Relationship between air pollution exposure and systemic inflammation in Canada

by

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Abstract

Research has shown that plausible links between air pollution exposure and both atherosclerosis and diabetes may exist through systemic inflammation. This present study quantified the association between particulate matter less than 2.5 μm and nitrogen dioxide with four biomarkers of inflammation (C-reactive protein, fibrinogen, white blood cells, and platelets) in a cross-sectional sample representative of the Canadian population aged between 18 and 79 (N=6322) from cycle 1 and 2 (2007-2012) of the Canadian Health Measures Survey. After adjusting for race, household income and temperature, results showed that daily and annual nitrogen dioxide (NO_2) was inversely associated with fibrinogen and the associations were slightly stronger among those taking statins, although not clinically significant. Although our results did not support our hypothesis, our findings raise new questions about other possible health effects behind the association between NO_2 exposure and fibrinogen.

Keywords: Canada; Air Pollution; Systemic Inflammation; Biomarkers; Environmental Exposure; Environmental Health

To my sweet mother and my beautiful son,
for their unconditional love and support throughout
this adventure. All along, you both walked beside me
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List of Acronyms

ATC	Anatomical Therapeutic Chemical
BRR	Balance Repeated Replication
CHMS	Canadian Health Measures Survey
CRP	C-Reactive Protein
DAG	Directed Acyclic Graph
MEC	Mobile Examination Center
NAPS	National Air Pollutant Surveillance
NO_2	Nitrogen Dioxide
$PM_{2.5}$	Particulate Matter less or equal of 2.5 μm
WBC	White Blood Cell
WHO	World Health Organization

Chapter 1.

Background, methods and rationale

1.1. Context for the proposed research and overview

Systemic inflammation is a major risk factor for development of diabetes and atherosclerosis (Libby, 2002; Wellen & Hotamisligil, 2005). These inflammatory diseases are increasing health problems in the developed as well as developing countries (Centre for Disease Control and Prevention (CDC), 2010; Weber & Noels, 2011) so research to identify the causes of these diseases is important. Diabetes is one of the risk factors for atherosclerosis while atherosclerosis is a risk factor for coronary heart disease and a leading cause of cardiovascular deaths (Watson, Harmel, & Matson, 2003; Weber & Noels, 2011). It was estimated that 285 million people worldwide suffered from diabetes in 2010 and it is predicted that 438 million people will have the disease by 2030 (CDC, 2010) while 16.7 million people worldwide died of cardiovascular complications in 2010 (Poole-Wilson, 2007). In Canada, 2.4 million people were living with diabetes in 2008/09, but it is predicted that 3.7 million could suffer from the disease by 2018/19 (Public Health Agency of Canada (PHAC), 2012) while in the United States as many as 1 in 5 to 1 in 3 adults could have diabetes by 2050 compared to 1 in 10 in 2010 (CDC, 2010). Atherosclerosis in its various forms causes one in five deaths in Canada (Pfizer, n.d.). It is difficult to project the costs of these diseases to the Canadian health care system, but for example, for treating diabetes as the population ages, when comparing a population with the disease to a population without it, the annual per capita health care costs are estimated to be 3 to 4 times higher (PHAC, 2010). Although diet, smoking and exercise are known major determinants of these diseases, the impacts of environmental pollution on them have received less research attention. If environmental pollutants increase the risks of diabetes and atherosclerosis, environmental policies could be used to reduce current and future burdens of these diseases.

A plausible link between air pollution and diabetes and atherosclerosis exists through the mechanism of systemic inflammation. A growing body of literature has linked air pollution to induction and maintenance of systemic inflammation in animals and humans (Frampton, 2006; Gomez-Mejiba et al., 2009; Hertel et al., 2010). Although the mechanisms are still being investigated, current knowledge suggests that air pollutants, such as fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2), cause irritation and inflammation in the alveolar space of the lung, which then leads to the release of inflammatory mediators, which increases the amount of inflamed cells in vascular circulation, leading to an acute (and possible chronic) systemic inflammatory state (Brook, Brook, & Rajagopalan, 2003; Gomez-Mejiba et al., 2009). Although levels of air pollution in Canada are mostly meeting the guideline levels set by the World Health Organization (2005), their report states that $PM_{2.5}$ exposure can have adverse health effects in urban settings even when these levels are barely above the background concentration (i.e. background from non-anthropogenic sources) and that NO_2 has been associated with adverse health effects at the guideline levels set by the WHO (WHO, 2005). This report not withstanding, other evidence seems to suggest that there is no threshold for the effects of air pollution and that negative health effects occur at concentrations below the WHO guideline concentrations (Brook et al., 2004, 2010; Dominici, Daniels, Zeger, & Samet, 2002; Pope, Brook, Burnett, & Dockery, 2010; Pope, 2000; Pope, C.A., & Dockery, 2006; Vedal, Brauer, White, & Petkau, 2003).

Thus the aim of this study was to investigate whether air pollution exposure of Canadian adults living in cities was associated with levels of inflammation. I hypothesized that in cities, daily and annual exposures of $PM_{2.5}$ or NO_2 would be positively associated with levels of inflammation indicated by four biomarkers of inflammation: C-reactive protein (CRP), fibrinogen, total count of white blood cells (WBC) and platelets. I also hypothesized that the daily and annual effects of $PM_{2.5}$ or NO_2 on inflammation would decrease the associations with the use of statins.

In this chapter, I will demonstrate the plausibility of these hypotheses and introduce the framework for the study. Specifically, I will first give background information on $PM_{2.5}$ and NO_2. Next, I will provide background information on inflammation. After that, I will introduce the four biomarkers of systemic inflammation used in this study. Then, I will describe the links between $PM_{2.5}$, NO_2 and systemic inflammation including previous studies that investigated these

associations. Finally, I will discuss what statins are, how they might provide a protective anti-inflammatory effect after air pollution exposure, and previous studies that have investigated this hypothesis.

1.2. Particulate Matter ($PM_{2.5}$) and Nitrogen Dioxide (NO_2)

Two pollutants, $PM_{2.5}$ and NO_2 are important air pollutants known for their effects on the inflammatory process (WHO, 2005). Particulate matter air pollution is an air-suspended mixture of organic and inorganic substances with both natural and anthropogenic sources (Anderson, Thundiyil, & Stolbach, 2012; WHO, 2003). The particles are solid and liquid and they vary in number, size, shape, surface area, chemical composition, and solubility (Pope, C.A., & Dockery, 2006).

The particles are classified based on their "aerodynamic equivalent diameter" (AED), which describes the particles' settling velocity (Anderson et al., 2012). They are subdivided based on their sources, toxicity, route of inhalation (oral or nasal) and area of deposition in the airways (Pope, C.A., & Dockery, 2006). The coarse particles, which measure between 2.5 and 10 μm, have their origins mainly from dust, soil, windstorms, and volcanoes as well as pollen, mold, and spores (Pope, C.A., & Dockery, 2006) and in normal individuals are generally easily filtered out by the nose and upper airway (Anderson et al., 2012). The fine particles, which are less than 2.5 μm in aerodynamic equivalent diameter, have been investigated extensively in epidemiological studies. They are believed to have harmful health effects because associations have been found between fine particulate matter and various health outcomes (Brook, 2008; Perrone, Gualtieri, & Consonni, 2013; Pope, C.A., & Dockery, 2006). These particles have the ability to penetrate into the alveoli deep in the lung (Perrone et al., 2013). Combustion processes, such as in car engines, wood burning, smelters, and mills are primary origins of these particles. The ultrafine particles, which are less than 0.1 μm, are found in largest number (WHO, 2003) and exposures primarily occur within a few hundred meters of the source, usually roads (Brook, 2008). Like the fine particles, their sources are primarily combustion-related and depend on photochemical reactions (Pope, C.A., & Dockery, 2006). Toxicological and physiological studies have suggested that fine particulate matter ($PM_{2.5}$) may have the greatest influence on human health (WHO, 2005). The reason is that $PM_{2.5}$ may be more toxic is partly due to their chemical composition, but also, that these particles can also be inhaled deeper into

the lungs, can remain suspended for longer periods of time, can penetrate more readily into indoor environments and can be transported over much longer distances (WHO, 2005).

When exposed to air, nitric oxide can be transformed to NO_2. This gas is easy to recognize because it is reddish brown and has a pungent odour (WHO, 2005). Because nitrogen dioxide exists as a gas, the only route of exposure is by inhalation whether it comes from indoor or outdoor sources (WHO, 2005). Although natural sources of nitrogen dioxide emission are more numerous than for anthropogenic emissions, their background concentrations are very minimal (WHO, 2005). The major sources of emission are anthropogenic fossil fuel combustion from both stationary and mobile sources, for example vehicles and ship engine emissions as well as appliances such as wood stoves and gas-fired appliances (Hesterberg et al., 2009). NO_2 is also a precursor for a number of harmful secondary air pollutants, like ozone, which happens through photochemical reaction (WHO, 2005). Although NO_2 is often seen as a surrogate measure for traffic emissions generally as well as a surrogate for one or more infrequently measured hazardous air pollutants due to its photochemical properties (Brook, 2008), this study is interested in the health effects of NO_2 specifically and not its effects as a traffic-related air pollutant.

In Canada, the Air Quality Management System (AQMS) is used to guide work on air emissions across the country. Part of the AQMS is the Canadian Ambient Air Quality Standards (CAAQS), which set the standards for fine particulate matter and ozone. The 2015 standards for $PM_{2.5}$ are 10 $\mu g/m^3$ (annual average) and 28 $\mu g/m^3$ (24-hour) (CCME, 2014). The CAAQS are now working on developing standards for NO_2. In the absence of these standards, the WHO's guidelines for NO_2 annual mean are 40 $\mu g/m^3$ or 0.02 ppm or 20 ppb and for hourly mean, 200 $\mu g/m^3$ or 0.22 ppm or 220 ppb (WHO, 2005).

1.3. Inflammation: definitions and background.

Inflammation is the body's natural defense to a threat or an injury. The inflammatory response usually starts in the barrier organs, at the epithelium in the skin, lungs, eyes and mouth as well as the gastrointestinal tract because they are the most exposed to environmental stressors (Ehlers & Kaufmann, 2010). These are examples of a localized type of inflammation, which is distinguished from systemic inflammation that is a more widespread an involves

multiple tissues and organs (Medzhitov, 2008). Inflammation is also distinguished by whether it lasts a short (acute) or long (chronic) time. An injury or an infection leads to acute inflammation. If the acute inflammatory state caused by an infection or injury fails to resolve, the inflammatory process can go on to show new characteristics consistent with chronic inflammation (Medzhitov, 2008; Roy, Bagchi, & Raychaudhuri, 2012). A chronic inflammatory state is usually characterized by the presence of macrophages, lymphocytes and plasma cells in the extravascular tissues (Frampton, 2006; Medzhitov, 2008).

The inflammatory process due to infections and injury is far better understood than systemic chronic inflammation in inflammatory diseases (Medzhitov, 2008). Although each chronic disease has its own inflammatory pathways, a generic model can be summarized (Libby, 2007). When the cells of an organ are injured, there is migration of several blood cell types into extravascular tissues, such as leucocytes and pro-inflammatory mediators. Once the blood cells are at the injured site, there is activation and interaction between the cells of the tissue; the leukocytes cross the vascular membrane. Signals are sent back and forth between pro-inflammatory molecules and lymphocytes, and macrophages arrive on site (Serhan, Ward, & Gilroy, 2010). Further on, I will explain the inflammatory process due to $PM_{2.5}$ and NO_2 exposure.

In this study, I will be focusing on systemic inflammation, which we define as an inflammatory state throughout the body characterized by the presence of biomarkers of inflammation which may be followed by an acute or a chronic inflammatory process, rather than localized inflammation even though I hypothesize that systemic inflammation due to $PM_{2.5}$ and NO_2 exposures would have its genesis in more localized inflammation of the lungs (see section 1.5). This specific focus is necessitated by a lack of useful biomarkers specific to lung inflammation (see section 1.4). Nonetheless, systemic inflammation is a meaningful outcome for this study because it is systemic inflammation that would indicate an exacerbation of exposure-induced lung inflammation sufficient to increase the risks of outcomes that are downstream of inflammation such as diabetes and atherosclerosis.

1.4. Biomarkers of systemic inflammation in this study.

It is possible to quantify systemic inflammation using factors (biomarkers) that are found in circulation during inflammation. In this study, I will use four of them. These biomarkers have been chosen to measure systemic inflammation because they are theoretically present as a consequence of systematic inflammatory response, have plausible biological roles in the inflammatory response due to exposure to $PM_{2.5}$ and NO_2 (see section 1.5) and are available in the Canadian Health Measures Survey (CHMS). Here I present brief overviews of each.

1.4.1. C-Reactive Protein (CRP) and Fibrinogen.

CRP is an acute-phase protein (or acute-phase reactant) that changes blood concentration during inflammation (Li, Rittenhouse-Olson, Scheider, & Mu, 2012). CRP is expressed primarily in the liver and adipose tissue in response to inflammatory cytokines (predominantly IL-6) and is released into circulation where it signals a inflammatory state (Ahmed, Jadhav, Hassan, & Meng, 2012). An increase of CRP levels can be detected 3-4 hours after injury or infection, but levels usually peak after 12 hours. For detecting systemic inflammation due to injury or infection, the best measurement is between 24 and 72 hours after injury or infection (Sandhu, Petroni, & George, 2005). CRP is a sensitive, but not a specific, indicator of systemic inflammation. A limitation of CRP as an indicator, though, is that it cannot distinguish between acute and chronic inflammation, inflammation due to injury or disease, or systemic inflammation generating from specific tissues or organs (Ho, 2009).

CRP has several qualities that are good for research purposes. It has long-term stability in stored biological specimens, a long half-life (around 19 hrs) in blood (which limits variability over time), a stable daily concentration in the absence of inflammation, and concentrations independent from age, sex or fasting status (Ho, 2009) and over the past decade can be measured at very low concentrations in serum. It is important to note, though, that some drugs, including statins, as well as physical activity and moderate alcohol consumption can decrease CRP levels whereas estrogen and oral contraceptives can increase them (Sandhu et al., 2005). These interactions need to be considered when developing models using CRP as the outcome.

CRP can be measured in plasma or serum but, in the CHMS, it is measured in serum. The diagnostic test to measure low levels of CRP is called high-sensitivity CRP (hs-CRP) and

can quantify concentrations as low as 0.04 mg/L (Statistics Canada, 2012). When using CRP levels to construct a dichotomous measure of "inflammation," hs-CRP levels below 0.01 mg/dl (1.0 mg/L) indicate no sign of inflammation while levels above 1.0 mg/L are a sign of systemic inflammation and/or injury (Sandhu et al., 2005). If hs-CRP level is higher than 3.0mg/L, a high risk of cardiovascular disease is indicated clinically (Sandhu et al., 2005). CRP can also be used as a continuous measure in research.

Fibrinogen is also an acute-phase protein (or acute reactant) that changes blood concentration during inflammation (Davalos & Akassoglou, 2012; Li et al., 2012). Fibrinogen is expressed primarily in the liver in response to inflammatory cytokines (especially IL-6) and glucocorticoids (Herrick, Blanc-Brude, Gray, & Laurent, 1999) and is released into circulation where it has roles in blood coagulation and inflammation (Davalos & Akassoglou, 2012). Fibrinogen levels increase several fold a few hours after injury or infection (Davalos & Akassoglou, 2012) but levels can take a few days before reaching a peak (Gabay & Kushner, 1999). Like CRP, fibrinogen is a sensitive and specific indicator of systemic inflammation with corresponding limitations (Ahmed et al., 2012). Interpretation of fibrinogen elevated levels has been found to be complicated (Kamath & Lip, 2003) but elevated levels have been associated with adverse outcome of acute coronary syndrome (Gil, Zarębiński, & Adamus, 2002).

Fibrinogen has some qualities that are good for research purposes. Its half-life in blood is about 4 days (Davalos & Akassoglou, 2012) which limits variability over time and concentrations can be quantified as low as 0.4 g/L (Health Canada, 2009). Environmental and genetic factors can influence fibrinogen levels as can many pharmaceuticals. Studies have found that fibrinogen concentration increases with body mass index, diabetes, and smoking but decreases with alcohol, and regular exercise while findings about the influence of hormone replacement are ambiguous (Kamath & Lip, 2003). Furthermore, it seems that seasonal variation affects plasma fibrinogen levels with a peak in winter but most importantly, studies have found that 20 to 51% of variation in plasma fibrinogen levels are due to genetic differences (Kamath & Lip, 2003).

Fibrinogen can be measured in plasma (but not serum) because it is a blood clotting factor. In healthy adults, plasma concentrations of fibrinogen vary from 2 to 4 g/L (Davalos & Akassoglou, 2012). In the CHMS, the method used to quantify fibrinogen levels in plasma was

based on the Clauss method, which converts the protein into fibrin and measures the clotting time of the diluted plasma, which is inversely proportional to the fibrinogen concentration (Health Canada, 2009). There is no accepted threshold for fibrinogen. Finally, fibrinogen can also be used as a continuous measure for research purposes.

Both CRP and fibrinogen are theoretically plausible biomarkers of systemic inflammation that is a consequence of air pollution exposure. It is hypothesized that after inhalation of air pollution particles, endothelial cells signal the production of cytokines, like IL-6 and IL-1β, which in turn signal the liver to start the synthesis of CRP and fibrinogen which will result in increased concentrations in blood by several fold (Davalos & Akassoglou, 2012; Pepys & Hirschfield, 2003). CRP has been widely used in risk assessment of cardiovascular disease (Pearson et al., 2003; Sandhu et al., 2005; Zakynthinos & Pappa, 2009) while fibrinogen has been used for prediction of atherosclerosis due to its impact on clot formation (Kampoli, Tousoulis, Antoniades, Siasos, & Stefanadis, 2009) but previous air pollution studies have used fibrinogen as a measure of inflammation in association with exposure (Elvidge, Matthews, Gregory, & Hoogendoorn, 2013; Li et al., 2012).

1.4.2. White blood cells (WBC) and Platelets.

My choice of WBC, also known as leucocytes, and platelets as biomarkers of systemic inflammation are based on their roles during inflammation. They are known to be produced and released by the bone marrow in response to inflammatory cytokine signalling (van Eeden & Hogg, 2002; van Eeden, Leipsic, Paul Man, & Sin, 2012). I hypothesize that similar to the process of cytokine signalling to the liver and expression of CRP and fibrinogen, WBC and platelets will be released into circulation due to cytokine signalling from endothelial cells after exposure to $PM_{2.5}$ and NO_2 (Poursafa & Kelishadi, 2010; Steenhof et al., 2014).

WBC are composed of five different cells: neutrophils, lymphocytes, monocytes, eosinophils and basophils (Cavenagh, 2007). Together they are our first line of defense against pathogens but also they remove damaged cells, toxins and other waste products (Ashton, 2010). Although some of the cells have the same functions, they also have their specific roles, and half-life during inflammation (Minors, 2004) which make it more difficult to interpret conclusions when using total WBC count as a biomarker. Like CRP, white blood cell count is a

sensitive and specific indicator of systemic inflammation, but as an indicator of inflammation, it cannot differentiate the underlying cause of inflammation.

Platelets, or thrombocytes, are small cell fragments that originate from the megakaryocytes in the bone marrow (O'Sullivan & Michelson, 2006), and their activation is responsible for the formation of clots when bleeding as well as regulating the process of inflammatory response (Ioannou, Kannan, & Tsokos, 2013) The sensitivity and specificity of platelets as a marker of systemic inflammation varies depending on the inflammatory process.

For research purposes, complete blood count (CBC), which include WBC and platelets level, is a common and inexpensive test to conduct, it does not require fasting, and many assays are available (Pearson et al., 2003). WBC and platelets levels have been found to increase with smoking and BMI (Huo & Ley, 2004; Madjid, Awan, Willerson, & Casscells, 2004).

WBC and platelets can be measured in whole blood. In the CHMS, the method used to quantify white blood cell and platelets levels was the Coulter method. Elevated levels in WBC count can be a sign of infection, inflammation, an immune system response or a blood disease (Ahmed et al., 2012) while, in platelets, it can be a sign of development of atherosclerosis (McNicol & Israels, 2008; Poursafa & Kelishadi, 2010). Normally, in a healthy individual, the WBC count ranges from 4,500 to 11,000 cells per microlitre of blood (Abbas & Lichtman, 2005) and the platelets count is around 150-400 $x10^{-9}$ platelets per liter of blood (Ioannou et al., 2013). There is no accepted threshold for WBC or platelets. Both can also be used as a continuous measure for research purposes.

1.5. Links between $PM_{2.5}$, NO_2 and systemic inflammation.

1.5.1. Lung Inflammation can plausibly lead to systemic inflammation.

The lung is the largest organ in the body that is unprotected from the environment as well as the one that has the most blood vessels going through it. Systemic inflammation due to air pollution is thought to start in the lung (Gomez-Mejiba et al., 2009). When environmental stressors, such as $PM_{2.5}$ or NO_2, are inhaled and pass the cilia in the nose, they reach the lung and make their way down to the alveolar space, which causes lung irritation and inflammation

(Brook et al., 2003). The irritation triggers the epithelial cells, which in turn activate pro-inflammatory mediators (cytokines) such as IL-6, TNF-α, and IL-1β. These pro-inflammatory cytokines can cross to the vascular system changing the epithelial permeability. The induction of chemokines and cytokines will, in turn, attract to the airways, inflammatory cells such as leucocytes, which act on the opening of endothelial cell tight junctions. The increased numbers of inflammatory cells augment the migration of neutrophils, which remove pathogens through phagocytosis and degranulation and, secondarily the monocytes, which after a few days become macrophages in the lung (Gomez-Mejiba et al., 2009). Most importantly, van Eeden & Hogg (2002) found that alveolar macrophages, when exposed to particulate matter, had a stronger effect on the production of pro-inflammatory response than the epithelial cells. With time, direct structural damage, either reversible or irreversible, will happen to the endothelial barrier. Resolution, repair and sometimes remodelling will happen if and when leucocytes are removed by the lymphocytes, by apoptosis or by the macrophages. However, resolution also depends on which cells were destroyed because enhanced levels of neutrophils and other cells associated with inflammation could be replaced by fibrous, collagenous scars (Serhan et al., 2010).

1.5.2. $PM_{2.5}$ exposure can plausibly lead to systemic inflammation.

The mechanisms by which irritation, inflammation and damage in the lungs due to $PM_{2.5}$ exposure could lead to more widespread systemic inflammation are not well understood (Tetrault (2004) cited in Mejiba et al. (2009)), but epidemiological and toxicological research suggest it is plausible. In a review, Brook et al., (2010) summarised that when particulate matter is inhaled, there are three different pathways that can mediate effects on the cardiovascular system. The first is through the autonomic nervous system. The translocation of particles along the olfactory nerve into the olfactory bulb lead the particles to interact with the lung receptors, which then send messages to the brain and create an imbalance in the autonomic nervous system (ANS) throughout the body. The second pathway is the translocation of particulate matter into the vascular system, which creates a systemic spill-over that produces a state of systemic oxidative stress and inflammation throughout the body. In the third pathway, the biological mechanism that this study is based on, the particles travel into the lung cells where there is a release of proinflammatory mediators from the lung endothelial cells. Actually, these pathways do not happen independently from each other; there is much overlap and interaction

between them (Pope et al., 2010). Li et al. (2012) conducted a systematic literature review of 44 epidemiologic studies looking at the change of CRP levels after particulate matter exposure. They found that the association between particulate matter exposure and CRP levels in humans need further studies to "better quantify the magnitude of CRP level changes in response to particulate matter". Their review took into account cross-sectional studies, longitudinal studies as well as randomized trials and included ultrafine, fine and coarse particle matter. Different populations were part of the studies: children, occupational population, healthy adults and adults suffering from cardiovascular diseases, or metabolic syndrome. From this review, the authors found that children were more susceptible to higher CRP levels after exposure to particulate matter exposure and that healthy adults with higher levels of particulate matter exposure had stronger responses in the CRP levels. They also found that changes in CRP levels among adults suffering inflammatory diseases were not significant possibly due to usage of medications.

Additionally, Bigert et al., (2008) conducted a study that was looking at different biomarkers indicative of cardiovascular diseases, like hsCRP and fibrinogen in relation to $PM_{2.5}$. The study group was comprised of 79 employees aged between 25 and 50 years who worked at the Stockholm underground. Of these employees, 44 wore portable monitors to measure their exposures. They also had different functions: 23 of them were platform workers (cleaners and ticket collectors) with high exposure to particles (cleaners had seven times higher exposure than for the control group and the ticket collectors, five times), 13 were train drivers with medium exposure (who had twice as high exposure than the control group), and 8 were ticket sellers with low exposure and served as the control group. Blood sampling was taken first after 2 non-working days and then, a second sample 48 hours after 2 days of work. The authors found that there was a significant increase of fibrinogen between the two samples for the drivers only (median (25^{th} and 75^{th} quartiles): sample 1: 2.77 (2.43; 3.23) sample 2: 3.03 (2.72; 3.48)). Most importantly, when comparing the three groups, it was found that hsCRP levels were higher for the platform workers compared to the two other groups in both samples (log mean (SD): platform workers: 1.44 (0.57), drivers: 1.35 (0.46), and sellers: 1.35 (0.42) ($p=0.25$)). It was also found that hsCRP levels in sample 2 were significantly higher in platform workers than train drivers (1.38 (0.68; 2.97) compared to 0.76 (0.33; 1.94)). This study suggested that acute exposure is possibly associated with hsCRP and fibrinogen levels.

Two cross-sectional studies have investigated these associations using short, medium and long-term exposure metrics in the same cohort. Hoffman et al. (2009) looked at long-term residential exposure to fine particulate matter and high traffic in association with hsCRP and fibrinogen levels. Their sample was part of a population-based prospective cardiovascular cohort study and included 4,814 participants aged 45 to 75 years. The pollutant annual average levels were estimated using a chemical transport model and distance between residence and major roads. In their analysis, the authors adjusted for short-term exposure by averaging PM_{10} levels from the 5 days preceding the blood draw. In crude analysis, the authors found that an increase in annual average $PM_{2.5}$ of 3.91 $\mu g/m^3$ was associated with an increase in baseline hsCRP of 16.7% (95%CI: 6.8; 27.5). The two different adjusted models that the authors conducted showed the same trend. As for the association with fibrinogen, the results showed, in the crude model, an increase in the biomarker of 2.4% (95%CI: 0.6; 4.2) in men only. Here again, the adjusted models showed the same trend. While Hoffman et al. looked at long-term residential exposure, Hertel et al. (2010) studied short and medium-term residential exposure in the same cohort. They used similar exposure data (but excluded traffic exposure) and limited their outcome to hsCRP levels only. They calculated exposure means up to three days prior to the blood draw (lags) and also modeled six different moving averages. The authors found no associations between $PM_{2.5}$ and hsCRP for single day exposures at any lag nor for the moving averages up to 21 days. However, an association was found for the 28-day mean (% change in hsCRP (95%CI): 7.06 (1.42; 13.02)).

Finally, in another study of short and medium-term exposure, Diez Roux et al., (2006) used the participants of the longitudinal "Multi-Ethnic Study of Atherosclerosis" with a total of 5,634 healthy subjects, aged 45 to 84 years. The authors wanted to investigate the relationship between $PM_{2.5}$ and CRP levels using cross-sectional data collected at baseline. $PM_{2.5}$ levels were obtained from monitors nearest to the subject's residence. The authors examined lags from the day prior to phlebotomy to the prior 60 days but constructed five exposures measures: prior day of blood draw, average of prior 2 days, average of prior week, average of prior month, and average of prior 2 months. They also analysed five different models using different variables. The results showed no clear evidence of a positive association between $PM_{2.5}$ and the odds of CRP being greater than or equal to 3 mg/l in the different models. However, in the same line as Hertel's study where an association was seen at the 28-day mean, here only the means of 30-day and 60-day exposure models showed a weak positive association, with wide

intervals between $PM_{2.5}$ and CRP levels: 3% increase for 30-day (95% CI:-2,10) and 4% for 60-day (95% CI:-3,11).

1.5.3. NO_2 exposure can plausibly lead to systemic inflammation.

The extent of research studies looking at inflammatory responses due to particulate matter is far greater than for NO_2 exposure. In this study, we are looking at NO_2 per se and not as a marker of traffic related air pollution. Studies with NO_2 usually seem to focus on lung function or respiratory disorders as the outcome rather than inflammation specifically. For NO_2, the evidence with regard to inflammation comes from experimental animal studies and it is still not known if the systemic inflammatory effects of NO_2 exposure seen in experimental animals also occur in humans (WHO, 2005). In toxicological studies, short-term NO_2 exposure to 3760 $\mu g/m^3$ (2.0 ppm) caused epithelial damage in BALB/c mice (Hussain, Jain, O'Shaughnessy, Businga, & Kline, 2004). In clinical studies, controlled chamber exposure has shown that four to six hours of exposure, including intermittent exercise, to 3760 $\mu m/m^3$ (2.0ppm) in healthy subjects caused an increased number of neutrophils (Azadniv et al., 1998) while an exposure of 7520 $\mu m/m^3$ (4.0 ppm) with intermittent exercise for 20 minutes a day every other day for 12 days caused a decreased number of mast cells, alveolar macrophages, and some lymphocytes and alveolar macrophages (Sandstrom, Helleday, Bjermer, & Stjernberg, 1992).

A small exposure study done in the Netherlands by Steenhof et al., (2014) exposed 31 healthy participants for 5 hours at five different locations with contrasting pollutant levels. Participants had in total three to seven exposures at different sites. The authors were interested in the associations between different pollutants, one of them being NO_2 concentration, and total blood counts. Blood draws were done before exposure, two hours after exposure and the next morning. The authors found no associations between WBC count and NO_2 exposure levels.

Alternatively, a cross-sectional study done in Tel-Aviv by Steinvil, Kordova-Biezuner, Shapira, Berliner, & Rogowski, (2008) looked at short-term exposures of urban pollution in a total of 3659 adult subjects. Air pollutants were measured by three monitoring stations throughout the city. They measured WBC count, fibrinogen and CRP for each participant and analysed each biomarker in association with each pollutant on consecutive days and up to seven days (lags) prior to blood draw. Similar to the results from Steenhof's study, they found

no significant associations between total WBC count or CRP and NO_2. However, they found a significant decrease in fibrinogen at different lags for NO_2 in men only. It is important to note that this study measured fibrinogen using the Clauss method

These results for fibrinogen were not consistent with a study by Rudez et al., (2009) that looked at long-term urban air pollution concentrations (NO_2) in association with fibrinogen and CRP levels in a small cohort study of 40 healthy participants living in Rotterdam, Netherlands. They collected a mean of 12.5 blood samples per participant throughout a period of one year and they used one monitoring station in the city centre for hourly measurement of the air pollutants. Their analyses included different lag periods before blood draw. Their results did not show any significant associations between any biomarkers and NO_2 levels.

Although the epidemiological evidence for associations between NO_2 exposure and biomarkers of inflammation mostly show no associations, I am including NO_2 in my study based on the few epidemiological studies that do show associations in combination with the toxicological evidence and biological plausibility that NO_2 exposure could lead to systemic inflammation.

1.6. Statins

Statins, or HMG-CoA reductase inhibitors, are a well-known class of medications used to reduce blood triglyceride and cholesterol levels. However, they have been found to also have pleiotrophic effects. Some studies have observed that statins may, in addition, reduce markers of inflammation in the blood (Arnaud et al., 2005a; Owens, 2012; Quist-Paulsen, 2010) through various mechanisms (Jain & Ridker, 2005). Experimental observations from cell culture and animal studies have shown that this effect may be happening in multiple cell types, such as endothelial and immune cells, and through multiple molecular mechanisms, for example by acting on HMG-CoA reductase in the cholesterol formation pathway (Jain & Ridker, 2005).

It is plausible that anti-inflammatory effects of statins could reduce or eliminate inflammatory responses after exposure to $PM_{2.5}$ and NO_2. Research by Miyata, Bai, Vincent, Sin, & Van Eeden (2013) looked at the effect of statins on New Zealand white rabbits which were exposed to either PM_{10} (1.0mg/kg) or saline three times a week for 4 weeks and received

either the statin lovastatin (5 mg/kg/d) or no treatment. It was found that lovastatin reduced the PM_{10}-dependent levels of inflammatory biomarkers IL-6 and IL-8 in bronchoalveolar lavage (BAL) fluid, the activation of macrophages in the lung tissues, as well as promoted the clearance of PM_{10} from the lung. The molecular mechanisms behind these effects were thought to come from the action of statins on production of IL-6, release of polymorphonuclear leukocytes (PMNs) from the bone marrow, and retention of PMNs by the lung tissue (Tasat & Yakisich, 2012). Although these findings cannot be directly generalized to our study due to differences in the exposure metric, they show that statins may provide protective effects during air pollution exposure including a reduction in inflammatory response due to exposure.

Few epidemiological studies have investigated whether statin usage can provide a protective effect with regard to inflammation caused by $PM_{2.5}$ and NO_2 exposure. Hertel et al. (2010), study that was mentioned earlier, was looking at short and medium-term exposure to $PM_{2.5}$ in association with CRP. A population-based prospective cardiovascular cohort study included 4 814 subjects aged 45 to 75 years. In this study, 422 participants were using statins. The authors conducted effect modification analysis by statin therapy and found an interaction for the 28-day mean where statins users didn't show an increase in CRP (in % change) after $PM_{2.5}$ exposure (1.00% (95% CI: -11.68; 15.50)) compared to non-statins users (7.98% (95% CI: 2.20; 14.09)). Contrarily, Hoffman et al. (2009) who used the same cohort study to look at long-term exposure of $PM_{2.5}$ and high traffic in association with CRP and fibrinogen did not find any effect in the effect modification between statins users and non-statins users. Despite Hoffman's results, Hertel et al. (2010)'s finding could possibly indicate that statins have inflammatory protective effects when exposed to fine particulate matter.

1.7. References

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Chapter 2.

Associations of residential ambient $PM_{2.5}$ and NO_2 with biomarkers of inflammation: a cross-sectional analysis of the Canadian Health Measures Survey

2.1. Introduction

The prevalence of inflammatory diseases such as diabetes and atherosclerosis are increasing in both developed and developing countries (CBC News, 2013; Weber & Noels, 2011). Although some determinants like obesity, lack of exercise, and hypertension are known to increase the risks of these diseases (Roger et al., 2012; Shoelson, Lee, & Goldfine, 2006), the impacts of environmental pollution have received less attention in research (Chuang, Chan, Su, Lee, & Tang, 2007; Sun, Wang, & Jin, 2005). If environmental pollutants increase the risks of inflammatory diseases, environmental policies could reduce future incidence of these diseases.

Plausible links between air pollution and both diabetes and atherosclerosis exist through the mechanism of systemic inflammation (Elvidge et al., 2013; Poursafa & Kelishadi, 2010; Sun et al., 2005). Experimental, observational and in vitro studies have shown that air pollution particles have the ability to trigger an inflammatory chain reaction in cells and tissues when inhaled leading to increased circulation of pro-inflammatory molecules and macrophages (Araujo, 2010; Barbara Hoffmann et al., 2009; Huttunen et al., 2012). Furthermore, many studies have been conducted on the associations of air pollution exposure with cardiovascular disease and mortality (Brook et al., 2010; Pope, C.A., & Dockery, 2006). There is now strong evidence that daily and long-term exposure to particulate matter increase cardiovascular mortality by one of three pathways: the release of proinflammatory mediators from endothelial cells in the lung, by activation of an imbalance in the autonomic nervous system or by translocation of particles into the bloodstream (Brook et al., 2010). Increased mortality might

also be the result of atherosclerosis, which can result from local inflammation, endothelial dysfunction and plaque formation (Paffen & DeMaat, 2006). Evidence drawn from a few studies have reported associations between long-term exposure to air pollution and atherosclerosis (Adar et al., 2013; Allen et al., 2009; Bauer et al., 2010; Diez Roux et al., 2008; Gan et al., 2014; Hoffmann et al., 2007; Künzli et al., 2005, 2010). Hence, there are plausible biological mechanisms through which air pollution exposure could lead to systemic inflammation and subsequent atherosclerosis and diabetes (Panasevich & Leander, 2009; WHO, 2005).

Systemic inflammation can be measured using inflammatory molecules in the blood. Four biomarkers indicative of inflammation were used in this study as outcome variables. C-reactive protein (CRP) is an acute-phase reactant that is a sensitive, but not specific, indicator of systemic inflammation (Sandhu et al., 2005). The most sensitive assay of CRP is called high-sensitivity CRP (hsCRP) and can give results as low as 0.1 mg/L serum (Statistics Canada, 2012). In healthy individuals, the median hsCRP concentration is 0.8 mg/L serum (Pepys & Hirschfield, 2003). hsCRP has been widely recognized as a predictor of cardiovascular diseases (Ben-Yehuda, 2007). Like hsCRP, fibrinogen is also a protein that is synthesized in the liver. In healthy adults, fibrinogen concentrations range from 2 to 4 g/L plasma. Fibrinogen is an acute-phase reactant, which plays a role in coagulation and inflammatory response. These dual roles imply an intricate interdependence between coagulation and inflammation (Davalos & Akassoglou, 2012). Fibrinogen has been found to be useful in the prediction of clinical atherosclerosis (Kampoli et al., 2009). White blood cells (WBC), or leukocytes, are well known as a first line of defense against pathogens, however they also remove damaged cells, toxins and other waste products (Ashton, 2010). In healthy individuals, the WBC count normally ranges from 4,500 to 11,000 cells per microlitre of blood (Abbas & Lichtman, 2005). Elevated WBC count can be a sign of infection, inflammation, immune system response or a blood disease (Abbas & Lichtman, 2005; Serhan et al., 2010). Platelets are regulators in the process of inflammatory response. In healthy people, the platelets count is around 150-400 $x10^9$ platelets per liter of blood (Ioannou et al., 2013). Their activation is responsible for the formation of blood clots and regulating the inflammatory response (Ioannou et al., 2013). They were also found to be responsible for a cascade of events during systemic inflammatory response due to their direct cell-to-cell contact (Smith & Weyrich, 2011).

Two exposure variables are used in this study – fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2). Due to its small particle size, $PM_{2.5}$ has the ability to penetrate deeper into the lungs than larger particles, remain suspended for longer periods of time, penetrate more readily into indoor environments and to be transported over much longer distances (WHO, 2005). $PM_{2.5}$ particles vary in chemical composition which may influence their toxicity and add to the complexity of studying their effects on inflammation (Pope, C.A., & Dockery, 2006). Many studies have found that an increase in particulate matter affects the concentration of inflammatory biomarkers in both short-term and long-term exposures however, when an association is found, the direction of the association is not consistent across studies (Bigert et al., 2008; Barbara Hoffmann et al., 2009; Li et al., 2012; Rückerl et al., 2006; Thompson et al., 2010; Tsai et al., 2012). Differences in geographic regions, however, as well as particle composition, time frame, duration, and susceptibility may play a role in the disparities found among the findings and among the direction of the association between exposure to $PM_{2.5}$ and inflammation.

Nitrogen dioxide (NO_2) is a gas that is produced by fossil fuel combustion. Inhalation of this gas irritates endothelial cells in the respiratory pathway. NO_2 is often seen as a surrogate for traffic, thus $PM_{2.5}$ and NO_2 can sometimes be correlated because traffic is a source for both (WHO, 2005). Toxicology studies have also found that nitrogen dioxide (NO_2) is toxic and that it produces significant health effects such as decreased pulmonary function, increased inflammation, and altered lung metabolism and structure (WHO, 2005). Clinical studies found that NO_2 exposure caused increased numbers of neutrophils (Azadniv et al., 1998), as well as decreased numbers of mast cells, alveolar macrophages and some lymphocytes (Sandstrom et al., 1992). Few epidemiological studies looked directly at the association between NO_2 exposure and inflammatory biomarkers (Hesterberg et al., 2009). Four studies (Johannesson, Andersson, Stockfelt, Barregard, & Sallsten, 2014; Rudez et al., 2009; Steenhof et al., 2014; Thompson et al., 2010) examining NO_2 exposure with different biomarkers found no association between NO_2 and any of WBC count, CRP, and fibrinogen levels while two studies found that NO_2 was positively associated with fibrinogen (Bind & Baccarelli, 2012; Pekkanen, Brunner, Anderson, Tiittanen, & Atkinson, 2000). Conversely, other studies found a decrease in fibrinogen levels after exposure to NO_2 (Forbes et al., 2009; Hajat et al., 2015; Schwartz, 2001; Steinvil et al., 2008).

Statins, or HMG-CoA reductase inhibitors, are commonly used medications for reduction of cholesterol levels. The Canadian Rx Atlas (Morgan et al., 2012) indicated that in 2012-13, more than 39.3 million prescriptions for cholesterol lowering drugs were filled by Canadians. Statins might have pleiotrophic effects beyond cholesterol reduction, including reducing biomarkers of inflammation (Arnaud et al., 2005b; Owens, 2012; Quist-Paulsen, 2010). This raises the interesting possibility that use of Statins might protect against any inflammatory effects of exposure to air pollution (Miyata et al., 2013; Tasat & Yakisich, 2012). Such an effect would be expected to reduce the association between air pollutants and biomarkers of inflammation among those who use Statins. A few previous studies have investigated this possibility. A study found that 28-day mean $PM_{2.5}$ was not associated with CRP among those using Statins, but it was among those not using Statins, however low power might have contributed to this result (Hertel et al., 2010). Another study, using the same cohort but looking at long-term exposure, did not find any modifying effect (Hoffmann et al., 2009). Other studies (Li et al., 2012; Ostro et al., 2014; Rückerl et al., 2007) are questioning whether the lack of association between $PM_{2.5}$ and CRP is possibly due to the high prevalence of Statins intake.

The aim of our research was to investigate whether residential daily average and annual average $PM_{2.5}$ and NO_2 were associated with biomarkers of inflammation in a nationally-representative sample of Canadian adults. We hypothesized that residential ambient $PM_{2.5}$ and NO_2 would be positively associated with C-reactive protein (CRP), fibrinogen, total count of white blood cell (WBC) and platelets. We also hypothesized that use of Statins would modify (reduce) the associations investigated here.

2.2. Methods

2.2.1. Data sources and linkage

This study used two cycles of the Canadian Health Measures Survey (CHMS), a nationally-representative, cross-sectional survey conducted every two years by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada. CHMS participants were sampled across the ten Canadian provinces and three territories. Persons living on reserves, institutions, or members of the Canadian Forces were excluded. Data collection for the first cycle was carried out from March 19, 2007 to February 25, 2009 and the

second cycle, from August 27, 2010 to November 30, 2011. Participation was voluntary and each participant was given information that explained the survey. Written consent to participate in all procedures in the study was given by each participant. Our data analysis was approved by Statistics Canada and the Research Ethics Board of Simon Fraser University.

The CHMS used a multistage sampling strategy aiming to produce national level estimates. The sampling method additionally ensured sufficient sample sizes by geographic region, population size, age, and sex. There were fifteen collection sites for cycle 1 and eighteen for cycle 2 selected from a sampling frame of potential sites covering 96.3% of the Canadian population. Households within 50 kilometers of the collection site in urban areas were eligible for selection (Statistics Canada, 2011). All participants living at home were invited to participate if they were between 6 and 79 years of age (cycle 1) or 3 to 79 years (cycle 2) (Statistics Canada, 2011, 2012). Excluded from blood sampling were those who received recent chemotherapy, double mastectomy, or were haemophiliac as well as participants who had acute or chronic conditions on both upper limbs (Statistics Canada, 2011, 2012). Fifty percent of participants were randomly selected to fast prior to blood draw. Fasting did not affect the biomarkers levels used in this study (Pearson et al., 2003). For each step and process during the survey, the CHMS tried to ensure quality control to reduce systematic bias. Staff training, clear protocols and procedures as well as data validation were just a few examples of methods used to achieve these goals. However, non-response from participants could happen at many levels. For cycle 1, a combined response rate of 52% was observed while cycle 2 had 56% (Statistics Canada, 2011, 2012).

We linked annual levels of $PM_{2.5}$ and NO_2 from a national land use regression model dataset (Hystad et al., 2011) which were estimated using land use regression models and by combining different measurements (derived satellite-based estimates of pollutant levels ($PM_{2.5}$ and NO_2) from the National Air Pollutant Surveillances (NAPS) monitoring stations, road length, population density, and proximity to large emitters) to predict pollutant concentrations (Brauer, Ainslie, Buzzelli, & Henderson, 2008; Hystad et al., 2011). Measurement of $PM_{2.5}$ was done with different monitor types: tapered element oscillating microbalances (TEOMs), dichotomous partisol samplers (Thermo Fisher Scientific Inc.), and beta-attenuation mass monitors (Met One Instruments Inc.). The models were used to estimate annual average $PM_{2.5}$ and NO_2 concentrations for each postal code, which were linked to the CHMS using the residential postal

code of participants at the time of the blood draw. Data for daily exposure levels of $PM_{2.5}$ and NO_2 were classified using the closest NAPS monitor to the residential postal code.

2.2.2. Variables

Variables in our models were selected based on a priori knowledge using a directed acyclic graph (Greenland, Pearl, & Robins, 1999), which is shown in Appendix B. Based on this, our models included three covariates to control for confounding: household income, expressed race, and outdoor temperature. Moreover, our analysis was limited to participants who were living in a census metropolitan area (CMA) or census agglomeration (CA) to reduce information bias caused by misclassification of exposure due to the fact that, in Canada, the air pollution monitoring network is predominantly urban and rural people tend to live a greater distance from monitors than urban people. We also investigated the use of Statins as an effect modifier of the association between $PM_{2.5}$, NO_2 and the four biomarkers of inflammation. Biomarkers of inflammation were assayed in blood samples collected using standardized venipuncture technique. Laboratory methods have been previously reported (Statistics Canada, 2011, 2012). Whole blood WBC and platelet counts were determined at the mobile examination centre (MEC) using the Beckman Coulter method with limits of detection (LOD} of 3.0 $x10^9$ /L and 50 $x10^9$ /L, respectively. Remaining whole blood was centrifuged and stored in the MEC laboratory then shipped once a week to a Health Canada laboratory for analysis. Briefly, serum hsCRP was measured using immunoturbidemetric assay LOD of 0.1 mg/L. Plasma fibrinogen was measured using a photo-optical clot detection method with an LOD of 0.8 g/L. Inflammation biomarkers were modeled as continuous variables. Annual and daily $PM_{2.5}$ and NO_2 were defined as described as above and used as continuous variables in models. Household income was defined as self-reported household income from all sources before taxes and deductions. For 23.8% of our sample, household income was imputed by Statistics Canada based on a number of other variables (Statistics Canada, 2012). We used six categories: less than $25,000, $25,000 to $50,000, $50,000 to $75,000, $75,000 to $100,000, $100,000 to $200,000, and greater than $200,000. Expressed race is defined as a racial category with which a person will self-identify especially to fit into a society's official race classifications (Veenstra, 2011). This type of racialized identity is often expressed in response to close-ended questions on government or institutional forms that collect information on race. Each respondent in the study was asked to self-identify their racial or cultural group between

Aboriginal, White, South Asian, Chinese, Black, Filipino, Latin American, Arab, Southeast Asian, West Asian, Korean, Japanese or Other. Respondents were able to choose up to four different racial identities. Due to very small samples sizes in certain categories, we reclassified expressed racial identity into six groups: White, Black, Chinese, South Asian, Aboriginal, and Other (including Multiple). Temperature was defined using NAPS data from the nearest monitor to the MEC on the day and at the time of the clinic visit of each participant. We categorized temperature in three categories: <0°C, 0- 18°C, and >18°C. The higher cut-off (18°C) is the usual base temperature for buildings, which determines when cooling is used. Statin usage was defined by self-report. Each participant was asked to name all prescribed medication, over-the counter or herbal medications that they were using. These medications were coded by CHMS staff based on the Anatomical Therapeutic Chemical (ATC) classification system (WHOCC, 2013). Statins were defined as ATC codes starting by A10BH, H01CB, C10AA, C10BA, and C10BX. One respondent who answered, "do not know" for medication use, was classified as not using Statins.

2.2.3. Statistical methods

We first evaluated the characteristics of study participants all together and stratified by Statin usage. We accounted for the complex sampling design to produce nationally-representative means and frequencies using SAS PROC SURVEYFREQ and PROC SURVEYMEANS. We then investigated the associations between annual average levels of $PM_{2.5}$ and NO_2 and levels of biomarkers hsCRP, fibrinogen, WBC, and platelets using eight separate multivariable linear regression models (PROC SURVEYREG). We also tested for non-linearity and we looked for influential points. We next modeled associations between daily average concentrations of $PM_{2.5}$ and NO_2 and levels of the four biomarkers for the day of the blood draw and the previous 35 days (separate models for each day). Study participants had to have complete data for all 36 daily average models to be included in any of them. For both annual and daily models, hsCRP was natural log-transformed to meet the assumption of normality of residuals, but other biomarkers were modeled using their original scales. All regression models were fit using SAS PROC SURVEYREG to account for the complex sampling design and produce nationally-representative parameter estimates. We then stratified the annual and daily average models by Statin usage and used an interaction term in separate models to generate a 2-sided p value for effect measure modification by Statin usage (*Y*

*biomarkers= β_1 + β_2 pollutant + β_3 pollutant * β_4 Statins + β_5 Statins + covariates*). Effect estimates were reported as associations corresponding to a 1 ug/m^3 increase in both annual average and daily changes $PM_{2.5}$ and 10 ug/m^3 increase in annual and daily changes NO_2. All data analyses were performed using SAS version 9.4 (Cary, NC).

2.3. Results

Our sample included 11387 participants from the combined cycles 1 and 2. From these participants, we selected 6455 subjects 18 years of age and older who lived in a CMA or CA. After excluding participants due to missing expressed racial identity data, our final sample for analysis included 6322 participants who had complete data for all three covariates (Appendix C). Beyond this, the number of participants varied across models according to missing annual or daily average $PM_{2.5}$ and NO_2 data.

Table 2.1 includes characteristics of our sample. Using sample weights, 2,362,581 out of 20,757,437 people (11%) were using Statins. Mean concentrations of the four biomarkers of inflammation were within normal ranges. The mean annual concentrations of $PM_{2.5}$ and NO_2 were higher (8.4 and 27.2 µg/m^3 respectively) than the mean daily concentration (6.6 and 22.2 µg/m^3) possibly reflecting seasons when participants were sampled and/or the inclusion of more predictor variables of $PM_{2.5}$ and NO_2 when calculating the annual concentrations (Hystad et al., 2011). More than a quarter of the sample did not have complete data for the daily concentrations of $PM_{2.5}$ and NO_2. The expressed race of the sample reflected the Canadian population with 79% white followed by 5% Chinese, 4% South Asian and 2% First Nations. The remaining expressed racial identity groups together (including Multiple) comprised 10%. Eleven percent of participants had household income less than $25,000 per year and 5% earned more than $200,000 per year.

Table 2.2 shows the results of regression models of annual average $PM_{2.5}$ associated with the four biomarkers of inflammation and adjusted for household income, expressed race, and temperature. A cross-sectional 1 µg/m^3 increase in annual average $PM_{2.5}$ was inversely associated with fibrinogen (β (95% confidence interval (CI)) = -0.09 (-0.18, 0.00) mg/L plasma)

among those using Statins. The 2-sided p value for effect modification by Statin use was <0.001 and the association between $PM_{2.5}$ and fibrinogen was not significant at alpha=0.05 among those not using Statins despite a larger sample size. None of the three remaining biomarkers of inflammation were significantly associated with annual average $PM_{2.5}$ in any model.

Table 2.3 shows the results of regression models of annual average NO_2 associated with the four biomarkers of inflammation and adjusted for household income, expressed racial identity, and temperature. A cross-sectional 10 $\mu g/m^3$ increase in annual average NO_2 was inversely associated with fibrinogen (β (95% CI) = -0.04 (-0.08, 0.00) g/L plasma). Although the 2-sided p value for effect modification was <0.001, the regression coefficients for annual NO_2 and fibrinogen did not differ by much among those using Statins or not. A cross-sectional 10 $\mu g/m^3$ increase in annual average NO_2 was inversely associated with WBC (β (95% CI) = -0.13 (-0.24, -0.01) $x10^9$/L blood) among those not using Statins, but was not associated among those using Statins. Also, a 10 $\mu g/m^3$ increase of annual average NO_2 was inversely associated with hsCRP in both groups however the associations were not significant: (β (95% CI) = -0.02 (-0.11, 0.07) mg/L plasma) for those using Statins and (β (95% CI) = -0.05 (-0.11, 0.02) g/L plasma) for those not using Statins.

The β's and 95% CI's for models of daily average $PM_{2.5}$ and NO_2 associated with the four biomarkers of inflammation are shown in figure 2.1. The major pattern was that daily NO_2 was inversely associated ($p<0.05$) with fibrinogen in 27 of 36 models using average NO_2 on the day of blood draw and the previous 35 days. There was also an apparent trend of an inverse association between NO_2 and hsCRP, however only 5 out of 36 models were statistically significant at 2-sided alpha=0.05. Among the remaining models (i.e. excluding NO_2-fibrinogen and NO_2-hsCRP models), only 11 out of 216 models (5%) had statistically significant associations, which is consistent with that expected by random error. The values for all of these models shown in figure 2.1 are included in Appendix D.

Table 2.4 shows models stratified by Statin usage for the associations of daily average NO_2 with fibrinogen and 2-sided p values for effect modification by Statin usage. Although p values for effect modification were all 0.001 or smaller, the values of the NO_2 parameter (to two decimal places) are similar across strata of Statin usage for each day. Differences in parameter estimates across Statin strata were only observable with more decimal places (data not shown).

The stratified models for the remaining air pollutant-biomarker models are shown in Appendix E. The results were similar to those for NO2 and fibrinogen: although the p values for effect measure modification were often statistically significant, the magnitudes of differences in the associations across strata are quite small and not necessarily clinically significant.

2.4. Discussion

Contrary to our hypothesis, we observed consistent inverse associations between daily and annual average residential ambient NO_2 concentrations and plasma fibrinogen in a cross-sectional sample that was representative of the non-institutionalized adult Canadian population living in cities. Inverse associations (2-sided $p < 0.05$) with daily NO_2 were observed in 27 of 36 models using average NO_2 on the day of blood draw and the previous 35 days. Annual average NO_2 was also inversely associated with fibrinogen. These associations did not appear to be meaningfully modified by Statin usage, although 2-sided p values for effect measure modification were all statistically significant (so small differences in the associations were observable). We also found a consistent trend toward inverse associations between hsCRP and daily average NO_2 on the day of the blood draw and the previous 35 days, but these associations mostly did not reach statistical significance. The other statistically significant associations that we observed could be explained by random error.

Although there is a growing body of empirical evidence that points to positive associations between ambient air pollution and biomarkers of inflammation (Brook et al., 2010; Chuang et al., 2007; Li et al., 2012; Pope et al., 2004; Rich et al., 2012; Riediker et al., 2004), our finding of inverse associations between NO_2 and fibrinogen are consistent with several previous studies (Forbes et al., 2009; Hajat et al., 2015; Panasevich & Leander, 2009; Steinvil et al., 2008). Although Forbes et al. (2009) and Panasevich & Leander (2009) did find a negative association between fibrinogen and NO_2, their results were not significant. On the other hand, Steinvil et al. (2008) who conducted a cross-sectional study in Tel Aviv including 3659 healthy participants (2203 males and 1456 females) with a mean of 46 years of age, found significant results in men only. The study looked at short-term exposure by using same day NO_2 levels and up to 7-day before the blood draw in addition to the week average. Levels of NO_2 were measured by three monitoring stations across the city. Their findings showed daily percentage change in fibrinogen between -1.16 to -2.26 while the week average showed -3.88%

changes after exposure to NO_2. Recently, Hajat et al. (2015) looked at short and long-term NO_2 exposure by conducting a retrospective study using 10,310 participants aged between 45 to 84 years (mean = 62) from the Multi-Ethnic Study of Atherosclerosis. To measure long-term exposure to NO_2, they used spatiotemporal prediction model and their results showed a -2.19 percentage difference for long-term exposure to NO_2 (results were not provided for short-term exposure).

The reasons for discrepant results between studies are uncertain. When comparing the results of the two previous studies with other studies that found positive associations between fibrinogen and NO_2, possible explanations can be speculated. In London, UK, Pekkanen et al. (2000) conducted a cross-sectional study based on the Whitehall II study participants. When using data from September 1991 and May 1993 to measure short-term exposure to pollutants, they found that the 24 hour mean NO_2 level was associated with increase in fibrinogen concentration. It is important to note that the mean NO_2 level was almost doubled (78.6 $\mu g/m^3$ or 41.8 ppb) what was measured in the previously mentioned studies. Likewise, Bind and Baccarelli (2012) looked at short-term exposure to air pollutants using a prospective cohort study including 704 elderly men, mean age of 73.2 years, from the Veterans Administration Normative Aging Study. When analysing the association between fibrinogen and 3-day moving average of NO_2, the authors found significant positive results. It is, however, well-known that children and older adults are populations more susceptible to the effects of air pollution exposure (Makri & Stilianakis, 2008; Solomon et al., 2011) which may explain the positive association. Finally, other studies (Johannesson et al., 2014; Rudez et al., 2009; Thompson et al., 2010) found no association between NO_2 exposure and fibrinogen. Their lack of findings may be due to small sample size that were lower than 45 participants.

A major strength of this study is that our sample was representative of the adult Canadian population living in cities, thus our results can be generalized broadly. However, we were unable to investigate these associations in subpopulations that might be more sensitive to any effects of ambient air pollution on inflammation biomarkers, such as those with existing metabolic disease (Dubowsky, Suh, Schwartz, Coull, & Gold, 2006; Janghorbani, Momeni, & Mansourian, 2014; Solomon et al., 2011). Although the low response rate in the CHMS indicates a potential for selection bias in this sample, we have no reason to suspect that agreement to take part in the study was systematically associated with exposure and outcome

in the source population. Nonetheless, the potential for selection bias is a limitation that should be considered when interpreting the results. We also chose to focus only on those living in cities to reduce information bias caused by misclassification of exposure. Air pollution is generally higher in Canadian cities relative non-urban