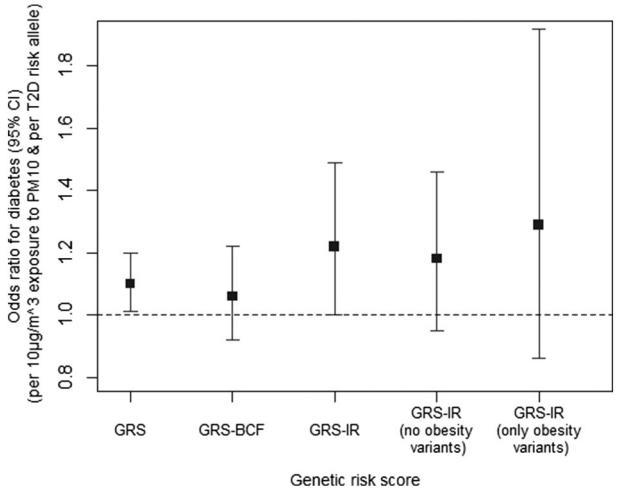

Fig. 1. Interactions between PM_{10} and count-GRS on prevalent diabetes in the SAPALDIA study. GRS: total genetic risk score; GRS-BCF: beta-cell function genetic risk score; GRS-IR: insulin resistance genetic risk score; GRS-IR (no obesity variants): insulin resistance genetic risk score excluding polymorphisms on *FTO* and *M4CR* with primary effect on obesity; GRS-IR (only obesity variants): insulin resistance genetic risk score including only polymorphisms on *FTO* and *M4CR* with primary effect on obesity; PM_{10}: particulate matter < 10 μm in diameter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults. Count-GRS was computed by summation of risk alleles. Odds ratios represent increase in odds of diabetes per 10 μg/m^3 exposure to PM_{10} and per risk allele. All associations were adjusted for obesity, age, sex, socio-economic status, smoking habits, consumption of alcohol, fruits and vegetables, physical activity and genome-wide population stratification. Study area was treated as a random effect in all models.

Our results also suggest that individuals with pre-existing inflammation like asthma or at risk of obesity are potentially most susceptible to air pollution increasing the risk for developing diabetes. We observed a stronger interaction of PM_{10} with insulin resistance variants among asthma cases which was even stronger when we restricted the score to the *FTO* and *M4CR* variants which are known to be causally related to higher BMI over the course of life (Perry and Frayling, 2008; Scott et al., 2014) (Fig. 1). Air pollution exposure has been linked to both asthma and obesity (Eze et al., 2015b; Jacquemin et al., 2015; Jerrett et al., 2014), and studies have linked asthma to obesity and insulin resistance (Husemoen et al., 2008; Sanchez Jimenez et al., 2014; Singh et al., 2013). While there is a consensus that obesity-related systemic inflammation likely contributes to the asthma etiology, epidemiological evidence on the relationship between asthma and diabetes is limited and conflicting. Some studies reported a link between asthma and diabetes (Ehrlich et al., 2010; Mueller et al., 2013) especially in obese people (Mueller et al., 2013), others did not (Rana et al., 2004).

While asthma and obesity are recognized inflammatory conditions and with experimental data from animal models corroborating that visceral adiposity-related inflammation may act as mediator for $PM_{2.5}$ to increase the risk for insulin resistance (Sun et al., 2009), other studies have shown discordance between systemic inflammation and severity of symptoms in obese asthmatics (Beuther et al., 2006; Haldar et al., 2008). Other lines of evidence suggest that non-inflammatory pathways might also link PM to insulin resistance (Brook et al., 2013), with experimental animal models providing evidence for insulin resistance in muscle tissues resulting from lipid and protein oxidation by-products upon acute exposure to ozone (Kodavanti, 2015; Vella et al., 2015). Hence, despite the strong evidence for a central role of pre-existing inflammation, e.g., due to asthma or being at genetic risk of obesity, other non-inflammation based mechanisms cannot be ruled out to underlie or contribute to the air pollution-diabetes association.

There is some evidences on the impact of environmental pollutants (including organophosphorus compounds, persistent organic pollutants and metals) on various aspects of beta-cell dysfunction that lead to diabetes (Hectors et al., 2011), but there is to date no experimental evidence on the impact of air pollutants on BCF. Although interactions with the polygenic risk involving the BCF variants in the 63-loci GRS were not significant, we observed some positive signals among non-asthmatics in the BCF pathway (Fig. 2) and nominally significant

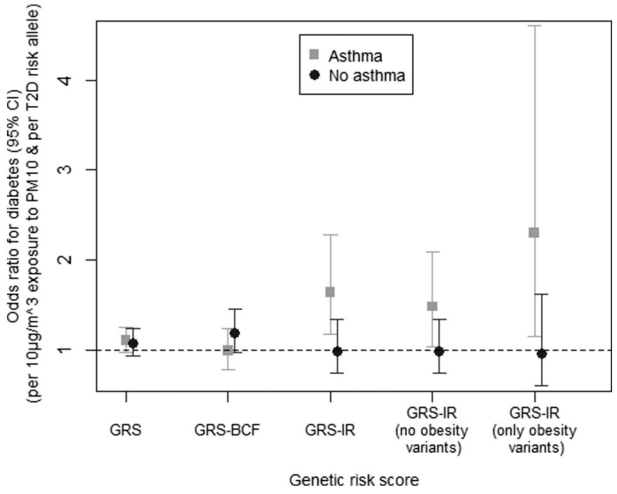

Fig. 2. Interactions between PM_{10} and count-GRS on prevalent diabetes in the SAPALDIA study, stratified by asthma status. GRS: total genetic risk score; GRS-BCF: beta-cell function genetic risk score; GRS-IR: insulin resistance genetic risk score; GRS-IR (no obesity variants): insulin resistance genetic risk score excluding polymorphisms on *FTO* and *M4CR* with primary effect on obesity; GRS-IR (only obesity variants): insulin resistance genetic risk score including only polymorphisms on *FTO* and *M4CR* with primary effect on obesity; PM_{10}: particulate matter <10 μm in diameter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults. Count-GRS was computed by summation of risk alleles. Odds ratios represent increase in odds of diabetes per 10μg/m^3 exposure to PM_{10} and per risk allele. All associations were adjusted for obesity, age, sex, socio-economic status, smoking habits, consumption of alcohol, fruits and vegetables, physical activity and genome-wide population stratification. Study area was treated as a random effect in all models. N (asthma) = 615; N (no asthma) = 909.

interactions with single variants in the BCF pathway (Table 2). This might indicate that PM may also have some impact on T2D through some alterations in the BCF.

This study has several strengths. It provides comprehensive evidence on the modifying effect of a polygenic risk score (including pathway-related components) on the association between ambient air pollution and DM. The SAPALDIA study contains a rich data set on well characterized participants including a large number of phenotypes and lifestyle characteristics, in addition to genomic data. We attempted to identify undiagnosed diabetes, using non-fasting blood tests, to limit outcome misclassification. Our estimates of air pollution derive from validated models, which have been applied to other SAPALDIA studies. These estimates were assigned to participants' residential address history, thus limiting exposure misclassification.

Despite these strengths, our study has also limitations. First is our inability to distinguish T1D and T2D. We assumed most of our diabetes cases to be type 2, since >90% of adult diabetes is type 2 (Alberti and Zimmet, 1998). We observed strong associations between the confirmed T2D risk alleles and our diabetes cases, in the range of published literature, thus strengthening our assumption of T2D. Moreover, when we limited the diabetes definition to either medication use or those without a diagnosis but increased non-fasting glucose levels, the associations with GRS remained unchanged. We had limited sample size (62% statistical power) for this analysis due to lacking genome-wide data. Assuming the observed effect is identical to the true effect, we would have needed twice the size of our sample to achieve 90% power for detecting this effect at the usual significance level of 5%. However, we made some salient findings, and IPW revealed no effect of potential selection bias in our study. This was a cross-sectional analysis, precluding any causal inferences. To limit this design bias, we focused on the 10-year mean of PM_{10} exposure, rather than on the mean during the year preceding the health assessment. Our study of genetic variation also limits this design bias to some extent considering that genetic variants remain unchanged throughout life. Furthermore, we studied PM_{10}, instead of $PM_{2.5}$, which may have stronger health effects due to its physical properties. Modeled $PM_{2.5}$ was not available for our study, but there is a high correlation between both pollutants across SAPALDIA study areas (R = 0.8) (Eze et al., 2014a). We would expect similar, if not stronger associations with $PM_{2.5}$. Lastly, our observations may be biased by the relationship between asthma (and its treatment) and diabetes, but we did not observe substantial differences in interactions between PM_{10} and total genetic risk, on stratification by asthma status.

Future studies should explore the possible role of air pollution in the impairment of BCF, and explore the role of unclassified T2D variants in disease etiology. Our present findings need confirmation and follow-up in diabetes cohort consortia. Consideration should also be given to ultra-fine particles, which can penetrate even further into the respiratory tract than $PM_{2.5}$ or PM_{10}.

In conclusion, our results indicate that polygenic risk of T2D may modify the effects of air pollutants on the risk of diabetes through alteration of insulin sensitivity among people with some existing background inflammation. This study is relevant given the need for the knowledge of genetic risk in disease prevention, and the importance of genotypes as research instrument in disentangling complexities and mechanisms in causality of modifiable risks.

Table 4
Sensitivity analyses using inverse probability weighting to assess for potential selection bias, and testing study area as a fixed effect, in the modification of associations between PM_{10} and diabetes by GRS in the SAPALDIA study.

Model	Interactions between PM_{10} and count-GRS on prevalent diabetes[a]	Interactions between PM_{10} and weighted-GRS on prevalent diabetes[a]
Inverse probability weighting for selection bias	OR (95% CI)	OR (95% CI)
63-loci GRS	1.10 (1.02, 1.20)[b]	1.08 (1.00, 1.16)[b]
GRS-beta cell function	1.07 (0.95, 1.20)	1.04 (0.93, 1.15)
GRS-insulin resistance	1.25 (1.03, 1.51)[b]	1.23 (1.03, 1.47)[b]
GRS-insulin resistance excluding obesity variants	1.21 (0.96, 1.53)	1.20 (0.95, 1.52)
GRS-insulin resistance (only obesity variants)	1.25 (0.86, 1.83)	1.54 (0.50, 4.69)
Study area as a fixed effect		
63-loci GRS	1.10 (1.01, 1.20)[b]	1.07 (0.99, 1.15)[b]
GRS-beta cell function	1.06 (0.92, 1.22)	1.03 (0.91, 1.15)
GRS-insulin resistance	1.22 (1.00, 1.50)[b]	1.21 (1.00, 1.48)[b]
GRS-insulin resistance excluding obesity variants	1.18 (0.95, 1.47)	1.17 (0.93, 1.47)
GRS-insulin resistance (only obesity variants)	1.29 (0.87, 1.91)	1.32 (0.90, 1.92)

Abbreviations: CI, confidence intervals; GRS, genetic risk score; OR, Odds ratio; PM_{10}, particulate matter <10 μm in diameter; SAPALDIA, Swiss cohort study on air pollution and lung and heart diseases in adults.

[a] ORs and 95%CIs represent increase in odds of diabetes per 10 μg/m^3 increase in exposure to PM_{10} and per unit risk allele. All models were mixed logistic regression with random intercepts for study areas, and adjusted for age, sex, educational attainment, neighborhood socio-economic index, smoking status, exposure to passive smoke and occupational vapors, dusts, gases and fumes, consumption of alcohol, fruits and vegetables, physical activity, body mass index and genome-wide population stratification.

[b] $P < 0.05$.

5. Current SAPALDIA team

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.04.032.

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SUPPLEMENTARY MATERIAL

Air pollution and diabetes association: modification by type 2 diabetes genetic risk score.

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Table of Content

Supplementary Table 1. Comparison of effect sizes of SNPs on the odds of diabetes in SAPALDIA with published data

RS number	CHR	Gene$_{(pathway)}$[a]	Risk allele	Risk allele frequency (SAPAL-DIA)	Risk allele frequency (published data)	OR effect size (SAPAL-DIA)[b]	OR effect size (published data)
rs10923931	1	*NOTCH2*	T	0.09	0.12	0.89	1.08
rs2075423	1	*PROX1*$_{(BCF)}$	G	0.64	0.62	0.71	1.07
rs780094	2	*GCKR*$_{(IR)}$	C	0.54	0.61	1.07	1.06
rs10203174	2	*THADA*$_{(BCF)}$	C	0.89	0.89	2.20	1.14
rs243088	2	*BCL11A*	T	0.48	0.45	0.95	1.07
rs7569522	2	*RBMS1*	A	0.47	0.44	1.16	1.05
rs13389219	2	*GRB14*$_{(IR)}$	C	0.62	0.60	0.85	1.07
rs2943640	2	*IRS1*$_{(IR)}$	C	0.66	0.63	1.19	1.10
rs1801282	3	*PPARG*$_{(IR)}$	C	0.89	0.86	0.75	1.13
rs1496653	3	*UBE2E2*$_{(BCF)}$	A	0.82	0.75	0.80	1.09
rs12497268	3	*PSMD6*	G	0.84	0.80	0.95	1.03
rs6795735	3	*ADAMTS9*	C	0.56	0.59	1.27	1.08
rs11717195	3	*ADCY5*$_{(BCF)}$	T	0.80	0.77	1.35	1.11
rs4402960	3	*IGF2BP2*$_{(BCF)}$	T	0.31	0.33	1.25	1.13
rs17301514	3	*ST64GAL1*	A	0.10	0.13	1.51	1.05
rs459193	5	*ANKRD55*$_{(IR)}$	G	0.74	0.70	0.99	1.08
rs6878122	5	*ZBED3*	G	0.30	0.28	1.00	1.10
rs7756992	6	*CDKAL1*$_{(BCF)}$	G	0.28	0.29	1.24	1.17
rs4299828	6	*ZFAND3*	A	0.72	0.79	0.96	1.04
rs3734621	6	*KCNK16*	C	0.03	0.03	1.03	1.07
rs17168486	7	*DGKB*$_{(BCF)}$	T	0.15	0.19	0.98	1.11
rs849135	7	*JAZF1*	G	0.50	0.52	0.87	1.11
rs10278336	7	*GCK*$_{(BCF)}$	A	0.60	0.50	1.01	1.07
rs17867832	7	*GCC1*	T	0.92	0.91	0.89	1.09
rs13233731	7	*KLF14*$_{(IR)}$	G	0.54	0.51	1.46	1.05
rs516946	8	*ANK1*$_{(BCF)}$	C	0.73	0.76	1.05	1.09

rs7845219	8	*TP53INP1*	T	0.50	0.52	1.06	1.06
rs3802177	8	*SLC30A8*$_{(BCF)}$	G	0.73	0.66	0.88	1.14
rs10758593	9	*GLIS3*$_{(BCF)}$	A	0.42	0.42	1.07	1.06
rs16927668	9	*PTPRD*	T	0.24	0.24	1.26	1.04
rs10811661	9	*CDKN2A/B*$_{(BCF)}$	T	0.80	0.82	1.54	1.18
rs17791513	9	*TLE4*	A	0.94	0.91	1.57	1.12
rs2796441	9	*TLE1*	G	0.61	0.57	1.08	1.07
rs11257655	10	*CDC123*$_{(BCF)}$	T	0.20	0.23	0.86	1.07
rs12242953	10	*VPS26A*	G	0.93	0.93	0.79	1.07
rs12571751	10	*ZMIZ1*	A	0.54	0.52	1.01	1.08
rs1111875	10	*HHEX/IDE*$_{(BCF)}$	C	0.61	0.58	1.03	1.11
rs7903146	10	*TCF7L2*$_{(BCF)}$	T	0.33	0.27	1.33	1.39
rs2334499	11	*DUSP8*	T	0.40	0.43	0.80	1.04
rs163184	11	*KCNQ1*$_{(BCF)}$	G	0.47	0.50	1.16	1.09
rs5215	11	*KCNJ11*$_{(BCF)}$	C	0.37	0.41	0.87	1.07
rs1552224	11	*ARAP1*$_{(BCF)}$	A	0.86	0.81	1.79	1.11
rs10830963	11	*MTNR1B*$_{(BCF)}$	G	0.27	0.31	1.28	1.10
rs11063069	12	*CCND2*	G	0.18	0.21	1.93	1.08
rs10842994	12	*KLHDC5*	C	0.82	0.80	1.26	1.10
rs2261181	12	*HMGA2*$_{(IR)}$	T	0.12	0.10	0.81	1.13
rs7955901	12	*TSPAN8*	C	0.47	0.45	1.24	1.07
rs12427353	12	*HNF1A (TCF1)*	G	0.80	0.79	1.39	1.08
rs1359790	13	*SPRY2*	G	0.73	0.72	0.96	1.08
rs4502156	15	*C2CD4A*$_{(BCF)}$	T	0.57	0.52	1.12	1.06
rs7177055	15	*HMG20A*	A	0.70	0.68	1.02	1.08
rs11634397	15	*ZFAND6*	G	0.65	0.64	1.24	1.05
rs2007084	15	*AP3S2*	G	0.93	0.92	1.26	1.02
rs12899811	15	*PRC1*	G	0.31	0.31	1.00	1.08
rs9936385	16	*FTO*$_{(IR)}$	C	0.42	0.41	1.35	1.13
rs7202877	16	*BCAR1*$_{(BCF)}$	T	0.90	0.89	2.31	1.12
rs2447090	17	*SRR*	A	0.64	0.62	0.83	1.04

rs11651052	17	*HNF1B (TCF2)*	G	0.49	0.44	0.92	1.10
rs12970134	18	*MC4R$_{(IR)}$*	A	0.28	0.27	1.08	1.08
rs10401969	19	*CILP2*	C	0.07	0.08	0.15	1.13
rs8182584	19	*PEPD$_{(IR)}$*	T	0.38	0.38	1.20	1.04
rs8108269	19	*GIPR*	G	0.32	0.31	1.02	1.07
rs4812829	20	*HNF4A*	A	0.18	0.19	1.16	1.06

BCF: Beta-cell function; IR: Insulin resistance; OR: Odds ratios; SAPALDIA, Swiss cohort study on air pollution and lung and heart diseases in Adults; SNPs: Single nucleotide polymorphisms. [a] All SNPs were genotyped in SAPALDIA using Illumina Human610Kquad BeadChip. [b] ORs from SAPALDIA were adjusted for age, sex, educational level, neighborhood socio-economic index, smoking status and pack years, passive smoke exposure, consumption of alcohol, fruits and vegetables, physical activity, body mass index and genome-wide population stratification.

Supplementary Table 2: Associations between PM_{10} and weighted-GRS on the odds of Diabetes in the SAPALDIA study

Model	Association with diabetes [a]	Interactions between PM_{10} and weighted-GRS on prevalent diabetes [b]
	OR (95% CI)	OR (95% CI)
Total weighted genetic risk score	1.09 (1.03, 1.14)[c]	1.07 (0.99, 1.16)[d]
Asthma	1.03 (0.95, 1.11)	1.08 (0.97, 1.20)
No asthma	1.16 (1.06, 1.26)[c]	1.07 (0.95, 1.21)
Weighted genetic risk score- IR	1.08 (0.95, 1.24)	1.21 (1.00, 1.46)[c]
Asthma	1.06 (0.87,1.28)	1.59 (1.16, 2.18)[c]
No asthma	1.12 (0.91, 1.38)	1.01 (0.76, 1.35)
Weighted genetic risk score- IR excluding *FTO* and *M4CR*	1.02 (0.87, 1.19)	1.17 (0.93, 1.46)
Asthma	0.99 (0.78, 1.25)	1.43 (1.00, 2.05)[c]
No asthma	1.07 (0.84, 1.36)	1.00 (0.71, 1.42)
Weighted genetic risk score- IR (only *FTO* and *M4CR*)	1.24 (0.95, 1.63)	1.33 (0.91, 1.94)
Asthma	1.26 (0.83, 1.91)	2.36 (1.22, 4.59)[c]
No asthma	1.24 (0.84,1.82)	0.98 (0.59, 1.62)
Weighted genetic risk score- Beta-cell function	1.12 (1.03, 1.23)[c]	1.01 (0.89, 1.13)
Asthma	1.06 (0.93, 1.22)	0.97 (0.81, 1.15)
No asthma	1.21 (1.06, 1.39)[c]	1.07 (0.90, 1.28)

CI: confidence intervals; GRS: genetic risk score; IR: insulin resistance; OR: Odds ratio; PM_{10}: particulate matter <10μm in daimeter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults. [a]ORs and 95%CIs represent increase in odds of diabetes per risk allele. [b]ORs and 95%CIs represent increase in odds of diabetes per 10ug/m^3 increase in exposure to PM_{10}. [a, b]All models were mixed logistic regression with random intercepts for study areas, and adjusted for age, sex, educational attainment, neighborhood cosio-economic index, smoking status, exposure to passive smoke and occupational vapors, dusts, gases and fumes, consumption of alcohol, fruits and vegetables, physical activity, body mass index and genome-wide population stratification. [c]$P<0.05$. [d]$P<0.1$.

Supplementary Table 3: Characteristics of participants in the present SAPALDIA study, stratified by Asthma status

Characteristics (% or Mean (SD))	Asthma N=615	No asthma N=909
Age (years)	51.0 (11.4)	52.5 (11.3) [a]
Females	51.5	50.4
Body mass index (kg/m^2)	26.4 (4.9)	25.6 (4.3) [a]
Formal education ≤ 9 years	6.8	6.4
Neighborhood socio-economic index (%)	63.5 (10.8)	63.4 (9.8)
Ever-smokers	56.9	56.5
Pack-years smoked	10 (17.7)	10.4 (18.5)
Exposure to passive smoke	48.9	47.2
Occupational exposure to vapours, gases, dusts and fumes	45.4	41.8
Alcohol consumption >1 glass/day	13.3	9.6 [a]
Consumption of fruits- Never/seldom	9.7	8.1
Consumption of raw vegetables- Never/seldom	2.4	2.0
150 minutes of moderate physical activity /week	46	47.6
Diabetes cases	5.9	3.7 [a]
10-year mean PM_{10} (μg/m^3)	22.1 (6.7)	22.1 (7.1)
Total count-GRS	67 (4.9)	67 (4.7)
Total weighted-GRS	66.4 (5.4)	66.5 (5.2)
Insulin resistance count-GRS	10.4 (2.1)	10.4 (1.9)
Insulin resistance weighted-GRS	10.4 (2)	10.4 (1.9)
IR (only *FTO* and *M4CR*) count-GRS	1.4 (0.9)	1.4 (0.9)
IR (only *FTO* and *M4CR*) weighted-GRS	1.5 (1.0)	1.5 (1.0)
Beta-cell function count-GRS	23.5 (2.9)	23.5 (2.8)
Beta-cell function weighted-GRS	19.9 (3.1)	19.9 (3.3)

GRS: genetic risk score; IR: insulin resistance; PM_{10}: particulate matter <10μm in diameter; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults. [a] Significant difference in proportion or mean between diabetes cases and participants without diabetes (P<0.05).

8. Article: A common functional variant on the pro-inflammatory interleukin-6 gene may modify the association between air pollutants and diabetes

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A common functional variant on the pro-inflammatory Interleukin-6 gene may modify the association between long-term PM_{10} exposure and diabetes

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Abstract

Background: Air pollutants have been linked to type 2 diabetes (T2D), hypothesized to act through inflammatory pathways and may induce interleukin-6 gene (*IL6*) in the airway epithelium. The cytokine interleukin-6 may impact on glucose homeostasis. Recent meta-analyses showed the common polymorphisms, *IL6* -572G > C and *IL6* -174G > C to be associated with T2D risk. These *IL6* variants also influence circulatory interleukin-6 levels. We hypothesize that these common functional variants may modify the association between air pollutants and T2D.

Methods: We cross-sectionally studied 4410 first follow-up participants of the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases (SAPALDIA), aged 29 to 73 years who had complete data on genotypes, diabetes status and covariates. We defined diabetes as self-reported physician-diagnosed, or use of diabetes medication or non-fasting glucose >11.1 mmol/L or HbA1c > 0.065. Air pollution exposure was 10-year mean particulate matter <10 μm in diameter (PM_{10}) assigned to participants' residences using a combination of dispersion modelling, annual trends at monitoring stations and residential history. We derived interaction terms between PM_{10} and genotypes, and applied mixed logistic models to explore genetic interactions by *IL6* polymorphisms on the odds of diabetes.

Results: There were 252 diabetes cases. Respective minor allele frequencies of *IL6* -572G > C and *IL6* -174G > C were 7 and 39 %. Mean exposure to PM_{10} was 22 μg/m^3. Both variants were not associated with diabetes in our study. We observed a significant positive association between PM_{10} and diabetes among homozygous carriers of the pro-inflammatory major G-allele of *IL6* -572G > C [Odds ratio: 1.53; 95 % confidence interval (1.22, 1.92); $P_{interaction\ (additive)} = 0.003$ and $P_{interaction\ (recessive)} = 0.006$]. Carriers of the major G-allele of *IL6* -174G > C also had significantly increased odds of diabetes, but interactions were statistically non-significant.

Conclusions: Our results on the interaction of PM_{10} with functionally well described polymorphisms in an important pro-inflammatory candidate gene are consistent with the hypothesis that air pollutants impact on T2D through inflammatory pathways. Our findings, if confirmed, are of high public health relevance considering the ubiquity of the major G allele, which puts a substantial proportion of the population at risk for the development of diabetes as a result of long-term exposure to air pollution.

Keywords: Particulate matter, Air pollution, Diabetes mellitus, Interleukin-6 gene, Gene-environment interactions, Single nucleotide polymorphisms, Cross-sectional epidemiology

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Background

Evidence suggests a positive association between ambient air pollution and risk of type 2 diabetes (T2D) [1–3]. This association is hypothesized to be mediated through inflammatory mechanisms. Experimental evidence [4, 5] suggests subclinical systemic inflammation occurring at several sites including adipose tissue, liver, skeletal muscles and the autonomic nervous system, with resultant insulin resistance, the hallmark of T2D. On the population level, acute and long-term exposure to ambient air pollution has been linked to raised markers of inflammation including circulating C-reactive proteins (CRP) [6, 7] IL-6, [8] fibrinogen [9, 10] vascular and intracellular adhesion molecules [11]. Indeed, air pollution is thought to accelerate pro-thrombotic state following lung inflammation through an IL-6 dependent pathway [12, 13]. Exposure to industrial particulate matter has been shown to induce IL-6 genes in human airway epithelial cells [14]. Likewise, exposure of mice to particulate matter induced the expression of genes involved in inflammation, lipid metabolism and atherosclerosis [8].

IL-6 in itself may be related to the development of type 2 diabetes [15]. Elevated levels of IL-6 were associated with higher incidence of type 2 diabetes [16, 17], and animal studies also showed IL-6 to inhibit insulin secretion from islet cells following glucose stimulation [18]. Other in vitro studies also showed negative impacts of IL-6 on insulin sensitivity through reduced insulin receptor expression [19] and adiponectin gene expression [20] in adipocytes. Among T2D patients, IL-6 was associated with whole-body insulin resistance and hyperglycemia [21]. Raised IL-6 levels were also associated with hyperinsulinemia in patients without T2D [22].

IL6 gene plays an important role in the regulation of systemic inflammatory pathways. Some common single nucleotide polymorphisms (SNPs), including *IL6* -572G > C and *IL6* -174G > C have been shown to influence the levels of circulatory IL-6 [23, 24], as well as circulating CRP [25]. In these studies, the major G alleles of both variants were identified as the pro-inflammatory alleles. Recent meta-analyses of 11,681 individuals of Asian and European descent showed the G allele of *IL6* -572G > C to be associated with increased risk of T2D [26] whereas another study of 22,626 individuals of European descent showed the C allele of *IL6* -174G > C to be associated with decreased risk of T2D [27].

Studying gene-environment interactions helps to better understand aetiologic mechanisms and causality of exogenous factors, in this case, air pollution, and to identify population at increased risk of adverse health effects of environmental exposures. We hypothesize, based on the above evidence, that the common functional SNPs, *IL6* -572G > C and *IL6* -174G > C, may modify the existing association between air pollutants and diabetes [28].

Methods

Study population

We studied participants of the Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults (SAPALDIA). This study has been described elsewhere in detail [29]. Briefly, it consists of a population-based sample of 9651 adults, aged 18–60 when they were recruited at baseline (1990/1991) from eight communities reflecting the diverse geographic and climatic features of Switzerland. Participants underwent health interviews and physical examinations. At first follow-up (2001/2002) which additionally included blood marker and genetic assays, 8047 participants completed at least the screening questionnaire [30]. 6212 participants at first follow-up, consenting to genetic assays were genotyped for *IL6* -572G > C and *IL6* -174G > C in those studies. For the present analysis, we studied 4410 follow-up participants, aged 29–73 years, with complete data on diabetes status, selected covariates, and population stratification data from genome-wide association studies. The algorithm for participant selection is shown on Fig. 1.

Participants provided informed consent for participation in the health interviews, physical examinations, blood marker and genetic assays. Ethical clearance for the SAPALDIA study was obtained from the Swiss Academy of Medical Sciences, the National Ethics Committee for Clinical research (UREK, Project Approval Number 123/00) and the Ethics Committees of the eight participating communities including Basel, Wald, Davos, Lugano, Montana, Payerne, Aarau and Geneva.

Identification of diabetes cases

Participants were identified as having diabetes if they 1) reported physician diagnosed diabetes or 2) use of diabetes medication in the past month or 3) had non-fasting blood glucose >11.1 mmol/L or 4) HbA1c >0.065. Non-fasting blood glucose was measured in all participants whereas HbA1c was only measured if non-fasting glucose >6.1 mmol/L [28]. The lack of data on diabetes status at baseline precluded the study of incident diabetes. Also, we could not exclude type 1 diabetes (T1D) and have assumed the majority of these cases to be T2D considering that on the average, >90 % of adult diabetes are T2D [31].

Individual assignment of air pollution exposure

We considered 10-year means of PM_{10} (particulate matter <10um in diameter) as our pollutant exposure measure of interest. In our previous studies on diabetes [2] and metabolic syndrome [32], associations with nitrogen dioxide (NO_2) disappeared in two-pollutant models that included NO_2 and PM_{10}, whereas those of PM_{10} remained unchanged. Therefore we did not consider NO_2 in this study. Estimates of PM_{10} exposure were

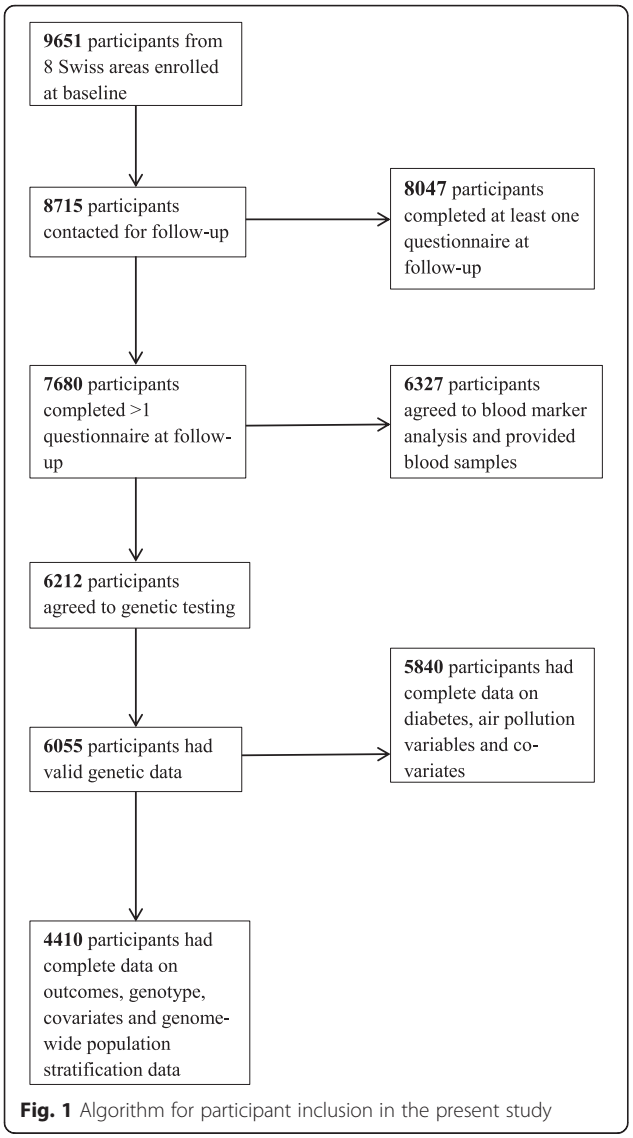

Fig. 1 Algorithm for participant inclusion in the present study

assigned to participants' residential addresses using dispersion models for the years 1990 and 2000 (respective years before baseline and follow-up) [33]. This model incorporated data from meteorology, topography and several emission inventories including industrial, agricultural, heavy equipment and traffic at a resolution of 200 × 200 m [34]. Annual PM_{10} levels measured at fixed monitoring stations across Switzerland and participants' residential histories were further used to derive estimates of mean PM_{10} exposure at participants' residential addresses over the 10-year period preceding the first follow-up health examination [28].

Genotyping of candidate SNPs, *IL6* -572G > C and *IL6* -174G > C.

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-buffered whole blood using PUREGENE DNA Purification Kit (GENTRA Systems, Minneapolis, USA) [30]. Genotyping for these polymorphisms was done using 5'-nuclease fluorescent polymerase chain reaction (Taqman) assay (Applera Europe, Rotkreuz, Switzerland). Detection of end-points was done using a 7000 ABI System detection device (ABI, Rotkreuz, Switzerland) [35]. Genotyping call rate was >97.5 %. A random selection of 638 samples (10 %) was genotyped twice for quality control and repeated genotypes were 100 % concordant ($R^2 = 1$; $P < 0.001$). Subsamples of 3015 and 1612 SAPALDIA participants had whole genome genotyping using the Illumina HumanOmniExomeExpress BeadChip and Illumina 610 K quad BeadChip (Illumina, San Diego, CA, USA) respectively. In this combined SAPALDIA subsample which are predominantly of Caucasian ancestry, we derived ten population stratification components using multidimensional scaling analysis (Plink v1.07 [36]) on 72,122 SNPs with MAF > 1 % and genotyping call rate >97 %, present on both genotyping arrays.

Potential confounders

We considered the following characteristics, based on our previous publication on air pollution and diabetes [28], as potential confounders: age (years; continuous), sex, years of formal education (≤9; >9), neighbourhood socio-economic index developed from a principal component analysis including occupation and educational level of household head, median rent and number of persons living in a household, expressed as a percentage [37]. We also considered smoking history (current, former and never smoker; and smoked pack-years computed by multiplying number of cigarette packs per year and number of smoking years), exposure to passive smoke (yes/no), occupational exposure to vapours gases, dusts and fumes (yes/no) daily consumption of at least one portion of fruits and vegetables (never; ≤3 days/week; >3 days/week respectively). We also considered alcohol consumption (including beers, wines, spirits and liquors: never; ≤1glass/day; >1glass/day); hours per week of vigorous physical activity defined as taking part in activities that make one sweat or out of breath (<0.5; ≥0.5), body mass index (BMI; kg/m^2, continuous) and genome-wide population stratification components.

Statistical analyses

We assessed linkage disequilibrium between *IL6* -572G > C and *IL6* -174G > C and tested both candidate SNPs for Hardy-Weinberg equilibrium (HWE) among the genotyped participants regardless of inclusion in the study. We computed an *IL6* genetic risk score (IRS) by summing up the risk alleles (number of G alleles coded as 0, 1 and 2) across both SNPs. We summarized the characteristics of 4410 included participants based on their genotype and also by inclusion and exclusion status. We applied a mixed

logistic regression model, with a random intercept by study area to explore the associations of diabetes with both SNPs, IRS and with PM_{10}. We generated interaction terms between PM_{10} and candidate SNPs (including IRS) and assessed their associations with prevalent diabetes. Interaction analyses with candidate SNPs involved additive, recessive and co-dominant models. Stratifying by genotype, we assessed the associations between PM_{10} and diabetes, to identify genotype-specific associations. We also stratified by groups of IRS indicating increased inflammatory risk. Since this study included 46 % of baseline participants which differed in several sociodemographic characteristics (Additional file 1: Table S1), we assessed the effect of potential participant selection bias on our results using inverse probability weighting by applying the inverse of the probability of participating in present study derived at baseline (using variables that significantly predicted participation in the present study), to the primary model in this study. All analyses were performed using a primary model which included, after stepwise selection, participants' age, sex, educational attainment, neighbourhood socio-economic index, smoking status, smoked pack-years, exposure to passive smoke, occupational exposure to vapours, gases, dusts and fumes, consumption of fruits and vegetables, vigorous physical activity, BMI and genome-wide population stratification components and applying a random intercept by study area.

We performed several sensitivity analyses. We defined diabetes based on each of the diagnostic criteria (excluding the cases identified only by alternative criteria). We repeated the interaction analyses using mixed logistic regression models with random slopes by study area to explore if study area influenced any of the observed interactions. All statistical analyses were performed with STATA software, version 14 (Stata Corporation, Texas).

Results

There were 252 diabetes cases in this study. Mean exposure to PM_{10} was 22.6ug/m^3 and mean IRS was 3 risk alleles. *IL6* -572G > C and *IL6* -174G > C were not in linkage disequilibrium ($R^2 = 0.02$; D' = 1.0). The results of the HWE test for *IL6* -572G > C and *IL6* -174G > C, which have respective minor allele frequency of 7 and 39 % in the SAPALDIA population are shown in Table 1. *IL6* -174G > C was in HWE in both cases and controls whereas *IL6* -572G > C only reached HWE among the diabetes cases ($P = 0.636$) and not among those without diabetes ($P = 0.006$). The overall HWE test for *IL6* -174G > C and *IL6* -572G > C was 0.408 and 0.006 respectively (Table 1). In the European study of ~6000 participants reporting an association between *IL6* -572G > C and T2D, this functional SNP (having MAF = 5 %) was also not in HWE [38]. Since only 30 participants carry the minor CC genotype of *IL6* -572G > C, we present the results for this SNP as GG vs. GC + CC, which yields better statistical power.

Table 1 Distribution of *IL6* -572 G > C and *IL6* -174 G > C genotypes and alleles by diabetes status

Genotype	Diabetes	No diabetes
	N = 286	*N* = 5554
IL6 -572 G > C*		
Genotype		
GG	248 (86.7)	4896 (88.2)
GC	36 (12.6)	623 (11.2)
CC	2 (0.7)	35 (0.6)
Allele		
G	532 (93)	10415 (93.7)
C	40 (7)	693 (6.3)
IL6 -174 G > C**		
Genotype		
GG	111 (39)	2081 (37)
GC	135 (47)	2614 (47)
CC	40 (14)	865 (16)
Allele		
G	357 (62)	6776 (61)
C	215 (38)	4344 (39)

Data are presented as absolute numbers (N) and relative numbers (%) in parentheses

*P-value for Hardy-Weinberg Equilibrium (HWE) test in diabetes cases = 0.585; no diabetes = 0.006; overall = 0.006. *P*-value for Fisher's exact test = 0.664

**P-value for Hardy-Weinberg Equilibrium test in diabetes cases = 0.918; no diabetes = 0.352; overall = 0.408. *P*-value of Chi-square test = 0.762

Compared to the carriers of the major GG genotype, carriers of the GC + CC genotype of *IL6*-572G > C had higher body mass index and PM_{10} exposure whereas carriers of CC genotype of *IL6* -174G > C smoked more, consumed more alcohol and had lower exposure to PM_{10} and there was a significant difference in genotype distribution across areas (Table 2). There was no significant difference in diabetes prevalence across genotypes for both polymorphisms (Table 2). Additional file 1: Table S1 shows the differences in these characteristics between the included and excluded participants. There were significant differences in most of the participants' characteristics including diabetes prevalence and PM_{10} exposure, but the prevalence of these characteristics were generally higher among the included participants compared to the excluded ones (Additional file 1: Table S1). The *IL6* -572G > C and *IL6* -174G > C genotypes, and IRS were similarly distributed between both groups (Additional file 1: Table S1).

The positive association between air pollutants and diabetes, which we previously observed in our previous study of 6392 participants at this follow-up study [28], persisted in the present sample. The adjusted odds of diabetes increased by 47 % (95 % CI: 1.21, 1.78) per

Table 2 Characteristics of participants by *IL6* -572 G > C and *IL6* -174 G > C genotypes

	IL6 -572 G > C			*IL6* -174 G > C			
	GG (*N* = 3891)	GC + CC (*N* = 519)	*P* (Chi^2)	GG (*N* = 1618)	GC (*N* = 2110)	CC (*N* = 682)	*P* (Chi^2)
Proportion (%)							
Females	48.4	49.1	0.768	48.3	48.1	50.6	0.509
Education ≥9 years	94.9	93.2	0.107	94.4	94.9	95.0	0.721
Never-smokers	44.3	45.6	0.490	42.4	44.8	48.1	0.036
Passive smoke exposure	46.4	46.6	0.927	48.6	45.4	44.4	0.078
Occupational VGDF exposure	42.8	42.4	0.845	43.9	42.6	40.9	0.398
Alcohol intake ≤1glass/day	91.0	91.4	0.620	89.8	91.2	93.0	0.050
Alcohol intake >1glass/day	9.0	9.6		10.2	8.8	7.0	
Portion of raw vegetables ≤3 days/week	18.2	20.8	0.150	17.4	19.1	19.4	0.351
Portion of raw vegetables >3 days/week	81.8	79.2		82.6	80.9	80.6	
Portion of fruits ≤3 days/week	35.8	35.6	0.923	35.0	36.2	36.7	0.657
Portion of fruits >3 days/week	64.2	64.4		65.0	63.8	63.3	
Portion of citrus fruits ≤3 days/week	64.2	64.0	0.909	64.2	63.9	64.8	0.917
Portion of citrus fruits >3 days/week	35.8	36.0		35.8	36.1	35.2	
Vigorous physical activity <0.5 h/week	35.5	37.2	0.449	37.3	34.6	34.2	0.082
Vigorous physical activity ≥0.5 h/week	64.5	62.8		62.1	65.4	65.8	
Diabetes cases	5.5	6.7	0.260	6.2	5.5	4.8	0.430
Areas: Basel	11.5	12.9	0.062	10.5	11.6	14.8	<0.001
Wald	19.1	19.1		19.0	18.6	20.8	
Davos	7.7	6.9		7.5	7.9	7.0	
Lugano	12.6	14.6		15.0	12.6	8.4	
Montana	11.1	6.9		9.2	11.2	12.0	
Payerne	13.1	12.7		13.3	12.1	15.2	
Aarau	16.6	19.3		16.9	17.6	14.4	
Geneva	8.4	7.5		8.6	8.4	7.3	
Means (SD)			*T*-test				ANOVA
Age (years)	51.8 (11)	51.3 (11)	0.269	51.8 (11)	51.8 (11)	51.5 (11)	0.732
BMI (kg/m^2)	25.8 (4.3)	26.3 (4.3)	0.025	26.0 (4.3)	25.8 (4.3)	25.9 (4.3)	0.152
Neighborhood SEI	63.7 (10)	63.8 (10)	0.827	63.5 (10)	63.9 (10)	63.8 (9)	0.455
10-year mean PM_{10} (μg/m^3)	21.8 (7.3)	22.8 (7.2)	0.005	22.3 (7.4)	21.9 (7.3)	21.5 (6.9)	0.030
Pack-years of smoking[a]	0 (14)	0.1 (16)	0.296	0.3 (16)	0 (16)	0 (14)	0.524

VGDF: vapours, gases, dusts and fumes; SD: standard deviation; BMI: body mass index; SEI: socio-economic index; PM_{10}: particulate matter <10 μm in diameter.
[a]values represent median (interquartile range) and *P*-values represent significance level of median test

10ug/m^3 of exposure to PM_{10}. We did not observe any significant association between the candidate *IL6* SNPs and diabetes, across three genetic models. We also did not observe significant associations between IRS and diabetes in our sample (Additional file 1: Table S2).

Stratified by genotypes, the association between PM_{10} and diabetes was most pronounced among carriers of the major GG pro-inflammatory alleles for both polymorphisms (Fig. 2). We observed significant interactions between PM_{10} and *IL6* -572G > C in the additive and recessive models which became more significant in the models accounting for potential selection bias by IPW and remained significant following Bonferroni correction at $P = 0.01$ (0.05/5) (Table 3). We did not observe any statistically significant interactions with *IL6* -174G > C across genetic models and adjustment for potential selection bias by IPW (Table 3). We observed a positive trend in the association between PM_{10} and diabetes across levels of IRS (Fig. 2). Carriers of four pro-inflammatory G alleles had the highest odds of diabetes per 10ug/m^3 increase in exposure to PM_{10} ($P_{interaction} = 0.10$) (Fig. 2). Compared to carriers of two pro-inflammatory G alleles,

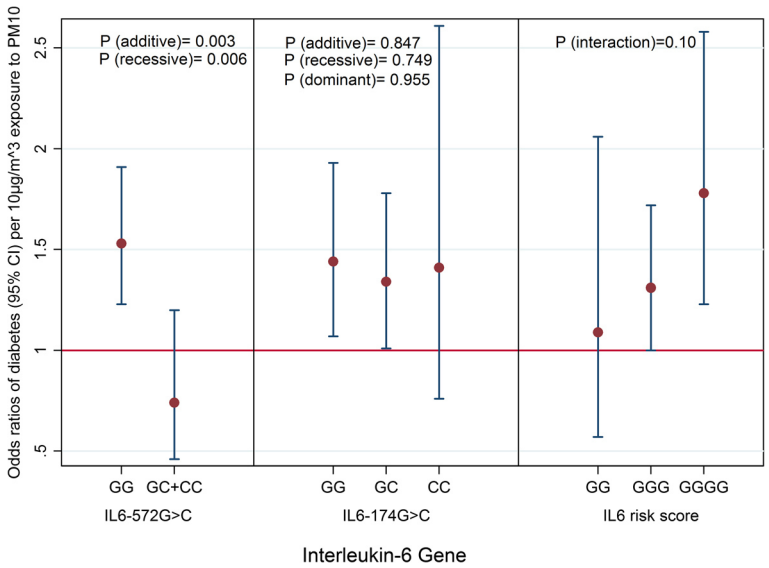

Fig. 2 Interaction between PM_{10} and *IL6* polymorphisms on odds of diabetes. Odds ratio values represent percentage increase of odds of diabetes per 10 μg/m^3 increase in PM_{10} exposure adjusted for potential selection bias. PM_{10}: particulate matter <10 μm in diameter. All associations are adjusted for body mass index, age, sex, socio-economic status, smoking habits, consumption of alcohol, fruits and vegetables, physical activity and genome-wide population stratification. Study area was treated as a random effect in all models

Table 3 Associations and interactions between PM_{10} and candidate SNPs on odds of diabetes, applying inverse probability weighting (IPW) to account for potential selection bias

Genotype	Genotype-specific PM_{10} and diabetes association OR (95 % CI)	*P*-value*	*P*-value of interaction**	*P*-value of interaction***	*P*-value of interaction****
IL6 -572G > C					
Adjusted model without IPW					
GG	1.53 (1.22, 1.92)	<0.001	0.031	*n.d.*	0.058
GC + CC	0.87 (0.51, 1.49)	0.618			
Adjusted model applying IPW					
GG	1.53 (1.23, 1.91)	<0.001	0.003	*n.d.*	0.006
GC + CC	0.74 (0.46, 1.20)	0.225			
IL6 -174G > C					
Adjusted model without IPW					
GG	1.49 (1.09, 2.04)	0.012	0.763	0.966	0.645
GC	1.35 (1.01, 1.80)	0.046			
CC	1.43 (0.80, 2.54)	0.226			
Adjusted model applying IPW					
GG	1.44 (1.07, 1.93)	0.016	0.847	0.955	0.749
GC	1.34 (1.01, 1.78)	0.044			
CC	1.41 (0.76, 2.61)	0.226			

Adjusted models include age, sex, educational attainment, neighborhood-level socio-economic status, smoking status, pack-years of smoking, exposure to passive smoke and occupational dusts and fumes, dietary fibre intake, alcohol consumption, physical activity, body mass index (BMI), PM_{10}. Study area was treated as random effects in all models. OR: odds ratio; CI: confidence intervals; OR values represent percent increase in odds of diabetes per 10 μg/m^3 increase in PM_{10} exposure. PM_{10}: particulate matter <10 μm in diameter. * *P*-value of genotype specific association between PM_{10} and diabetes. ** additive model (per G allele); ***dominant model (GG + GC vs. CC); ****recessive model (GG vs. GC + CC); *n.d.*: not done due to the few number of CC allele carriers

carriers of four risk alleles had 60 % (95 % CI: −15 %, 197 %) higher odds of diabetes per 10ug/m^3 increase in exposure to PM_{10}. These interactions were largely stable to confounder adjustments.

Sensitivity analyses showed very similar results. Defining diabetes according to each of the classification criteria showed very similar results with significant interactions in the additive and recessive genetic models (Table 4). Odds of diabetes identified through blood tests among GG genotype carriers remained positive but less significant (Table 4). Applying a logistic regression model with random slopes by study area also did not change estimates of the interaction between PM_{10} and the candidate SNPs on prevalent diabetes, but changed the estimated association between PM_{10} and diabetes among the pro-inflammatory GG genotype carriers (Table 4). In a sub-sample of 2825 non-asthmatic participants who were genotyped using the Illumina Human Omni Exome Express Bead Chip, where *IL6* -572G > C was in HWE (Pearson's correlation R^2 between both SNPs =1), associations persisted among pro-inflammatory GG carriers [OR: 1.64 (1.24, 2.16)] and interactions persisted in the models accounting for potential selection bias ($P_{(\text{additive})}$ =0.053; $P_{(\text{recessive})=}$ 0.048).

Discussion

We found a modifying effect of *IL6* -572G > C polymorphism on the association between air pollutants and diabetes, where carriers of the pro-inflammatory GG genotype were most susceptible. These associations were highly stable to confounder adjustments and remained robust across several sensitivity analyses. The lack of interaction with *IL6* -174G > C is supported by the fact that both SNPs are not in linkage disequilibrium. Combining both SNPs into an IRS showed increased association among participants at high genetic risk of inflammation.

The lack of association between PM_{10} and DM among the GG genotype carriers in the fixed effect model could be attributed to variation of PM_{10} constituents across different areas (Pearson's R for PM_{10} crustal components across four SAPALDIA areas = 0.34) [39]. Due to the fact that the likelihood-ratio tests for interactions between PM_{10} and study area (also between SNP and study area) were non-significant ($P > 0.2$), and that the goal of SAPALDIA air pollution studies is to capture the between area differences in health effects which cannot be explained by the fixed factors in the models, we performed the main analyses using area as a random covariate. In addition, applying the fixed effects model did not change our estimates of interaction between *IL6* polymorphisms and PM_{10} which is our main interest in this study. The reduced significance of interactions among those reporting the use of diabetes medication is most likely to be due to under-reporting of medication use. The absence of any significant association between the *IL6* polymorphisms and diabetes in our sample may be due to our inability to differentiate T1D from T2D, or the relatively small number of cases compared to other studies [26]. Thus, our observation of a significant interaction is surprising given the limited number of cases in our study.

To our knowledge, this is the first evidence on gene-air pollution interaction in adult diabetes. Until now, gene-air pollution interaction studies focused on respiratory and cardiovascular outcomes, exploring diverse candidate genes or polymorphisms, pollutants and outcomes [40–42]. Many of the interacting genes including *IL6*, regulate systemic oxidative stress and inflammatory pathways [41, 43]. *IL6* is one of the genes involved in systemic inflammation by regulating or inducing the production of inflammatory cytokines such as IL-6 and CRP. In-vitro studies also show that exposure to particulate matter induces *IL6* and *CRP* gene expression in epithelial and macrophage cell lines [14, 44, 45]. In addition, polymorphisms on *IL6* have been shown to interact with acute exposure to carbon monoxide and nitrogen dioxide, in eliciting plasma IL-6 response [43].

Our results support the hypothesis that exposure to air pollution may contribute to diabetes aetiology through inflammatory pathways. Since we have analysed two C/G polymorphisms located in the promoter region of *IL6* in our study, a potential mechanism of action could be related to changes in DNA methylation at these sites, affecting *IL6* gene expression. Air pollution exposure was positively associated with methylation of *IL6* in elderly men [46]. Hypomethylation of *IL6* was associated with raised levels of serum IL-6 in patients with rheumatoid arthritis [47], and with body weight among diabetes patients [48]. In contrast, increased methylation of *IL6* was associated with risk of obesity [49] and body weight among patients without diabetes [48]. Furthermore there is suggestive evidence on a potential link between epigenetic changes at inflammatory genes (including *IL6*) and diabetes [50, 51]. While the evidence supports a role of *IL6* methylation with regard to both, air pollution and diabetes, the relevance of hyper- versus hypomethylation and the association with the *IL6* SNPs studied here needs further clarification.

Our study has major strengths. It provides first evidence to our knowledge on gene-air pollution interactions on diabetes risk. It derives from the large database of the population-based SAPALDIA cohort, with well characterized phenotypes, genotypes and lifestyle characteristics. Our air pollution estimates were assigned to participants' residences and derived from validated models which have been applied to other studies. [33] By taking into account residential histories of participants, we could compute individual estimates of long-

Table 4 Other Sensitivity Analyses

Sensitivity analysis	Genotype	Adjusted OR (95 % CI)	*P*-value*	*P*-value**	*P*-value***	*P*-value****
Diabetes defined as self-reported physician diagnosis and medication use [N(diabetes) = 196]	*IL6* -572G > C					
	GG	1.41 (1.11, 1.79)	0.005	0.001	*n.d.*	0.004
	GC + CC	0.63 (0.38, 1.04)	0.070			
	IL6 -174G > C					
	GG	1.23 (0.88, 1.71)	0.222	0.931	0.797	0.794
	GC	1.33 (0.98, 1.81)	0.065			
	CC	1.17 (0.58, 2.35)	0.669			
Diabetes defined as self-reported physician diagnosis only [N(diabetes) =193]	*IL6* -572G > C					
	GG	1.43 (1.13, 1.83)	0.003	0.008	*n.d.*	0.003
	GC + CC	0.63 (0.38, 1.04)	0.072			
	IL6 -174G > C					
	GG	1.23 (0.88, 1.71)	0.226	0.881	0.749	0.694
	GC	1.38 (1.01, 1.88)	0.041			
	CC	1.16 (0.57, 2.33)	0.680			
Diabetes defined as self-reported use of diabetes medication only [N(diabetes) =125]	*IL6* -572G > C					
	GG	1.25 (0.95, 1.66)	0.113	0.008	*n.d.*	0.031
	GC + CC	0.49 (0.22, 1.10)	0.085			
	IL6 -174G > C					
	GG	1.10 (0.72, 1.66)	0.666	0.954	0.600	0.803
	GC	1.24 (0.86, 1.78)	0.246			
	CC	0.90 (0.36, 2.28)	0.829			
Diabetes cases identified from blood tests only [N (diabetes) = 184]	*IL6* -572G > C					
	GG	1.65 (1.27, 2.13)	<0.001	0.002	*n.d.*	0.006
	GC + CC	0.70 (0.39, 1.25)	0.223			
	IL6 -174G > C					
	GG	1.46 (1.04, 2.06)	0.030	0.738	0.553	0.962
	GC	1.41 (1.00, 1.99)	0.052			
	CC	1.83 (0.86, 3.90)	0.118			
Model applying random slopes for study areas [N(diabetes) = 252]	*IL6* -572G > C					
	GG	0.92 (0.44, 1.92)	0.819	0.004	*n.d.*	0.008
	GC + CC	0.44 (0.19, 1.05)	0.065			
	IL6 -174G > C					
	GG	0.86 (0.41, 1.83)	0.704	0.814	0.928	0.687
	GC	0.78 (0.36, 1.70)	0.540			
	CC	0.85 (0.33, 2.20)	0.735			

Adjusted models include age, sex, educational attainment, neighborhood-level socio-economic status, smoking status, pack-years of smoking, exposure to passive smoke and occupational dusts and fumes, dietary fibre intake, alcohol consumption, physical activity, body mass index (BMI), PM_{10}. Study area was treated as random effects in all models except the model with random slopes for study area.OR: odds ratio; CI: confidence intervals; OR values represent percent increase in odds of diabetes per 10 μg/m3 increase in PM_{10} exposure. PM_{10}: particulate matter <10 μm in diameter; *n.d*: not done due to very low sample size for CCgenotype. *P-value of genotype specific association between air pollutant and diabetes. **additive model (per C allele); ***dominant model (GG + GC vs. CC); ****recessive model (GC + CC vs.GG)

term exposure to air pollution, which is a crucial in understanding disease development and progression attributable to air pollution. We minimized outcome misclassification by identifying undiagnosed diabetes cases through additional blood tests.

Our study also has limitations. It has a cross-sectional design hence we cannot infer causality of observed associations. We tried to improve this by estimating air pollution exposure in the ten years prior to the survey where diabetes was assessed. In addition, an exploratory

analysis excluding 17 diabetes cases who reported to have started using diabetes medication before 1991 gave very similar results. We could not differentiate T1D from T2D cases and therefore have misclassified a few cases (on the average <10 % as T2D instead of T1D). [31].

One of our functional SNPs of interest, *IL6* -572G > C, was not in HWE (Table 1). SNPs may deviate from HWE due to genotyping error, population stratification or population selection [52, 53]. Therefore, we assessed quality control by genotyping a subsample of our participants using an alternative genotyping array. There was no indication of genotyping errors since both SNPs had a perfect correlation between the two genotyping rounds. Also, we adjusted for population stratification in our study population using genome-wide principal components. It has been shown that SNPs may still deviate from HWE despite controlling for the aforementioned reasons [53]. It is also important to note that *IL6* -572G > C was not in HWE in a study linking it to T2D in Europeans [38] and the minor alleles were similarly distributed between the reference study (MAF = 5 %) and our study (MAF = 7 %). Furthermore, the genotype frequencies were also similarly distributed [Diabetes cases- GG: 90.6 %, GC: 9 %, CC: 0.4 %; No diabetes - GG: 86.7 %, GC: 12.6 %, CC: 0.7 % vs. Table 1). We additionally assessed public databases to identify any potential interference on our PCR probe by nearby SNPs but found no evidence for such. We did not measure plasma IL-6 concentrations in our participants due to lack of funds, precluding our assessment of association between candidate SNPs and serum IL-6 levels, but there was a positive correlation between both SNPs and mean high-sensitivity C-reactive proteins (hs-CRP) measured at this first follow-up (*IL6* -572G > C-CC: 1.28 g/l, GC: 1.45 g/l, GG: 1.58 g/l and *IL6* -174G > C-CC: 1.58 g/l; GC: 1.53 g/l; GG: 1.60 g/l). The larger differences in hs-CRP levels associated with *IL6* -572G > C agrees with its larger interaction effect. Lastly, our study had sample size limitations, especially among the CC genotype carriers of *IL6* -572G > C, limiting the statistical power to detect more associations. Despite this, we made some statistically significant observations.

Conclusions

Our findings suggest that homozygous carriers of the common pro-inflammatory major 'G' allele of *IL6* -572G > C polymorphism may be more susceptible to the diabetogenic effects of particulate matter, supporting the relevance of inflammatory pathways in the relationship between air pollution and diabetes. If confirmed, our results are of high public health relevance considering the ubiquity of the major G alleles, which put a substantial proportion of the population at risk for the development of diabetes as a result of exposure to air pollution. Our results therefore call for replication by other longitudinal population-based studies with adequate air pollution, genotype and diabetes information.

Additional file

Additional file 1: Table S1. Characteristics of included and excluded participants. **Table S2.** Association between functional *IL6* polymorphisms and diabetes. (DOCX 27 kb)

Abbreviations

BMI: body mass index; DNA: Deoxyribonucleic acid; EDTA: ethylenediaminetetraacetic acid; HbA1C: Glycosylated haemoglobin; hs-CRP: high-sensitivity C-reactive proteins; HWE: Hardy-Weinberg equilibrium; *IL6*: Interleukin-6 gene; IL-6: Interleukin-6 cytokine; NO_2: nitrogen dioxide; PM_{10}: Particulate matter with diameter less than 10 microns; SAPALDIA: Swiss cohort study on air pollution and lung and heart diseases in adults; SNP: single nucleotide polymorphism; T1D: Type 1 diabetes; T2D: Type 2 diabetes..

Competing interests

All authors declare no actual or potential financial and non-financial interests. The funders played no role in the design or the outcome of this study.

Authors' contributions

All authors contributed equally to the conception of this study and the development of this manuscript. All authors read and approved the final manuscript.

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ADDITIONAL FILE

A common functional variant on the pro-inflammatory Interleukin-6 gene may modify the association between long-term PM_{10} exposure and diabetes

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Table A1: Characteristics of included and excluded participants

Proportion (%)	Included (N=4410)	Excluded % (N)	P-value (Chi^2)
Females	48.5	57.6 (5241)	<0.001
Education ≥9 years	95.0	91.0 (5241)	<0.001
Never-smokers	44.4	41.4 (5241)	0.026
Passive smoke exposure	46.5	47.6 (5241)	0.414
Occupational VGDF exposure	42.8	41.0 (5241)	0.180
Alcohol intake ≤1glass/day	90.0	90.9 (2976)	0.908
Alcohol intake >1glass/day	9.0	9.1 (294)	
Portion of raw vegetables ≤3 days/week	18.5	21.7 (752)	0.003
Portion of raw vegetables >3 days/week	81.5	78.3 (2518)	
Portion of fruits ≤3 days/week	35.8	33.0 (1112)	0.030
Portion of fruits >3 days/week	64.2	67.0 (2158)	
Portion of citrus fruits ≤3 days/week	64.1	65.4 (2142)	
Portion of citrus fruits >3 days/week	35.9	34.6 (1128)	0.320
Vigorous physical activity <0.5 hour/week	35.7	45.5 (1488)	
Vigorous physical activity ≥0.5 hour/week	64.3	54.5 (1782)	<0.001
Diabetes cases	5.7	3.2 (75)	<0.001
IL6-572 G>C: GG	88.2	88.0 (1494)	0.417
GC	11.1	11.6 (197)	
CC	0.7	0.4 (7)	
IL6-174 G>C: GG	36.7	38.9 (690)	0.219
GC	47.8	45.4 (808)	
CC	15.5	15.8 (281)	
Areas: Basel	11.8	15.4 (970)	<0.001
Wald	19.0	15.9 (676)	
Davos	7.6	8.9 (408)	
Lugano	12.8	18.3 (743)	
Montana	10.6	6.2 (326)	
Payerne	13.0	15.7 (919)	
Aarau	16.9	11.2 (551)	
Geneva	8.3	8.4 (632)	
Means (SD) [N]			P-value (T-test)
Age (years)	51.8 (11.1)	53.1 (12.0) [5241]	<0.001
BMI (kg/m^2)	25.9 (4.3)	25.9 (4.7) [3270]	0.874
Neighborhood SEI	63.7 (9.9)	62.9 (10.6) [3270]	0.005
10-year mean PM_{10} ($\mu g/m^3$)	22.0 (7.2)	23.2 (7.2) [5241]	<0.001
IL6 risk score	3.1 (0.7)	3.1 (0.7) [1698]	0.222
Pack-years of smoking [a]	10.4 (18.0)	11.8 (19.1) [5241]	0.069

VGDF: vapours, gases, dusts and fumes; SD: standard deviation; BMI: body mass index; SEI: socio-economic index; PM_{10}: particulate matter <10μm in diameter. [a] values represent median (interquartile range) and P-values represent significance level of median test.

Table A2: Association between functional *IL6* polymorphisms and diabetes

Model	Reference genotype		Crude OR (95% CI)	Adjusted model OR (95% CI)	Adjusted model+PM_{10} OR (95% CI)
IL6-572G>C					
Additive	Per G allele	Per G allele	0.91 (0.66,1.26)	0.94 (0.64,1.39)	0.95 (0.64,1.40)
Co-	CC	GC	0.99 (0.23,4.29)	1.23 (0.20,7.72)	1.23 (0.20,7.23)
dominant	CC	GG	0.89 (0.21,3.73)	1.13 (0.18,7.00)	1.14 (0.19,7.01)
Dominant	CC	GG+GC vs. CC	0.90 (0.21,3.78)	1.16 (0.19,7.15)	1.17 (0.19,7.18)
Recessive	GC+CC	GG vs. GC+CC	0.90 (0.63,1.28)	0.93 (0.61,1.40)	0.93 (0.62,1.41)
IL6-174G>C					
Additive	Per G allele	Per G allele	1.04 (0.87,1.24)	1.07 (0.87,1.31)	1.07 (0.87,1.31)
Co-	CC	GC	1.09 (0.76,1.56)	1.20 (0.78,1.86)	1.22 (0.79,1.88)
dominant	CC	GG	1.10 (0.76,1.59)	1.19 (0.76,1.86)	1.21 (0.77,1.88)
Dominant	CC	GG+GC vs. CC	1.09 (0.77,1.54)	1.20 (0.79,1.81)	1.21 (0.81,1.83)
Recessive	GC+CC	GG vs. GC+CC	1.03 (0.81,1.32)	1.03 (0.77,1.38)	1.03 (0.78,1.38)
IL6 genetic risk score					
	Per G allele	Per G allele	1.01 (0.86,1.20)	1.05 (0.86,1.27)	1.05 (0.86,1.28)
	2 G alleles	3 G alleles	1.12 (0.81,1.54)	1.14 (0.78,1.66)	1.16 (0.80,1.68)
	2 G alleles	4 G alleles	1.05 (0.74,1.48)	1.11 (0.74,1.67)	1.13 (0.75,1.69)

Adjusted models include age, sex, educational attainment, neighborhood-level socio-economic status, smoking status, pack-years of smoking, exposure to passive smoke and occupational dusts and fumes, dietary fibre intake, alcohol consumption, physical activity, body mass index (BMI), PM_{10}. Study area was treated as random effects in all models. OR: odds ratio; CI: confidence intervals; OR values represent percent increase in odds of diabetes across different genetic models. PM_{10}: particulate matter <10μm in diameter.

PART V DISCUSSION, CONCLUSIONS & APPENDICES

9. Discussion and Conclusions

9.1 Main findings in a general context

The findings from this work provide new insights into the relationship between exposure to air pollutants and risk of T2D. This work has applied several epidemiologic methods and genetic epidemiology to try to reach its ultimate goal, which is to quantify and elucidate the mechanisms underlying the potential role of air pollution in diabetes aetiology. Genetic epidemiology seeks to contribute to causal understanding of the links between genomic variations, environmental influences and phenotypic variability. By identifying disease mechanisms and susceptibilities, the research field of genetic epidemiology helps to improve understanding of disease mechanisms and preventive measures against modifiable risk factors, and identify biomarkers for risk screening.

This PhD thesis incorporates the principles of Swiss TPH built around the triangle of innovation, validation and application. Although most of the objectives of this work center on innovation [chapters 6-8] and validation [chapters 4-8] [Table 2], it contributes to application in three ways. First, this work demonstrates the relevance of air pollution and physical activity interaction for health-in-all policy framework. Second, this work has paved the way for capacity building and its application in diabetes projects in Africa. Third, SAPALDIA is importantly influencing air quality policies in Switzerland and Europe (Brunekreef et al., 2015, Adam et al., 2015, Downs et al., 2007, FOEN, 2015) [see http://www.sapaldia.ch/en].

Table 2: Contribution of the objectives of this thesis to the nexus of Swiss TPH built around the triangle of innovation, validation and application. Shaded areas represent the specific areas of contribution by the respective objective.

Objective	Innovation	Validation	Application
Association between ambient air pollution and diabetes in Europe and North America: Systematic review and Meta-analysis			
Long-term air pollution exposure and diabetes in adults			
Long-term exposure to air pollution and metabolic syndrome in adults			
A common functional variant in the pro-inflammatory Interleukin-6 may modify the association between air pollutants and diabetes			
Air pollution and diabetes association: Modification by type 2 diabetes genetic risk score			
Consideration of objectives in the overall impact of SAPALDIA research			
Human development in the frame of developing and NCD research agenda in the South.			

In the following sections, this work discusses the findings made in its constituent projects including associations between air pollutants and diabetes, associations between air pollutants and markers of cardiometabolic syndrome and the modifying effects of genetic variations on the relationship between air pollutants and diabetes. Also discussed are other relevant potential modifiers identified in the course of this work, its strengths and limitations and research outlook in this growing field of epidemiology.

9.1.1 Relationship between air pollutants and impaired glucose homeostasis

The meta-analysis pooling the effects of $PM_{2.5}$ and NO_2 presented in chapter 4 provides summarized quantitative evidence linking both pollutants to the risk of T2D. This work synthesized data from three studies on $PM_{2.5}$ and four studies on NO_2 and analyzed them in a random-effects meta-analysis [Chapter 4]. Interestingly, the studies on $PM_{2.5}$ were quite homogenous despite variations in design and population and presented stronger effect

estimates compared to NO_2. Despite the potential for high risk of bias which calls for cautionary interpretation, pooled estimates were remarkably robust across sensitivity analyses (Eze et al., 2015a). This meta-analysis identified methodological gaps including the need to identify undiagnosed diabetes cases, and for consideration of neighborhood-level socio-economic status and noise exposure as potential confounders [Chapter 4]

In the SAPALDIA cohort, the results of our meta-analysis were validated, demonstrating a positive significant relationship between exposure to 10-year mean ambient PM_{10} and NO_2, and prevalent diabetes [Chapter 5]. In an improved analytic framework as suggested in chapter 3, this study identified undiagnosed diabetes cases through additional blood tests, and explored the roles of neighborhood-level socio-economic status and noise exposure as potential confounders [Chapter 5]. Estimates of this association were remarkably stable to confounder adjustments and also were not affected by the definition of diabetes or potential selection bias assessed using inverse probability weighting (Eze et al., 2014a).

Since the publication of the above evidence, additional mixed evidence on the impact of air pollutants on T2D have emerged. Park et al. (2015) found a positive significant relationship between one-year mean $PM_{2.5}$ and NOx, and prevalent diabetes among American residents from six cities. Observed associations between both pollutants and incident T2D in the same study were positive but non-significant (Park et al., 2015). Another study by To et al. (2015) found a positive association between 18-year mean $PM_{2.5}$ and prevalent diabetes among women resident in Ontario. In Germany, a 5-year longitudinal study reported a positive association between two-year mean PM_{10} and incident T2D. A positive but non-significant relationship was reported for $PM_{2.5}$ (Weinmayr et al., 2015). In the first evidence from Australia using data from the Australian Longitudinal Study on Women's Health, a positive but non-significant association was observed between exposure to 3-year mean NO_2 and prevalent diabetes (Lazarevic et al., 2015). An update of the previously reported meta-analysis

[Chapter 4] including this additional evidence, done by this work, demonstrates a positive relationship between $PM_{2.5}$ and NO_2, with risk of T2D [Appendices 1 and 2].

Before the hypothesis of a potential aetiologic link between air pollution and T2D based on thinking towards the interrelationships between cardiovascular diseases and T2D (Elkeles, 2000, Bagg et al., 2000), there was more interest in the impact of air pollutants on cardiovascular morbidity and mortality as demonstrated by several epidemiological studies (Brook et al., 2004, Brook et al., 2010). It became imperative that to understand mechanisms and disentangle this complexity, a first step would be to identify the pathway that could be driving cardiovascular diseases and T2D constituting the cardio-metabolic syndrome.

This work attempted to answer this question by investigating associations with metabolic syndrome and its components [Chapter 6] in the SAPALDIA cohort. While other studies explored associations of air pollution exposure [in different populations] with individual components of metabolic syndrome for instance Fuks et al. (2014), Chuang et al. (2011), and (Xu et al., 2010) or the modifying effect of this syndrome in air pollution health effects (Krishnan et al., 2013, Devlin et al., 2014, Chen and Schwartz, 2008), this work investigated all the components of metabolic syndrome at the same time in the same population, providing a first evidence in this regard toward understanding the more likely pathways for cardio-metabolic effects of air pollutants. In this study, we reported a positive association across all three metabolic syndrome phenotypes, but the relationship was strongest with the glucose-dependent phenotype (Eze et al., 2015c).

Across the individual components, we also found positive relationships with hypertension, replicating findings from another study (Fuks et al., 2014), and obesity, replicating a similar finding that was made only in children (Jerrett et al., 2014). Although we defined metabolic syndrome using a self-reported fasting time of 4 hours, our results are considered valid as a 4-hour fasting time can be used in emergencies or outpatients to identify diabetes cases or its

intermediate phenotypes (Troisi et al., 2000). On the other hand, non-fasting lipids have been shown to predict the risk of cardiovascular events comparably to fasting lipids, in women (Bansal et al., 2007). In addition, we replicated our main findings among ~400 participants reporting 8 hours fasting time [Chapter 6]. A recent population-based study also found an association between short-term exposure to NO_2 and fasting serum glucose (Sade et al., 2015). Taken together, it appears that a major pathway through which air pollution exposure impacts on cardio-metabolic health could be through alterations in glucose homeostasis.

9.1.2 The culprit: Is it the physical or chemical properties of pollutants?

Traffic–related air pollution represents a common and major source of ambient air pollution. Markers of traffic-related air pollution include NO_2, NOx, $PM_{2.5}$, ultrafine particles, carbon monoxide, sulphates etc. derived from combustion processes in vehicles. Non-traffic related air pollutants may include particles like PM_{10} which are derived from natural or agricultural sources. Unlike the gases which are mainly derived from combustion processes, particulate matter has multiple sources and characteristics including morphology, solubility, surface area, count, stability, oxidative potential which mostly constitute its physical properties and combine to determine their toxicity (Brook et al., 2010). A large proportion of particulate matter may consist of organic sources including fungal spores and pollen (Yin et al., 2005), which are easily recognized by the macrophage and other immune cells, activating innate immune responses (Goto et al., 2004) and eliciting inflammation. Brook et al. (2010) suggested that traffic might lead to more toxic combinations of PM than natural PM or other sources, contributing to a stronger activation of inflammatory pathways, and an expert citation suggests that carbonaceous particles may be more toxic than nitrates and sulphates (Tuomisto et al., 2008). Thus, it is expected that particles, being a mixture of various components, are more implicated in the air pollution effects.

Much of the research onrespiratory health impacts of air pollution exposure have focused on particulate pollutants which could occur as fine secondary acid-coated particles (Schlesinger, 1995, Zelikoff et al., 1997), soluble fine particles with oxy-reactive components (Murphy et al., 1998, Adamson et al., 1999), insoluble ultrafine particles (Roth et al., 1998, Ziesenis et al., 1998), or bio-organics (Behrendt et al., 1997, Knox et al., 1997). An experimental study on the impact of oxidation [by NO_2 and O_3] on the physical and chemical properties of soot, and their influence on inflammatory markers demonstrated that despite the increased cytotoxic properties of oxidized compared to non-oxidized soot, there was no difference in the IL-8 concentration produced by both (Holder et al., 2012), suggesting the relative importance of the physical properties of soot in eliciting inflammatory reactions.

Epidemiological evidence from the European Study of Cohorts for Air Pollution Effects demonstrate that particulate matter may be more relevant than NOx in eliciting inflammatory responses (Hampel et al., 2015, Mostafavi et al., 2015). A positive association was demonstrated between C-reactive proteins [CRP] and markers of traffic-related pollution including traffic density and coarse PM. Although this (Lanki et al., 2015) found a positive association between NOx and CRP, Mostafavi et al. (2015) could not replicate this finding in their study which focused on healthy adults. In another study focusing on PM constituents, a positive relationship was observed between copper and iron contents of PM and CRP; and between zinc content of PM and fibrinogen (Hampel et al., 2015). Studies from the US have also demonstrated consistent evidence on the positive link between iron and sulphate content of PM and ischaemic heart disease mortality (Ostro et al., 2010, Lippmann et al., 2013) .

Experimental research in the aspects of insulin resistance has mainly focused on fine particles (Sun et al., 2005, Xu et al., 2011, Rajagopalan and Brook, 2012, Liu et al., 2013, Rao et al., 2015) and demonstrating insulin resistance due to PM-induced inflammation. Population-based epidemiologic evidence from this work presented in chapters 5 and 6 supports this PM

hypothesis and showed the relative importance of particulates in PM_{10} models additionally accounting for NO_2 exposure. In fact, the effect of NO_2 was completely lost when considering intermediate phenotypes [Chapter 6]. This work applied multi-pollutant models because both pollutants were testing independent hypothesis and had some positive correlation with each other, so it was useful to see which was more relevant. However multi-pollutant models can be limited by co-variation of pollutants, the possibility of complex interactions among pollutants, potential confounding by unmeasured pollutants, the subtlety of association of interest, exposure uncertainty and power issues (Tolbert et al., 2007). Despite the apparent importance of particles in air pollution-related health effects, it is expected that most health effects should occur in situations of combined particle-gaseous pollutant exposure as demonstrated in some studies (van Bree and Cassee, 2000, Brook et al., 2010).

9.1.3 Are women at greater risk?

Sex differences in health outcomes remain an issue in environmental epidemiology. Several studies have explored susceptibility due to sex in investigating the health impacts of environmental exposures including air pollutants (Clougherty, 2010). It is thought that being male or female influences one's exposure to dose, from dose to effective dose and from effective dose to health outcomes (Clougherty, 2010).

Exposure to dose could be determined by the sex-differences in the respiration of air-borne pollutants due to sex-dependent characteristics of lung function (Jones and Lam, 2006) and gas-blood barrier permeability (Brauner et al., 2009). Dose to effective dose could be determined by the hormonal differences between both sexes, for instance, the oestrogenic influence on some environmentally-derived compounds (Morris et al., 2003), the higher propensity for mercury retention in kidneys in women (Hultman and Nielsen, 2001) and the potential change in exposure patterns due to pregnancy (Nethery et al., 2009). At the target organs, sex differences could influence the translation from effective dose to health outcomes.

Sex-linked hormonal differences influenced alterations in vascular endothelium (Prisby et al., 2008) and it was demonstrated that women had more urogenital pathologies than men in areas with high arsenic exposure (Concha et al., 1998).

Of additional interest is the role of gender- a social construct which includes norms, roles and behaviours shaped by relations among the sexes (Krieger, 2003)- in shaping observed health outcomes due to air pollution exposure. Gender determines people's activity patterns including leisure and work-related activities. Thus, an analysis of gender will inform about concentration of exposure (Clougherty, 2010). Studies from developing countries showed that women were more likely to suffer respiratory problems due to indoor fossil fuels, more frequently than men, attributed to the fact that women generally perform the cooking at home (Behera and Balamugesh, 2005). This may be explained by the fact that estimates of air pollution exposure at home will better capture the actual exposures of people who spend more time at home than others (Brook et al., 2008).

In the meta-analysis presented in chapter 4, we found significant sex differences in the risk of T2D due to air pollution exposure. While this appears to be an initial support for the sex and gender hypotheses, it is important to note that an update of this meta-analysis, done in this work, and incorporating five additional studies published after this meta-analysis (Eze et al., 2015a, Park et al., 2015, To et al., 2015, Weinmayr et al., 2015, Lazarevic et al., 2015) showed comparable estimates between males and females, obliterating the initially observed differences [Appendices 1 and 2]. This pattern did not change for both pollutants when we limited the inclusion criteria to longitudinal studies [Appendices 1 and 2].

9.1.4 The role of physical activity- implication for health-in-all policies?

Physical activity leads to physiological changes that may enhance the health effects of air pollution. These may include changes in breathing pattern, pollution dose, and nasal defenses

(Giles and Koehle, 2014, Niinimaa et al., 1980). As exercise intensity increases, ventilation increases and breathing switches from predominantly nasal to oral, bypassing the nasal filtration and increasing the inhaled dose of pollutants (Niinimaa et al., 1980). Acute exposure to PM2.5 was shown to induce platelet activation, raised markers of inflammation and oxidative stress and DNA damage (Brauner et al., 2007, Vinzents et al., 2005) during exercise. Chronic exposure to ultra-fine particles during exercise in an urban setting was also associated with increased leukocyte and neutrophil counts compared to exercise in a rural setting (Bos et al., 2013). Evidence for reduction in exercise performance (Kargarfard et al., 2011, Giles et al., 2012), lung inflammation (Alfaro et al., 2007, Rundell et al., 2008) and reduction of lung function (Giles et al., 2012, Brauner et al., 2009), on exposure to air pollutants during exercise, has been mostly positive (Giles and Koehle, 2014). Exposure to air pollutants during exercise was also associated with angina and myocardial ischemia (Lanki et al., 2006, Pekkanen et al., 2002) an effect potentially mediated by vasoconstriction and endothelial dysfunction through autonomic disturbances (Brook et al., 2010).

The findings from this work presented in chapters 5 and 6 support a role for physical activity in exacerbating the glycemic effects of air pollution. Other population-based studies investigating the air pollution-diabetes relationship also reported stronger associations among the physically active (Andersen et al., 2012, Weinmayr et al., 2015). However, this pattern was not observed in our study on the relationship between passive smoke and diabetes in adult never-smokers (Eze et al., 2014c). Taken together, these findings demonstrate the importance of considering air pollution in the promotion of physical activity. On the other side, evidence suggests that physical activity led to less retention of PM in the lung and higher bronchial clearance some hours after exercise, compared to at rest (Bennett et al., 1985). Another study demonstrated that high intensity cycling obliterates the differences in physiological parameters observed with low-intensity cycling with filtered air and with diesel exhaust (Giles et al., 2014). In addition, exposure to NO_2 did not significantly modify the protective effect of

physical activity on mortality, with this effect occurring across both high and low levels of NO_2 exposure (Andersen et al., 2015). Overall, despite precautionary measures towards exercising in polluted areas, the long-term benefits of physical activity towards preventing mortality may outweigh the risks associated with air pollution exposure during physical activity (de Nazelle et al., 2011), hence it is important for environmental and health policies to interact in this regard.

9.1.5 The roles of inflammation and insulin resistance: evidence from genetics

Inflammation plays a major role in the aetiology of metabolic disorders (Hotamisligil, 2006). In obese individuals, activated M1 macrophages produce various pro-inflammatory cytokines that suppress insulin signaling in adipose tissue, whereas activated M2 macrophages found in lean individuals produce interleukin-10 which enhances insulin signaling in adipocytes (Hirosumi et al., 2002, Cai et al., 2005). In mice, CD11c knockout of M1 macrophages reduced obesity-induced inflammation in the adipose tissue and insulin resistance (Patsouris et al., 2008).

Induction of systemic inflammation by air pollutants (Figure 7) begins in the lungs where the pollutants may: activate innate immune cells directly or through the generation of reactive oxygen species (Goto et al., 2004, Kampfrath et al., 2011); activate adaptive immunity through oxidation by antigen-presenting cells; cause the overflow of reactive oxygen species into the systemic circulation, leading to inflammatory response (Dominici et al., 2007, Kampfrath et al., 2011) and additionally activating the central nervous system, following the overflow of inflammatory markers into the systemic circulation (Liu et al., 2013, Rajagopalan and Brook, 2012, Rao et al., 2015). Other hypothesis includes a rapid and direct translocation of ultrafine particles and soluble compounds from the alveoli to the capillary circulation (Simkhovich et al., 2008).

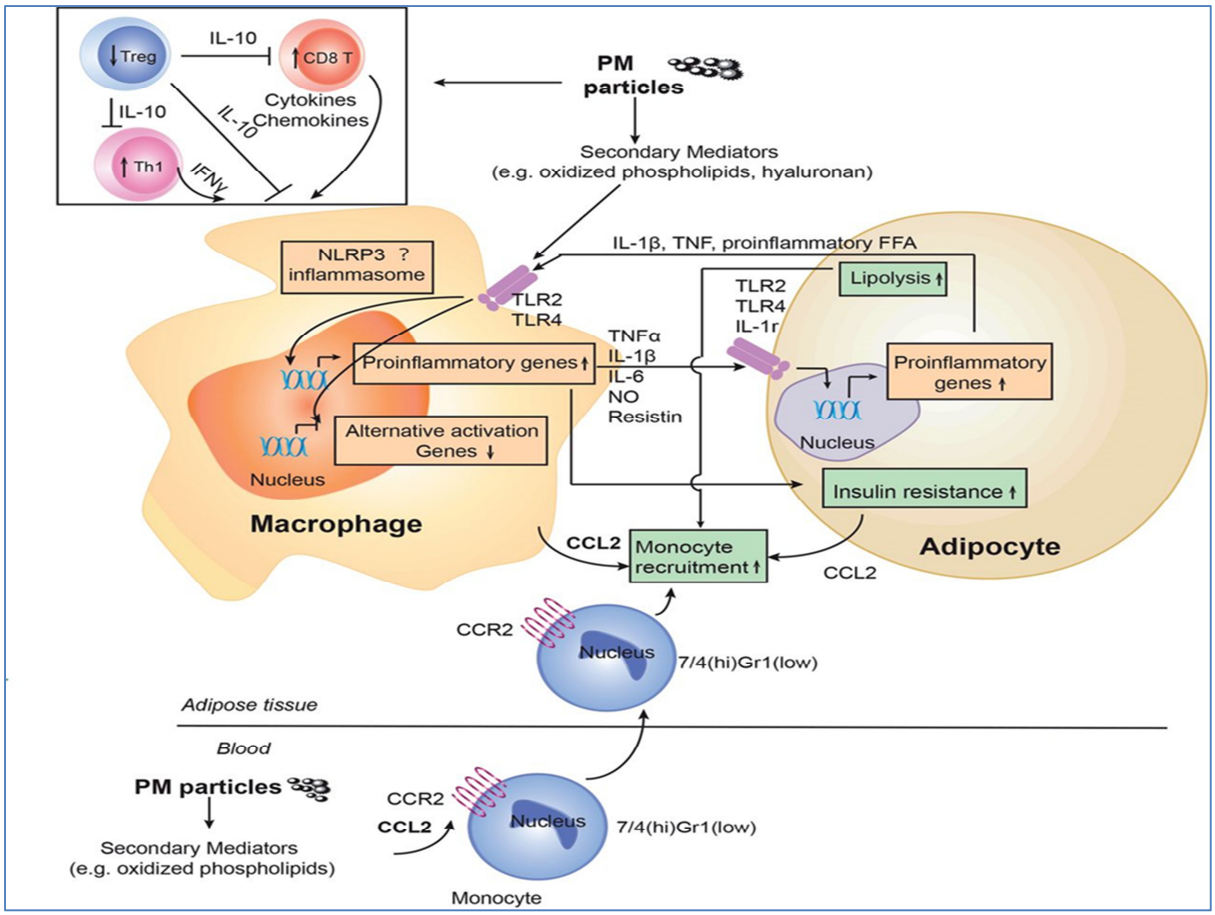

Figure 7: Mechanisms involved in inflammatory response activation by air pollutants (Rao et al., 2015)

Much of this evidence has been from experimental animal models. The need for replication in human population requires conditions where several inflammatory processes are measured with controlled air pollution exposure, which may be quite expensive, considering issues with statistical power and optimizing confounder control. Given limited resources, a good and valid tool to assess the role of immune system and inflammation-related processes would be through the use of genetic variations [obtained through a combination of genotyping and imputation using reference genomes] as indicators of functional parameters relevant to the understanding of systemic impacts of environmental toxicants.

Genetic epidemiology applies the principle of Mendelian randomization by using genetic variants in observational studies (Lawlor et al., 2008). Mendelian randomization has been likened to natural randomized controlled trials (Hingorani and Humphries, 2005, Davey Smith

and Ebrahim, 2005), and implies that the inheritance of one trait is independent of the inheritance of other traits. Although not always true for genes located close to each other on homologous chromosomes (i.e., in linkage disequilibrium) (Morgan TH, 1915), the randomization principle still controls bias when relating genetic variants to disease outcomes at a population level because alleles are generally unrelated to confounding factors (Bhatti et al., 2005, Davey Smith et al., 2007); there is no reverse causality since diseases do not alter genotype; there is less associative selection bias (Berkson, 2014), less regression dilution bias (Davey Smith and Phillips, 1996), and causal inference can be made since the association between a genotype and a modifiable risk mostly remains throughout life (Davey Smith and Ebrahim, 2004). This approach requires the knowledge of the functions of genetic variants, which is then applied as an instrument for the modifying factor of interest (Lawlor et al., 2008).

As presented in chapter 7, we applied a 63-locus polygenic risk score for T2D to assess its impact in the air pollution-diabetes relationship (Eze et al. 2016a). As expected, this score associated well with diabetes status, validating our assumption of T2D. Other studies have also reported the predictive power of T2D gene risk scores on T2D (Andersson et al., 2013, Cornelis et al., 2009, Langenberg et al., 2014, Talmud et al., 2015, Vassy et al., 2014), all demonstrating a positive relationship. Exploring the mediating role of pathway-specific scores (insulin resistance and beta-cell function), while not measuring insulin sensitivity and beta cell function parameters in this work, allowed a better understanding of the mechanisms in this relationship. Additional observation of interactions with asthma phenotype on PM_{10}-diabetes relationship further strengthened our understanding of the involvement of inflammatory pathways in this complex relationship (Eze et al., 2016a).

Moreover, the results presented in chapter 8 contribute more evidence on the involvement of inflammatory mechanisms in the PM_{10}-diabetes relationship (Eze et al., 2016b). By using a

functional *IL6* polymorphism known to regulate the production of circulating IL-6 (Brull et al., 2001, Sanderson et al., 2009), one of the inflammatory risk markers of T2D (Pradhan et al., 2001, Kristiansen and Mandrup-Poulsen, 2005, Spranger et al., 2003), without measuring plasma IL-6, this work was able to demonstrated that homozygous carriers of the pro-inflammatory major 'G' allele were most susceptible to PM_{10}. Raised plasma concentrations of IL-6 are associated with having a GG genotype (Brull et al., 2001, Sanderson et al., 2009), thus using the polymorphism as proxy has clearly demonstrated the impact of environmental toxicants, in inducing production of IL-6, and potentially increasing the risk of diabetes in a substantial proportion of the population with the risk allele. Although we did not have plasma IL-6 measures to corroborate our findings, levels of high sensitivity C-reactive proteins, which is also regulated to an extent by *IL6* polymorphisms (Sainz et al., 2008), was found to correlate positively with this polymorphism in this study (Eze et al., 2016b).

9.1.6 Strengths of study

The specific strengths of the studies constituting this work are presented in chapters 4-8. Taken together, this work makes great scientific contributions toward identifying and understanding the mechanism involved in the hypothesized relationship between exposure to air pollution and T2D. Taking optimal advantage of the research potential of the well-characterized SAPALDIA database which includes information of socio-demography, health, lifestyle factors, home and work exposures, and genomics [Figure 8], this work demonstrates in great depth the relevance and potential of big data in disentangling the complexities of non-communicable disease aetiology. The relevance of studying interactions also point to the need for much larger cohorts and biobanks such as the United Kingdom biobank.

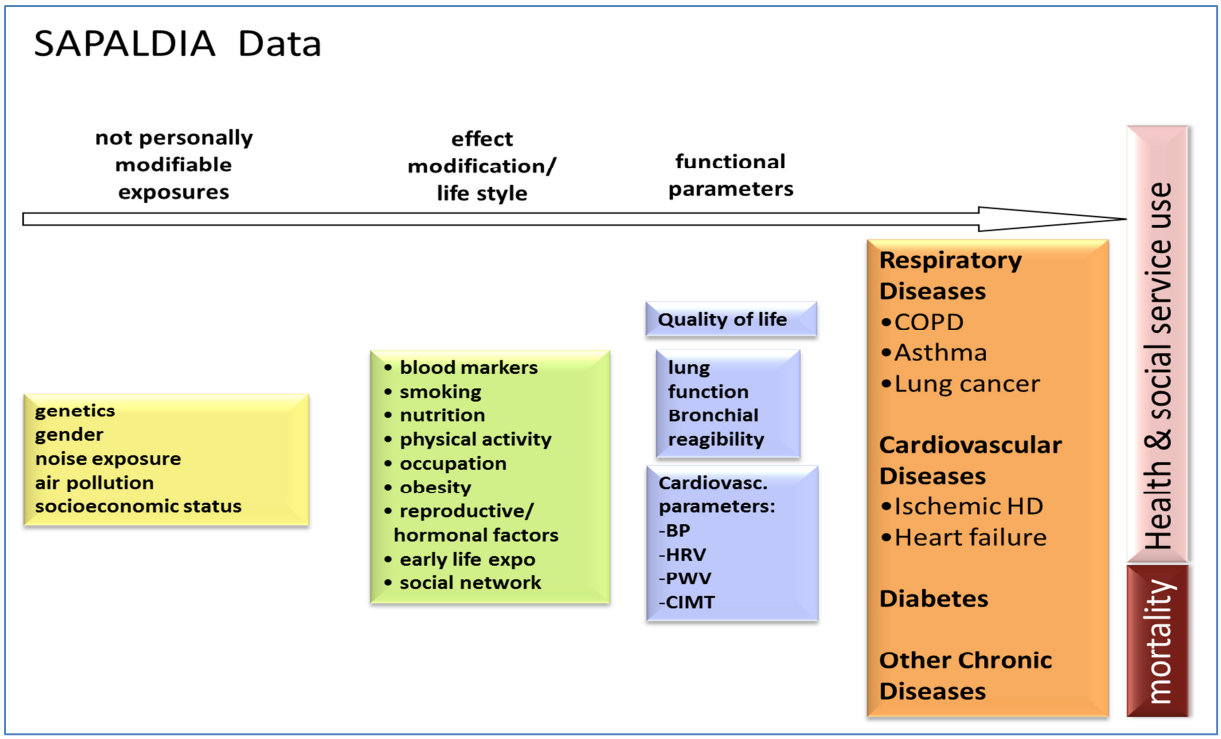

Figure 8: Data collected longitudinally in SAPALDIA over 25 years

This work was done using validated air pollution models which predicted well air pollution exposures, both at home and traffic sites using combinations of Gaussian dispersion models and Land-use regression models (Liu et al., 2007, Liu et al., 2012). In the frame of the SAPALDIA study, this work identified prevalent diabetes cases at first follow-up (Eze et al., 2014a) and confirmed them by the use of genetic variants [Chapter 7]. This work also identified cases of metabolic syndrome following the prediction and validation of waist circumference measures which were not measured at the first follow-up of the SAPALDIA study (Eze et al., 2015c). Using a population-based approach, this work has also demonstrated the importance of gene-environment interactions in understanding the mechanisms and susceptibilities of exposure to environmental toxicants, and further strengthened the relevance of genotypes as research instruments in understanding causality [Chapters 7 and 8]. Lastly, we applied adequate statistical methods across studies, performing several sensitivity analyses including inverse probability weighting and multiple imputations to assess the relevance of potential selection bias and robustness of our findings [Chapters 5-8].

9.1.7 Limitations of study

As discussed in chapters 4-8, the main limitation of this work was the cross-sectional approach in all its studies which limits the inference of causality. This was mostly due to data availability and also affected our identification of incident T2D cases. As discussed in chapters 5, 7 and 8, we used 10-year means of pollutants up to the year prior to first follow-up when diabetes cases were identified, and we expect <10% of these diabetes cases to be T1D (Alberti and Zimmet, 1998). Air pollution measures were derived from measurements captured at a resolution of 200x200m (Liu et al., 2007). A higher resolution of the air pollution exposure would also potentially limit misclassification and lead to stronger results.

Another limitation is that we considered PM_{10} as our particulate matter of interest, instead of $PM_{2.5}$ or ultra-fine particles, which may penetrate further into the respiratory tract through translocation. At the start of this work, only PM_{10} was available for analysis, which was later shown to correlate well with $PM_{2.5}$ across SAPALDIA areas (R~0.8). Ultra-fine particles were measured at the second follow-up and replication studies are planned with diabetes.

Compared to diabetes cohort consortia, we had relatively small sample size of diabetics across our studies. While this may have been a limitation, this work presented very relevant findings which are expected to even be stronger and more significant in bigger studies having all the necessary exposure, phenotype and genomic data.

9.2. Outlook: Disentangling the complexities of non-communicable disease aetiology

In attributing aetiologic roles to environmental stressors in the development of T2D, future studies should focus on disentangling complexities involved in the inter-relationships of these constituent or potential risk factors and conduct studies in different contexts.

9.2.1. Disentangling air pollution and noise effects and understanding interactions

Noise is a well-known environmental stressor, an unwanted sound, which also has adverse health effects through annoyance, sleep disturbances, cognitive and emotional disturbances (Muzet, 2007). Traffic noise is a common type of environmental noise especially in urban areas and growing evidence from noise epidemiology has demonstrated its impact on cardiovascular health (Babisch, 2006, Babisch, 2011). Recently, the impact of noise on diabetes has been receiving some attention, and is thought to act through stress-related pathways impacting on glucose homeostasis (Cappuccio et al., 2010, Dzhambov, 2015). On the other hand, traffic is a combined source of both air pollution and noise making both exposures to potentially confound each other in common outcomes.

Different studies have investigated the spatial distribution of both traffic-related air pollution and noise to understand their correlation patterns e.g. Allen et al. (2009). Correlations were observed to be dependent on area and time, and night-time correlations appeared to be strongest (Kim et al., 2012). In addition, Foraster et al. (2011) described a positive substantial correlation between annual NO_2 and 24-hour noise measures which was dependent on urban structures and traffic patterns. These data indicate that noise could confound health associations observed with air pollution, hence the importance of disentangling associations of both exposures.

In their review of the air pollution-noise confounding on cardiovascular outcomes, Tetreault et al. (2013) concluded with caution that since the degree of confounding in the nine pooled studies was low, it may not be necessary to control for noise in studies on health effects of air pollution. In identifying the determinants for varying degrees of confounding, Foraster (2013) suggested that while this conclusion may be true for some settings, it cannot be generalized due to the fact that the correlations between noise and air pollution varied across areas, and confounding could be relevant in some studies. On the other hand, the use of different noise

and air pollution indicators and lack of information of exposure data quality may have also led to the low confounding. Furthermore, the inability to account for indoor noise exposure- which is more relevant for health effects- might have also biased the noise estimates (Foraster, 2013).

Attempts at disentangling both exposures have been made in some studies on the health effects of air pollution and noise exposures. Studies on air pollution have adjusted for noise [Chapter 4], whereas studies on noise have adjusted for air pollution exposure (Coogan et al., 2012, Sorensen et al., 2013). In both situations, the associations of health outcomes with pollutants of interest were sustained despite adjusting for the potentially confounding exposure. Lack of confounding, like in our study, may have been due to the use of low resolution noise data, which precluded the correct assessment of confounding, and may have led to residual confounding in the study (Kunzli, 2013). All these estimates have been from outdoor exposures. In relation to noise and its health effects, it is particularly important to measure indoor levels at home to refine exposure because of the impact of shielding on noise and the importance of sleep on the noise-related health effects. Therefore, adjustment for outdoor traffic noise levels would not truly capture the individuals' exposure and will hinder the independence of the effects of traffic-related air pollution from those of road traffic noise (Foraster et al., 2014). In fact, Foraster et al. (2014) demonstrated that estimating indoor noise exposure reduces the correlation with outdoor air pollution and better disentangles relationship between noise, NO_2 and blood pressure, which could not be achieved by using only outdoor noise estimates. It is also essential that indoor air pollution is assessed for a more global overview of noise and air pollution exposures in this regard.

Until now, noise epidemiology research has relied on A-weighted sound pressure levels [LAeq] which represent average noise levels over different day periods or the 24 hours (Dzhambov, 2015, Rylander et al., 1986). Also of interest is the number of events which

reflects the number of times noise levels are above a threshold at a given time (Fields, 1984). Advances in noise research have resulted in refined metrics that may better capture the temporal noise variations and their health effects. The metric termed intermittency ratio expresses the proportion of the acoustical energy contribution in the total energetic dose that is created by individual noise events above a certain threshold (Wunderli et al., 2015).

Analytical approaches to disentangle the noise-air pollution confounding in studies using well characterized estimates for both exposures may include stratification by area or using area-weighted regression (Foraster et al., 2014) and generating interaction terms between both exposures. With the development of indoor noise exposures, intermittency ratios, and indoor air pollution exposures, applying these analytic approaches to these exposure metrics in parallel will contribute to the understanding of independent effects of noise and air pollution exposure measures in future research.

9.2.2. The role of comparative research in disentangling complexities

Comparative epidemiology seeks to answer research questions using comparisons across species, phenotypes, settings etc. By comparing spatial and temporal trends and relationships across defined characteristics, new concepts, hypotheses and theories may evolve. Comparative epidemiology improves epidemiological studies and allows reaching valid conclusions through a better understanding of the complexities and the background of the subject under study.

A. <u>Considering air pollutants from different sources and at different levels</u>

As demonstrated in chapter 4, epidemiological and experimental evidence from the health effects of air pollution regarding diabetes has been from Europe and North America. Even additionally published evidence [following the meta-analysis] has also been from these regions, including Australia. Advancements in air quality monitoring available in these

western countries are lacking in the developing settings like Africa. Switzerland, for instance, covering an area of ~42000 km^2 has 16 monitoring stations according to the Swiss Agency for Environment, Forests and Landscape (SAEFL, 2003), whereas Nigeria having an area of ~920,000 km^2 has only one monitoring station, according to the Nigerian national Air Quality Monitoring Programme (NNAQMP, 2015). Pollution levels have been demonstrated to be higher in the developing countries of Africa and Asia, compared to the areas with better regulation facilities [Figure 9]. A recent review demonstrated that most countries with high pollution levels do not even have regulatory limits for air pollutants (Kunzli et al., 2015), demonstrating the general lack of interest in air quality issues. Also the lack of epidemiological cohorts in the developing areas of the world also limits the generation of evidence from these areas (Lelieveld et al., 2015).

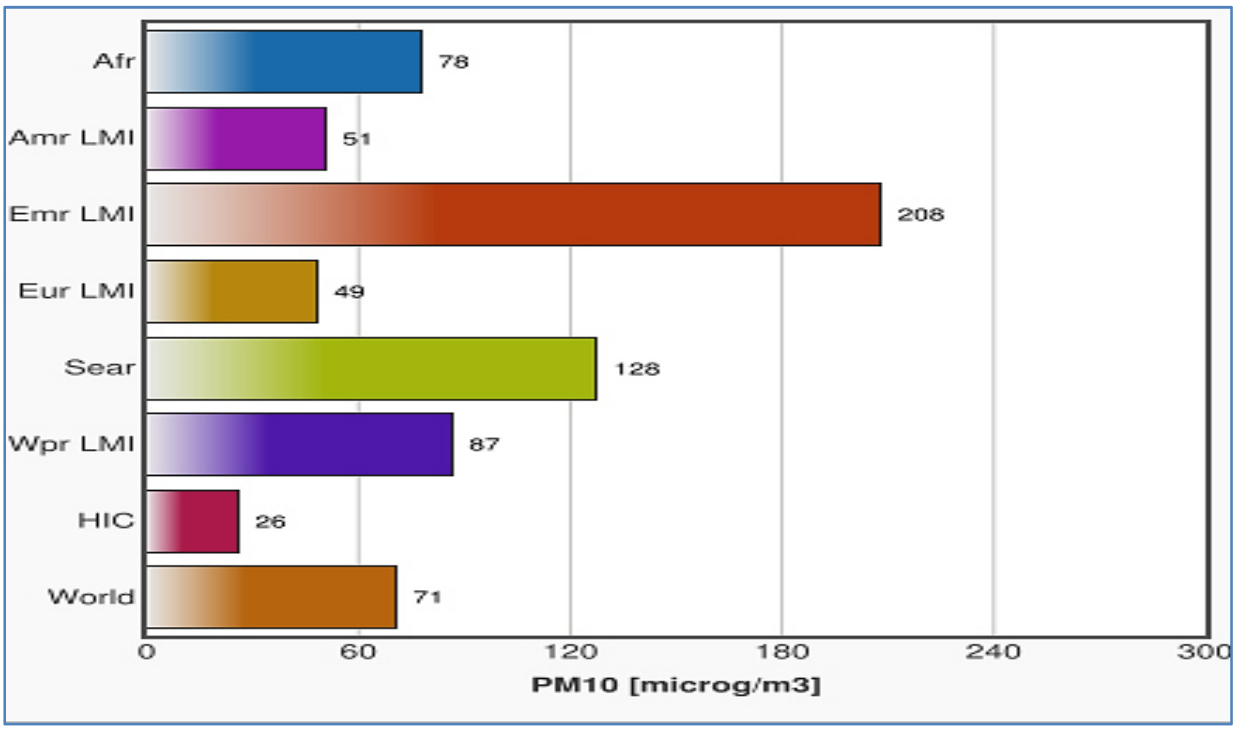

Figure 9: PM_{10} levels by region for any year in the period 2008-2012. Amr: America, Afr: Africa; Emr: Eastern Mediterranean, Sear: South-East Asia, Wpr: Western Pacific; LMI: Low- and middle-income; HI: high-income. PM_{10} values are regional urban population-weighted (WHO, 2014).

Environmental toxicants depend on geography and meteorology, and this holds particularly true for particulate matter which occurs as a mixture of several particles. In the SAPALDIA

cohort, the ratio of $PM_{2.5}$ to PM_{10} is ~0.8, implying the possibility of extension of results observed with PM_{10} to $PM_{2.5}$. In contrast, 2013 field studies in Nigeria showed the ratio of $PM_{2.5}$ and PM_{10} to range from 10-33% across six cities with varying residential and traffic densities (NNAQMP, 2015). One may therefore expect different patterns of associations of PM_{10} and $PM_{2.5}$ with health outcomes compared to those observed in Switzerland.

Countries in the South face a dual burden of pollutant exposure – ambient pollutants and indoor pollutants mainly from biomass fuel, both of which contributed >6 million global deaths in 2010 (Lim et al., 2012). Figure 10 demonstrates the regional distribution contributions of various pollutant sources to mortality.

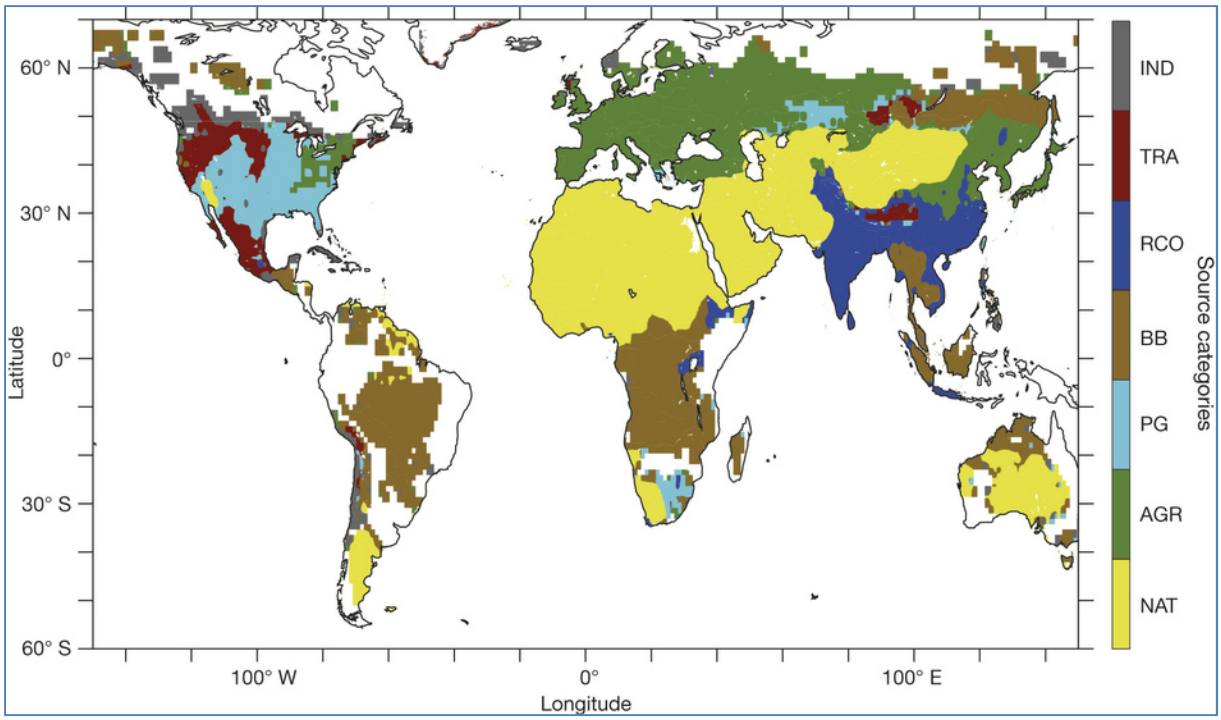

Figure 10: Distribution of source categories responsible for largest impact on mortality due to outdoor air pollution. IND, industry; TRA, land traffic; RCO, residential and commercial energy use; BB, biomass burning; PG, power generation; AGR, agriculture; and NAT, natural. In the white areas, annual mean $PM_{2.5}$ is below the concentration–response threshold (Lelieveld et al., 2015).

Biomass burning contributes about 70% to $PM_{2.5}$ in Brazil and up to 90% in Angola (Lelieveld et al., 2015). A Chinese study showed that the use of biomass greatly increased PM concentrations in the home (Jiang and Bell, 2008). $PM_{2.5}$ from biomass burning are mainly carbonaceous particles (Tuomisto et al., 2008) comprising pro-oxidative organic

hydrocarbons that activate inflammatory pathway and cause DNA damage (Bhatnagar, 2006). Exposure to PAH from cooking in particular was linked to oxidative DNA damage in restaurant workers (Pan et al., 2008). Indoor air pollution from biomass fuels was also linked to adverse respiratory outcomes including tuberculosis and lung cancer (Behera and Balamugesh, 2005, Ezzati and Kammen, 2001, Hernandez-Garduno et al., 2004), cardiovascular diseases, diabetes (Lee et al., 2012) and increased mortality from lung cancer and cardiovascular diseases (Lippmann et al., 2013). Thus, considering air pollution from biomass, in addition to ambient air pollution, by extending NCD research to the South, will add to the understanding of the degree of their health impacts, towards a more targeted control (Kunzli et al., 2015).

B. Considering air pollution effects in a context of high burden of infections and therefore, inflammation.

Infectious diseases are associated with inflammatory responses which usually resolve with appropriate treatment. Individuals mounting strong inflammatory responses to certain infections have been shown to be more susceptible to age-related inflammatory morbidity later in life (Koopman et al., 2012, Gurven et al., 2008). Koopman et al. (2012) demonstrated a higher inflammatory profile in a Ghanaian population compared to a western population, and a lower prevalence of cardio-metabolic risk factors and diseases in the Ghanaian population. Corroborating this finding, higher level of CRP was observed among Bolivians compared to Americans across all ages despite having lower cases of NCDs (Gurven et al., 2008). The presence of chronic infections may lead to cell and organ damages and derangements in the immune system which may impact on susceptibilities to environmental toxicants. This is particularly true in the LMIC where infections are endemic and exhibit chronicity. In fact, a substantial proportion of disability-adjusted life years attributed to

cardiovascular diseases in LMIC are due to inflammatory precursors such as rheumatic fever and neglected infections of poverty (Moolani et al., 2012).

While it is evident that inflammation plays a role in T2D and diabetes puts individuals at high risk to develop infections, the role of infections in developing diabetes is poorly understood. Exposure to particulate matter was related to the development of pneumonia in children (MacIntyre et al., 2014), and outdoor sulphates and indoor biomass exposure were related to tuberculosis (Hwang et al., 2014, Lin et al., 2007), both conditions being strong activators of the immune system. Engaging LMIC in NCD research incorporating environmental stressors will aid the understanding of the role of cellular and immune changes due to sustained infections in the development of NCDs.

C. Considering different gender contexts

The importance of sex and gender in environmental epidemiology has been previously discussed. While it would seem obvious to disentangle these two concepts, it has not been very practical to clearly differentiate exposures by sex and gender. In addition, conflation of both terms in epidemiologic research due to the fact that they are intertwined has also not helped (Krieger, 2003). An understanding of the gendered environment can improve exposure assignment, and help to better identify biologic responses. It can also provide a model for examining other social effect mediators of health outcomes in relation to environmental exposures (Clougherty, 2010, Clougherty and Kubzansky, 2009).

Apart from stratifications and analyses using sex-based interaction terms (Chapters 4-6), other analytic approaches to improve our understanding may include population-specific exposure modeling clarifying gendered exposure differences (Maziak et al., 2005); temporally refined exposure assessment incorporating gendered activities in probabilistic models (Zidek et al., 2005); propensity analysis incorporating predictive modeling for exposures and responses, and allowing the prediction of likelihood of exposure, given population exposure distributions

and possibly, pre-exposure characteristics (Kurth et al., 2006); and variants of multilevel modeling to disentangle between- and within sex variations (Phillips, 2005). It is also important to note the issue of competing risks and differences in the background rates of disease when interpreting sex- or gender-based risk estimates (Perneger, 2001).

9.2.3. The role of exposome approaches in disentangling complexities

A. Limitations of genetics

Apart from the inherent problems in genetic epidemiology studies including issues with population stratification, where differences in genetic ancestry lead to population heterogeneity (Davey Smith and Ebrahim, 2003); issues with genetic pleiotropy, where a gene may regulate related phenotypes (Newcomer et al., 1978); and issues with canalization, where developmental adaptation due to genetic predisposition reduces or prevents phenotypic expression (Davey Smith and Ebrahim, 2003), genetic variation remains insufficient for capturing the full mechanistic or biological changes in response to air pollution and underlying susceptibilities. This is mainly due to the complexities in the downstream result of genetic expression, where post-transcriptional and post-translational events contribute to metabolic end-points, which can be considered closer to the phenotype (Hollywood et al., 2006). Thus metabolite profiling can identify biomarkers of air pollution exposure or disease.

B. Omics biomarker and molecular networks

Omics technology today allows capturing molecular patterns in their full complexity. Easily accessible blood samples can be measured for various omics profiles that reflect full patterns of molecular networks. Much can be learnt about molecular networks mediating the impact of air pollution on diabetes or other health outcomes by applying the meet-in-the-middle concept which combines, within a population-based study, the prospective search for intermediate

biomarkers elevated in subjects that develop a disease and a retrospective search for links of these biomarkers to environmental exposures [Figure 11].

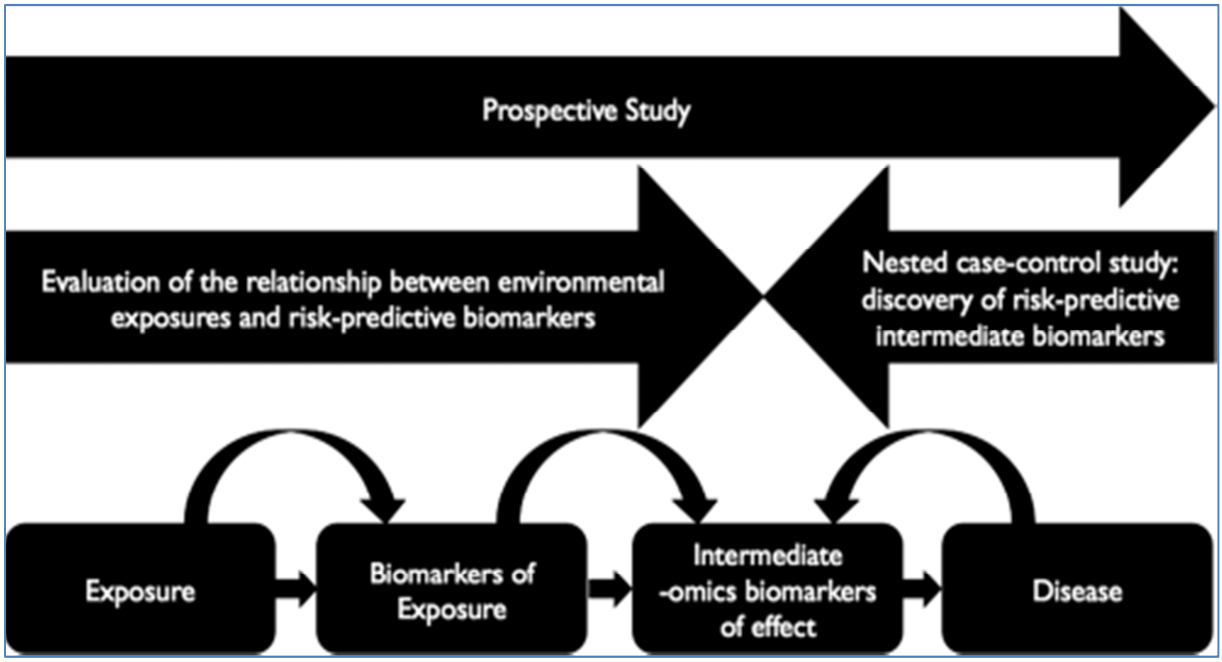

Figure 11: Schematic representation of the meet-in-the-middle concept of exposomics (Vineis et al., 2013)

This meet-in-the-middle concept suggests the inference of causality if an association is found within the component steps including first, investigating association between exposure and disease; second, investigating association between (biomarkers of) exposure and intermediate omics biomarkers of early effects; and investigating association between disease outcomes and intermediate omics biomarkers of early effects (Vineis et al., 2013).

C. Exposome-wide approaches for disentangling complexities

The exposome includes a combination of reactive electrophiles, metals, endocrine disrupters, immune modulators and receptor-binding proteins representing intermediate markers of early effect, which can be measured on a body fluid or tissue sample using the various omics technologies (Wild, 2012). At the exogenous risk factor level, research is also moving from looking at single exposures to a large number of exposures combined by applying exposome-wide association studies [EWAS] which uses the same approach as in GWAS. A practical

example of EWAS applying the meet-in-the middle concept in studying the molecular links between ambient PM [$PM_{2.5}$ and PM_{10}] and lung function across 280 metabolites identified through non-targeted metabolomics profiling found circulating levels of eight metabolites to be associated with both exposure to PM and lung function, and observed the strongest association with alpha-tocopherol (Menni et al., 2015), the biologically active form of antioxidant vitamin E which also regulates *VEGF* expression (Zingg et al., 2015). In doing this, this study was able to demonstrate the importance of oxidative pathways in the PM and lung function relationship. Similar approaches with PM in relation to diabetes will contribute to mechanistic understanding of this relationship.

A first attempt at environment-wide association study on type 2 diabetes assessed the association between 266 biomarkers of exposure to environmental agents among participants of the National Health and Nutrition Examination Survey in 2010 (Patel et al., 2010). In this study biomarkers of exposure were measured from urine and blood samples and positive associations with prevalent T2D were observed between pesticide-derivative heptachlor epoxide and vitamin gamma-tocopherol, and a protective effect of beta-carotenes was also demonstrated (Patel et al., 2010). Future studies that include measures of PM and applying the meet-in-the-middle concept will provide a better understanding of the roles of these metabolites in the relationship between PM and T2D.

In the context of epigenome-wide association studies, markers of DNA methylation are capable of capturing long-term exposure to environmental toxicants including tobacco smoke (Zeilinger et al., 2013, Breton et al., 2014) and particulate matter (Madrigano et al., 2012, Breton and Marutani, 2014), and could also be of interest in capturing a history of infections, or as a disease risk marker (Beyan et al., 2012). Epigenome-wide association studies have been applied in diabetes and CVD-related phenotypes in population-based studies (Rakyan et al., 2011, Hidalgo et al., 2014). In addition, individuals with T2D were shown to exhibit

epigenetic and transcriptional changes in adipose tissue that are relevant to disease development (Nilsson et al., 2014, Dayeh et al., 2014). Taken together, application of exposome-wide approaches in the framework of the meet-in-the-middle concept in future studies will improve our understanding of aetiologic relationships between environmental exposures and T2D.

9.3. Conclusions

9.3.1. Brief summary of main findings

- This work has presented compelling evidence supporting a positive relation between exposure to air pollutants and T2D.
- Particulate matter may be a more relevant marker of air pollution relevant for diabetes
- Individuals at high genetic risk for type 2 diabetes may be more susceptible to the diabetogenic effects of air pollutants.
- Alterations in insulin sensitivity may be a more relevant pathway through which air pollutants lead to T2D.
- The presence of back ground inflammation may potentiate the contribution of genetic risk to air pollutant susceptibility.
- Physical activity presented another pathway through which air pollutants may impact on type 2 diabetes. The modifying effect of physical activity should be confirmed and appropriate measures taken in the promotion of physical activity.
- Future studies should explore the potential impacts of air pollutants on beta-cell function, and the contributions of non-inflammatory pathways in the health effects of air pollutants

9.3.2. Public Health relevance of findings

- From the northern perspective, the findings from this work demonstrate the contribution of ambient air pollution to diabetes burden in Switzerland. PM also contributed to the burden of impaired fasting glycaemia and central obesity, which are risk factors for diabetes and some of the major contributors to global burden of disease (Lim et al., 2012). Although effect estimates were moderate to small, and may have a small impact at the individual level, exposures may be unavoidable and affects a substantial proportion of the population. If our association is causal, air pollution control policies targeting the general population would contribute to the reduction of diabetes prevalence, benefitting millions of people. Thus there is need for continuous update of air quality guidelines following evidence from research. In line with our finding of associations at mean concentrations below current guidelines, exposure to air pollutants at levels below air quality guidelines in Sweden was related to gestational diabetes and preeclampsia (Malmqvist et al., 2013). First, the European Union should adopt the WHO limits, and both organizations should unanimously develop evidence-based lower limits adapted to national and local conditions (Brunekreef et al., 2015). It is important to note that although studies have not been able to demonstrate lower thresholds at which exposure to air pollutants may not have health effects, making it difficult to set these limits, sustained efforts should be made at lowering the current standards. Appropriate dose-response studies are therefore needed to demonstrate a lower threshold of air pollution effects, for evidence-based information of air quality policies.
- The strength of evidence on the impact of physical activity on the susceptibility to air pollutants raises cause for concern. While this calls for well-designed studies for confirmation, efforts should be made to encourage physical activity in settings with

less air pollution. For instance, individuals in polluted cities may have to exercise indoors or go to less polluted areas for outdoor physical activity. In order not to discourage physical activity in general, it is important to note once more that air pollution may be unavoidable and the health benefits of physical activity will most likely outweigh the risks due to air pollution exposure (Andersen et al., 2015, Giles et al., 2014). There is thus a need for interactions between health and environmental policies towards sustaining and promoting physical activity in achieving better overall health (de Nazelle et al., 2011).

- From the southern perspective, the lack of evidence from this area calls for an urgent need for comparative evidence, considering that air pollution can reach very high levels [a combination of ambient air pollution and indoor pollution from biomass fuels] and NCD burden is increasing, and reaching levels of infectious diseases, due to epidemiologic transition. The wide gradient in air pollution exposures may enable the study of dose-response relationships and the variety of exposures will generate evidence for targeted policies.

- The development of NCD research agenda in the South, incorporating environmental exposures, will provide evidence in the local southern contexts which will inform policy in these countries, and stimulate serious interests in air quality regulation which are presently lacking (Kunzli et al., 2015).

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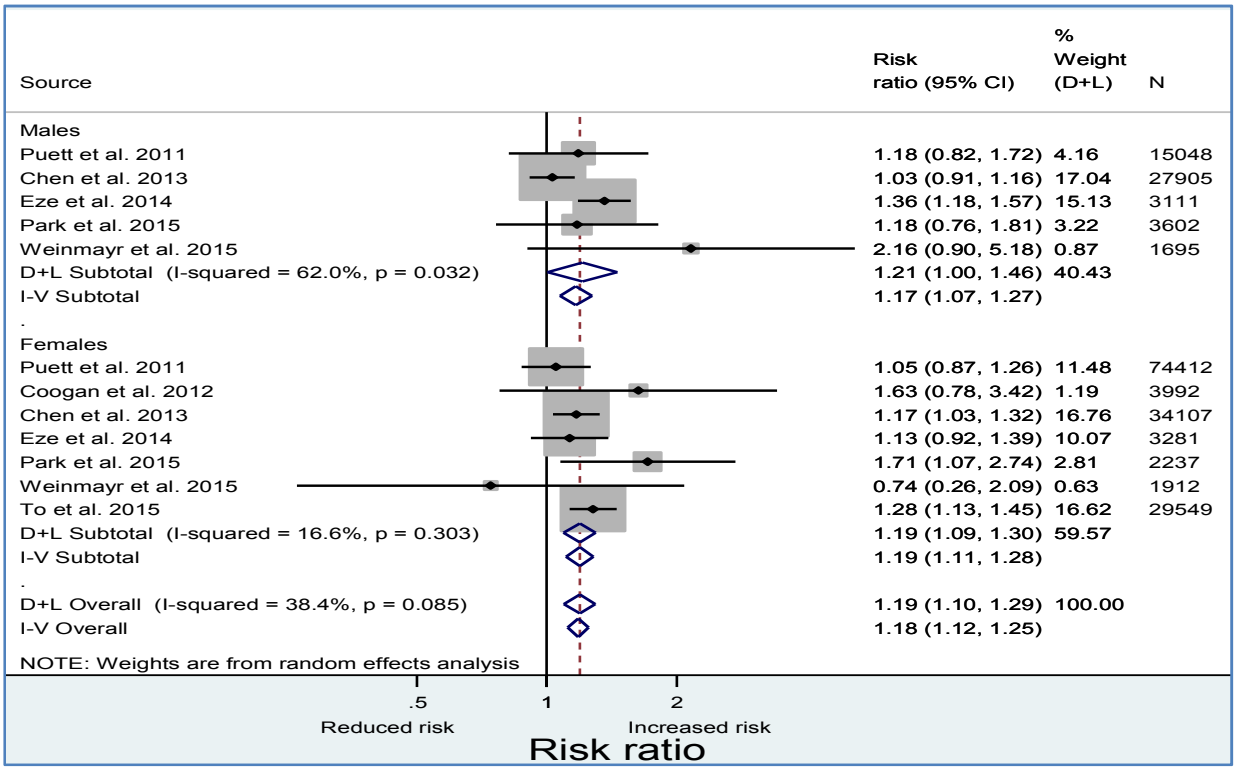
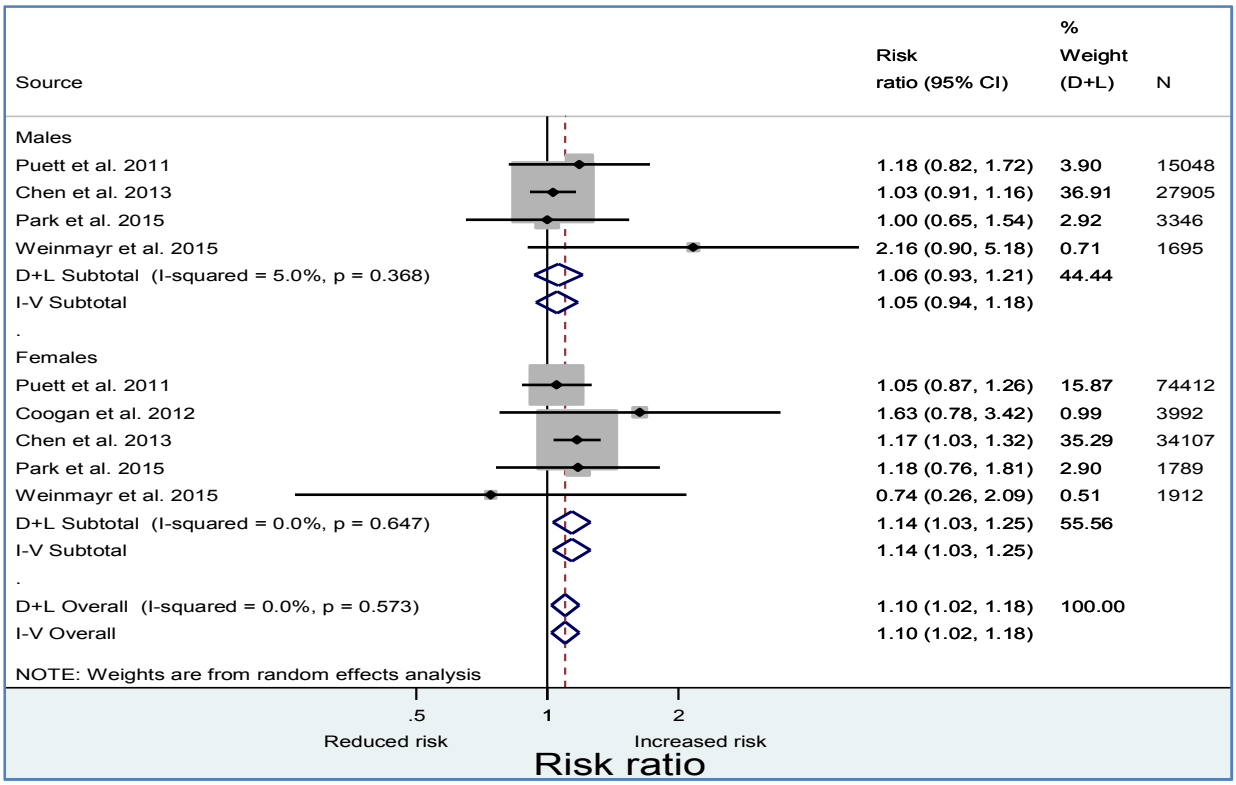
Appendix 1: Updated meta-analysis of $PM_{2.5}$ and risk of T2D.

A. Meta-analysis combining longitudinal and cross-sectional studies

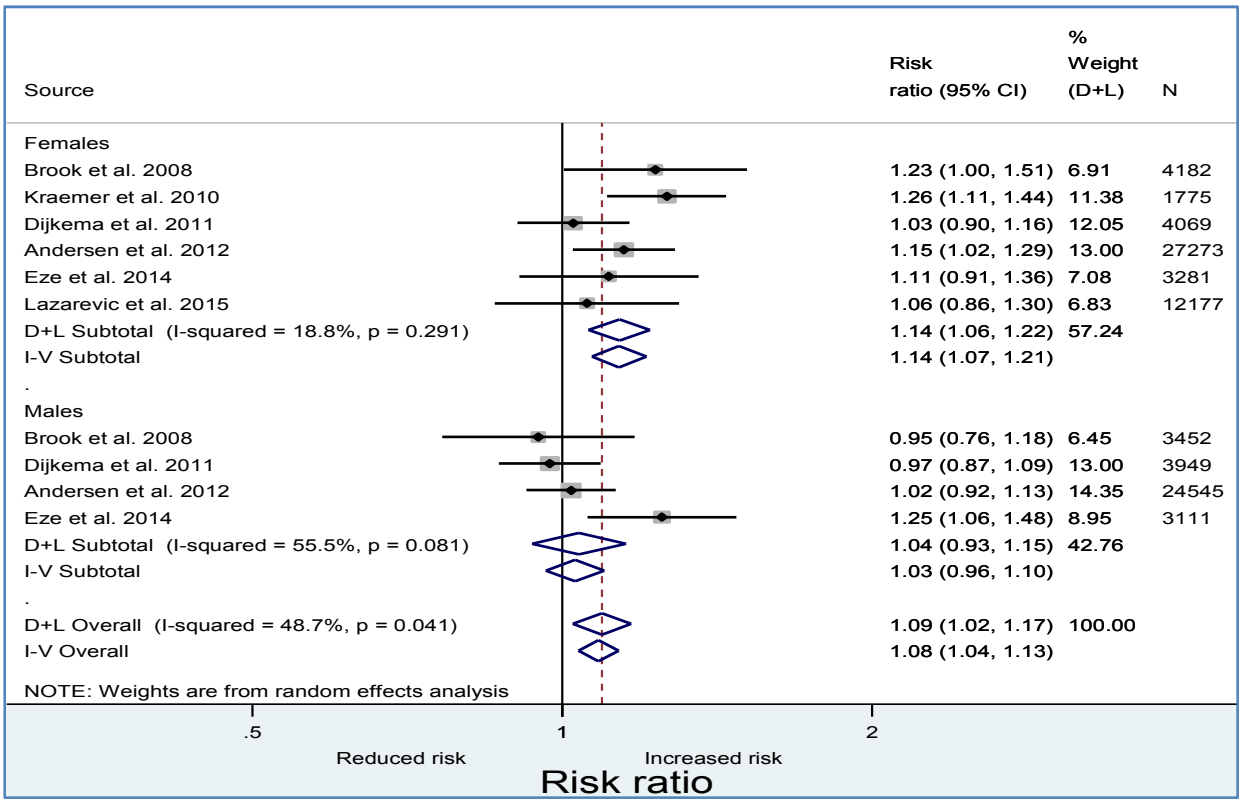


B. Meta-analysis limited to only longitudinal studies

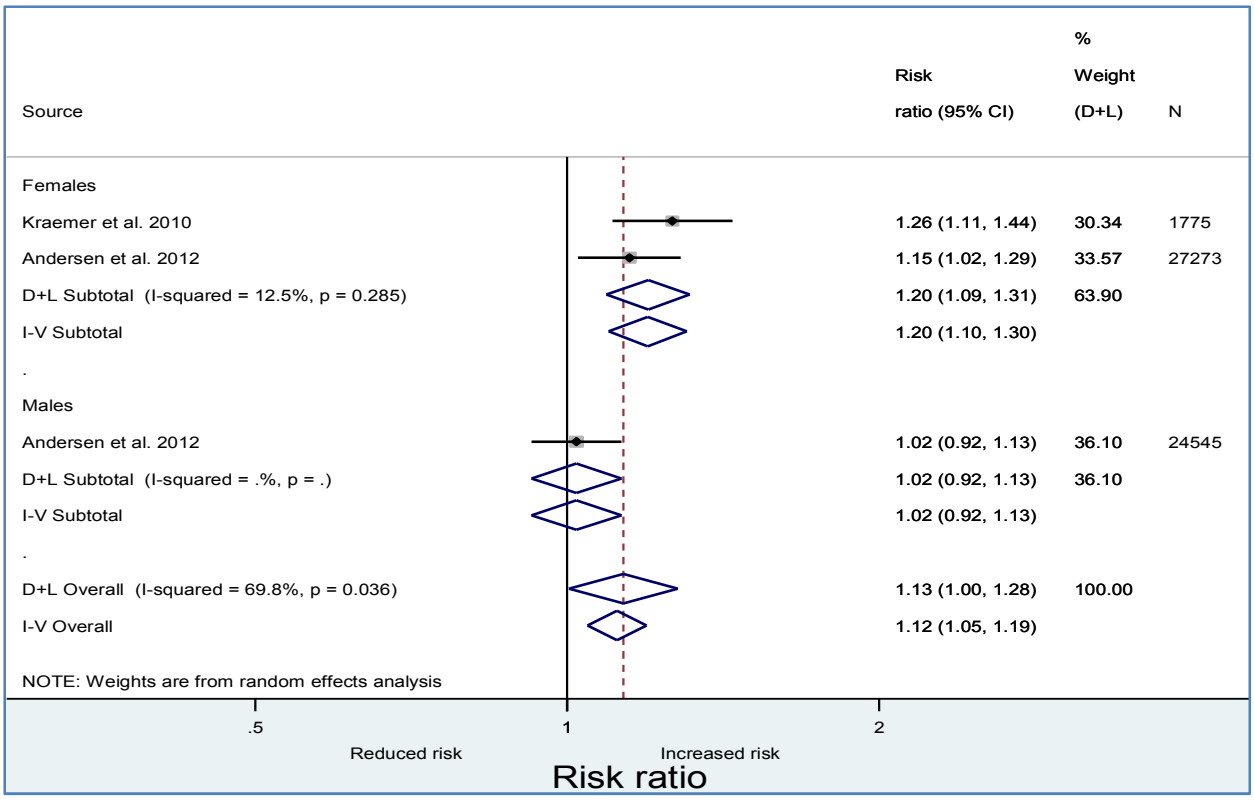


Appendix 2: Updated meta-analysis of NO_2 and risk of T2D.

A. Meta-analysis combining longitudinal and cross-sectional studies



B. Meta-analysis limited to only longitudinal studies



Appendix 3. Short version of SAPALDIA 2 health questionnaire (German)

Question number	Question
	☐ Answers
T_H00010	Haben Sie in den letzten 12 Monaten irgendwann ein pfeifendes Atemgeräusch in der Brust gehabt?
	☐ nein ⟶ *gehen Sie bitte zu Frage T_H00040, S. 1*
	☐ ja
	☐ weiss nicht
	☐ Weigerung
T_H00020	Haben Sie in den letzten 12 Monaten Mühe gehabt mit Atmen, wenn Sie dieses pfeifende Atemgeräusch in der Brust gehabt haben?
	☐ nein
	☐ ja
	☐ weiss nicht
	☐ Weigerung
T_H00030	Haben Sie in den letzten 12 Monaten dieses pfeifende Atemgeräusch gehabt, ohne dass Sie gleichzeitig erkältet waren?
	☐ nein
	☐ ja
	☐ weiss nicht
	☐ Weigerung
T_H00040	Sind Sie in den letzten 12 Monaten irgendwann aufgewacht mit einem Druckgefühl oder Engegefühl in der Brust?
	☐ nein
	☐ ja
	☐ weiss nicht

☐ Weigerung

T_H00050 Haben Sie in den letzten 12 Monaten tagsüber einen Anfall von Atemnot gehabt, wenn Sie ruhig waren? (gemeint ist "in Ruhe")

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00060 Haben Sie in den letzten 12 Monaten einen Anfall von Atemnot nach körperlicher Anstrengung gehabt?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00070 Sind Sie in den letzten 12 Monaten jemals aufgewacht, weil sie einen Anfall von Atemnot gehabt haben?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H00100, S. 2*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00080 Sind Sie in den letzten 3 Monaten durchschnittlich mindestens einmal in der Woche mit einem Anfall von Atemnot aufgewacht?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00100 Sind Sie in den letzten 12 Monaten jemals wegen eines Hustenanfalles aufgewacht?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00110 Husten Sie normalerweise morgens nach dem Aufstehen?

☐ nein ——— *gehen Sie bitte zu Frage T_H00130, S. 2*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00120 In welchen Jahreszeiten husten Sie normalerweise morgens nach dem Aufstehen?

☐ unabhängig von der Jahreszeit

☐ nur im Winter

☐ nur im Frühling, Sommer oder Herbst

☐ weiss nicht

☐ Weigerung

T_H00130 Husten Sie normalerweise tagsüber oder nachts?

☐ nein ⟶ *wenn T_H00110 „nein“* ***und*** *T_H00130 „nein“*

gehen Sie bitte zu Frage T_H00170, S. 3

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00140 Husten Sie so an den meisten Tagen während mindestens 3 Monaten im Jahr?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00150 In welchen Jahreszeiten husten Sie normalerweise tagsüber oder in der Nacht?

☐ unabhängig von der Jahreszeit

☐ nur im Winter

☐ nur im Frühling, Sommer oder Herbst

☐ weiss nicht

☐ Weigerung

T_H00160 Seit wie vielen Jahren?

_____(Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H00170 Haben Sie normalerweise Auswurf morgens nach dem Aufstehen?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H00190, S. 3*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00180 In welchen Jahreszeiten haben Sie normalerweise Auswurf morgens nach dem Aufstehen?

☐ unabhängig von der Jahreszeit

☐ nur im Winter

☐ nur im Frühling, Sommer oder Herbst

☐ weiss nicht

☐ Weigerung

T_H00190 Haben Sie normalerweise tagsüber oder nachts Auswurf?
☐ nein ⟶ *wenn T_H00170 „nein“* ***und*** *T_H00190 „nein“*

gehen Sie bitte zu Frage T_H00310, S. 4

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00200 Haben Sie normalerweise an den meisten Tagen während mindestens 3 Monaten pro Jahr solchen Auswurf?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00210 In welchen Jahreszeiten haben Sie normalerweise tagsüber oder nachts Auswurf?

☐ unabhängig von der Jahreszeit

☐ nur im Winter

☐ nur im Frühling, Sommer oder Herbst

☐ weiss nicht

☐ Weigerung

T_H00220 Seit wie vielen Jahren?

_____(Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H00310 Haben Sie jemals Asthma gehabt?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H00500, S. 5*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00320 Wurde dies von einem Arzt bestätigt?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00370 Haben Sie in den letzten 12 Monaten einen Asthmaanfall gehabt?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H00430, S. 4*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00380 Wie viele Asthmaanfälle haben Sie in den letzten 12 Monaten gehabt?

_____(Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H00390 Wie viele Asthmaanfälle haben Sie in den letzten 3 Monaten gehabt?

_____(Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H00430 Nehmen Sie zur Zeit irgendwelche Medikamente gegen Asthma (auch Inhalationsmittel, Aerosole oder Tabletten)?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00500 Haben Sie allergischen Schnupfen oder Heuschnupfen?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H00520, S. 5*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00640 Haben Sie in diesem Jahr schon Heuschnupfen gehabt?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00520 Hatten Sie jemals Probleme mit Niesen oder mit einer laufenden oder verstopften Nase, ohne erkältet zu sein oder eine Grippe zu haben?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H00730 Haben Sie eine chronische Erkrankung, die Sie in irgendeiner Weise einschränkt?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

Haben Sie etwas von dem Folgenden?

T_H00740 Arthritis

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00741 Hoher Blutdruck

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00742 Schwerhörigkeit

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00743 Krampfadern

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00744 Grauer Star (Linsentrübung)

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00745 Herzkrankheiten

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00746 Depression

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00747 Diabetes/Zuckerkrankheit

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00748 Migräne/oft auftretende

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00749 Krebs (Stellen Sie die Frage so: Haben Sie Krebs gehabt?)

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00750 Schlaganfall

☐ nein

☐ ja, aber nicht vom Arzt diagnostiziert

☐ ja, vom Arzt diagnostiziert

☐ weiss nicht

☐ Weigerung

T_H00880 Mit welchem Alter haben Sie Ihre vollzeitliche Ausbildung abgeschlossen?

(0 entspricht hauptberuflich Student)

_____ (Zahl einfüllen)

☐ weiss nicht

☐ Weigerung

Was machen Sie zur Zeit?

T_H00890 voll erwerbstätig

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00891 teilweise erwerbstätig

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00892 Hausfrau/Hausmann

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00893 in Ausbildung

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00894 pensioniert/Rentner

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00895 arbeitslos

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00896 längerer Militärdienst (z.B. RS), längere Ferien (z.B. nach Schulabschluss oder zwischen zwei Stellen)

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00897 krank oder invalid

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H00898 mache etwas anderes

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H01000 Haben Sie jemals in einem Beruf gearbeitet, bei dem Sie Dampf, Gas, taub oder Rauch ausgesetzt waren?

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H01340 Leben Sie in derselben Wohnung/Haus wie in der letzten Untersuchung?

☐ Nein

☐ Ja

☐ weiss nicht

☐ Weigerung

T_H01720 Welche Aussage beschreibt Ihre Wohnsituation am besten? Ich wohne

☐ im Stadt/Dorfzentrum an stark befahrener Strasse

☐ im Stadt/Dorfzentrum an wenig bis mässig befahrener Strasse

☐ im Aussenquartier/am Dorfrand an mässig bis stark befahrener Strasse

☐ im Aussenquartier/am Dorfrand an wenig befahrener Strasse

☐ in alleinstehenden Haus auf dem Land

☐ weiss nicht

☐ Weigerung

T_H01730 Wie gross ist werktags das Verkehrsaufkommen auf der Strasse, an welcher Sie wohnen?

☐ Stark befahrene Strasse/ununterbrochener Verkehrsfluss

☐ Mässig befahrene Strasse/viele Autos fahren vorbei

☐ Wenig befahrene Strasse/nur ab und zu ein paar Autos

☐ weiss nicht

☐ Weigerung

T_H01740 Wie oft fahren an Wochentagen Lastwagen durch die Strasse, an welcher Sie wohnen?

☐ nie

☐ selten

☐ öfter am Tag

☐ fast den ganzen Tag

☐ weiss nicht

☐ Weigerung

T_H02040 Haben Sie schon einmal mindestens ein Jahr lang geraucht?

(„Ja“ heisst mindestens 20 Zigarettenpackungen oder 360g Tabak im ganzen Leben ODER: mindestens 1 Zigarette pro Tag, oder eine Zigarre pro Woche für ein Jahr).

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02150, S. 10*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02050 In welchem Alter haben Sie angefangen, regelmässig zu rauchen?

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02060 Rauchen Sie zur Zeit (im letzten Monat)?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02105, S. 9*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02070 Wie viel rauchen Sie jetzt im Durchschnitt?

Anzahl Zigaretten pro Tag

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02072 Anzahl Zigarren pro Woche

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02073 Pfeifentabak in Gramm pro Woche

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02105 In welchem Alter haben Sie aufgehört zu rauchen?

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02110 In der gesamten Zeit, in der Sie rauchten, haben Sie durchschnittlich wie viel geraucht?

Anzahl Zigaretten pro Tag

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02150 Sind Sie in den letzten 12 Monaten regelmässig Tabakrauch ausgesetzt gewesen? (regelmässig heisst, an den meisten Tagen oder Nächten)

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02280, S. 10*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02160 Sie selber nicht mitgezählt, wie viele Personen rauchen in Ihrem Haushalt?

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02170 Rauchen an Ihrem Arbeitsplatz andere Personen regelmässig?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02190 Wie viele Stunden sind Sie täglich dem Tabakrauch von anderen Leuten ausgesetzt?

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02280 Haben Sie seit der letzten Untersuchung jemals inhalierbare Glucocorticoide (Kortison) benutzt? (Liste zeigen)

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02390 Haben Sie in den letzten 12 Monaten die Notfallstation eines Spitals aufgesucht wegen Atemproblemen?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02420, S. 11*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02400 War dies wegen Asthma, Mühe mit der Atmung oder wegen des pfeifenden Atemgeräusches?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02410 Wie oft in den letzten 12 Monaten?

_____ (Zahl einfüllen)
☐ weiss nicht

☐ Weigerung

T_H02420 Haben Sie in den letzten 12 Monaten die Notfallstation eines Spitals aufgesucht wegen Herz-Kreislaufproblemen?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02480, S. 11*

☐ ja

☐ weiss nicht

☐ Weigerung

War dies wegen

T_H02430 Angina pectoris

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02431 Herzinfarkt

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02432 Herzrhythmusstörungen

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02480 Haben Sie in den letzten 12 Monaten eine Nacht in einem Spital verbracht wegen Atemproblemen?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02520, S. 12*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02490 War dies wegen Asthma, Mühe mit der Atmung oder pfeifender Atmung?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02520 Haben Sie in den letzten 12 Monaten eine Nacht in einem Spital verbracht wegen Herz-/Kreislaufproblemen?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02590 Sind Sie in den letzten 12 Monaten von einem Arzt untersucht worden wegen Atembeschwerden oder wegen Mühe mit der Atmung?

☐ nein ⟶ *gehen Sie bitte zu Frage T_H02650, S. 12*

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02600 War dies wegen Asthma, wegen Mühe mit der Atmung oder wegen eines pfeifenden Atemgeräusches?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H02650 Sind Sie in den letzten 12 Monaten von einem Arzt untersucht worden wegen Herz-/Kreislaufbeschwerden?

☐ nein

☐ ja

☐ weiss nicht

☐ Weigerung

T_H03030 Wie viele Tage konnten Sie in den letzten 12 Monaten wegen Asthma, wegen Mühe mit der Atmung oder wegen pfeifender Atemgeräusche nicht zur Arbeit gehen?

_____ (Zahl einfüllen)

☐ weiss nicht

☐ Weigerung