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Associations between ambient air pollution and blood markers of inflammation and coagulation/fibrinolysis in susceptible populations

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ABSTRACT

The pathophysiological pathways linking particulate air pollution to cardiovascular disease are still not fully understood. We examined the association between ambient air pollutants and blood markers of inflammation and coagulation/fibrinolysis in three potentially susceptible populations.

Three panels of non-smoking individuals were examined between 3/2007 and 12/2008: 1) with type 2 diabetes mellitus (T2D, n = 83), 2) with impaired glucose tolerance (IGT, n = 104), and 3) with a potential genetic predisposition which could affect detoxifying and inflammatory pathways (n = 87) defined by the null polymorphism for *glutathione S-transferase M1 (GSTM1)* in combination with a certain single nucleotide polymorphism on the *C-reactive protein (CRP)* or the *fibrinogen* gene. Study participants had blood drawn up to seven times every four to six weeks. In total, 1765 blood samples were analysed for CRP, interleukin (IL)-6, soluble CD40 ligand (sCD40L), fibrinogen, myeloperoxidase (MPO), and plasminogen activator inhibitor-1 (PAI-1). Hourly mean values of particulate air pollutants, particle number concentrations in different size ranges and gaseous pollutants were collected at fixed monitoring sites and individual 24 hour averages calculated. Associations between air pollutants and blood markers were analysed for each panel separately and taking the T2D panel and the IGT panel together, using additive mixed models adjusted for long-term time trend and meteorology.

For the panel with potential genetic susceptibility, CRP and MPO increased for most lags, especially with the 5-day average exposure (% change of geometric mean and 95% confidence interval: 22.9% [12.0;34.7] for CRP and 5.0% [0.3;9.9] for MPO per interquartile range of $PM_{2.5}$). Small positive associations were seen for fibrinogen while sCD40L, PAI-1 and IL-6 mostly decreased in association with air pollution concentrations. Except for positive associations for fibrinogen we did not see significant results with the two other panels.

Participants with potential genetic susceptibility showed a clear association between inflammatory blood biomarkers and ambient air pollutants. Our results support the hypothesis that air pollution increases systemic inflammation especially in susceptible populations which may aggravate atherosclerotic diseases and induce multi-organ damage.

Abbreviations: APHEA, Air Pollution and Health, a European Approach; BC, black carbon; BMI, body mass index; CHD, coronary heart disease; CO, carbon monoxide; CRP, C-reactive protein; DE, diesel exhaust; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GSTM1, glutathione S-transferase M1; Hs, high sensitivity; IL-6, interleukin 6; IGT, impaired glucose tolerance; KORA studies, Cooperative Health Research in the Region Augsburg; MPO, myeloperoxidase; NC, number concentration; NO, nitric oxide; NO_2, nitrogen dioxide; OGTT, oral glucose tolerance test; PAI-1, plasminogen activator inhibitor-1; $PM_{2.5}$, particulate matter (mass) with a size range of <2.5 μm in aerodynamic diameter; PM_{10}, particulate matter (mass) with a size range of <10 μm in aerodynamic diameter; PNC, particle number concentration; REVIHAAP, Review of evidence on health aspects of air pollution; sCD40L, soluble CD40 ligand; SNP, single nucleotide polymorphism; SOA, secondary organic aerosols; sP-selectin, soluble platelet selectin; T2D, type 2 diabetes; UFP, ultrafine particles, particle number concentration, with a size range of <0.1 μm in aerodynamic diameter; WHO, World Health Organisation.

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1. Introduction

Epidemiological studies show that air pollutants have adverse effects on human health (Ruckerl et al., 2011) including the cardiovascular system (Brook et al., 2010). Also, several studies have identified human subgroups that are more susceptible to the harmful effects of particulate air pollution than the general population. They show that the obese, the elderly and patients with diseases such as chronic obstructive pulmonary disease, previous myocardial infarction or diabetes have a heightened risk of experiencing an acute exacerbation of their diseases compared with the general population at the same air pollution concentration (Brook et al., 2010).

Recent studies have drawn special attention to the adverse effects of ambient air pollution on patients with type 2 diabetes (T2D) who seem to be particularly sensitive to pollution-triggered cardiovascular events (Dubowsky et al., 2006; O'Neill et al., 2005, 2007; Pearson et al., 2010; Schneider et al., 2010, 2011; Zanobetti and Schwartz, 2002). One hypothesis is that the same biological mechanisms that link air pollution and atherogenesis promote the development of T2D. Insulin resistance, the main biological pathway that causes T2D, is triggered by oxidative stress and pro-inflammatory mediators. Thus, T2D and the vascular effects of PM may share common pathways and interact to enhance responsiveness of diabetic patients to air pollutants (Brook et al., 2008). Bhatnagar (2009) therefore suggested that more effort be put into studies investigating the link between air pollution and diabetes in human populations. In a recent review Liu et al. (2013) concluded that the evidence from epidemiological studies, animal and toxicological experiments supports the hypothesis that inflammation caused by environmental factors is the key pathway which explains the recent increase in metabolic disorders such as T2D. For the study described here, we not only included participants with T2D, but also participants with impaired glucose tolerance (IGT), a prediabetic stage. These participants have an enhanced risk of T2D, yet they are still typically not on hypoglycaemic drug treatment.

This second group was selected as some medications may protect patients from the adverse effects of ambient air pollution. In a European study on myocardial infarction survivors, for example, we found no association between air pollutants and high sensitivity (hs) C-reactive protein (CRP), possibly due to the high intake of statins in up to 89% of the study population (Ruckerl et al., 2007a).

Other studies further indicate that people with unfavourable genotypes related to oxidative stress react more strongly to ambient air pollution (Baja et al., 2010; Chahine et al., 2007; Park et al., 2006). Subjects with a deletion on the *GSTM1* gene, for example, have more difficulties coping with oxidative stress and may therefore be more responsive to agents that increase oxidative stress such as ambient particles. To follow up on the idea of oxidative stress as a possible pathway linking air pollution and cardiovascular disease, we chose subjects with potential genetic predisposition on detoxifying and inflammatory pathways but without manifest diabetes/IGT. In addition to a *GSTM1* deletion, participants with two major alleles of the re1205-SNP on the *CRP* gene were selected as a strong association with the blood CRP concentration was demonstrated in a previous European-wide study showing a negative association for at least one minor allele (Kolz et al., 2008). Moreover, this SNP has been associated with worse lung function in the same population (Sunyer et al., 2008), indicating a possible link to air pollution. The SNP rs1800790 on the β-chain of the *fibrinogen* gene was found to affect blood fibrinogen levels, with clear positive associations between having at least one minor allele and plasma fibrinogen levels (Jacquemin et al., 2008). We chose participants with at least one minor allele on this SNP. In addition, an adverse relationship with particulate matter has been shown with this SNP (Peters et al., 2009).

Potential pathways linking air pollution and cardiovascular diseases have been established by looking at blood markers of inflammation and coagulation/fibrinolysis in association with air pollution. Associations of such blood markers and air pollution have been examined intensively, but results are not always coherent (Ruckerl et al., 2011). Overall, however, most of these studies indicate that air pollutants can induce oxidative stress and inflammation, as well as prothrombotic responses by vascular endothelial cells and platelets with expression of inflammatory cytokines, cellular adhesion molecules and coagulation factors. It is therefore of interest to examine the effects of particulate air pollution on blood markers, especially in susceptible subgroups.

Depending on the blood marker, an acute-phase response which involves a de novo synthesis in the liver takes about two (e.g. for CRP) and up to ten days (e.g. for fibrinogen) to reach its peak (Gabay and Kushner, 1999), so delayed effects are common for some acute phase proteins. As IL-6 is the chief stimulator of the production of acute phase proteins (Heinrich et al., 1990) and has a very short half-time of less than 6 h (Ridker et al., 2000) one would expect a short lag time. And indeed, IL-6 has been shown to respond to air pollutants within hours in some epidemiological studies (Ruckerl et al., 2007a; Tsai et al., 2003) and in some more experimentally focused studies (Hilt et al., 2002; Krishnan et al., 2013; Swiston et al., 2008). However, epidemiological panel studies generally showed more delayed effects from lag 1 up to lag 9 (Delfino et al., 2008, 2009; Schneider et al., 2010; Thompson et al., 2010). We therefore expected responses in particular for ambient air pollutant concentrations averaged over the 24 h and the five days before blood withdrawal, depending on the blood biomarker.

2. Materials and methods

2.1. Study design and study population

In a prospective panel study in Augsburg, Germany, individuals with T2D or IGT and individuals without T2D or IGT but with a potential genetic predisposition on detoxifying pathways were recruited from a large ongoing study (KORA — Cooperative Health Research in the Region of Augsburg). Between March 19th 2007 and December 17th 2008, participants were invited to take part in up to seven examinations scheduled every four to six weeks on the same weekday and the same time of the day. Exclusion criteria were current smoking (participants had to be non-smokers or ex-smokers for at least twelve months), ntake of anti-platelet drugs except for acetylsalicylic acid, myocardial infarction (MI) and/or interventional procedures (e.g. percutaneous transluminal coronary angioplasty, bypass surgery) less than six months before the start of the study and a chronic inflammatory disease such as Crohn's disease or ulcerative colitis.

An oral glucose tolerance test (OGTT) was performed on participants who had not been identified as having T2D either by a physician or by typical medication use. Participants with a fasting glucose level >125 mg/dl or a 2 h OGTT glucose level ≥200 mg/dl were defined as having T2D and included in the first panel in addition to those with a physician's diagnosis or respective medication intake. IGT was specified as 2 h OGTT glucose levels ≥140 mg/dl but <200 mg/dl and participants who met these criteria were included in the second panel.

Participants without T2D or IGT but with the null polymorphism for *glutathione S-transferase M1* (*GSTM1*) and either of two major alleles of the single nucleotide polymorphism (SNP) rs1205 located on the *CRP* gene (n = 41) or at least one minor allele of the SNP rs1800790 located on the *fibrinogen* gene *FGB* (n = 29) were included in the third panel. Sixteen participants had both polymorphisms.

Written informed consent was given by all participants. The study protocol was approved by the local Ethics Committee ("Bayerische Landesaerztekammer").

2.2. Clinical measurements

At the first visit, data on health status, medication use, disease and smoking history was collected. At each visit a short questionnaire on health status was given and subsequently venous blood samples were drawn (Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged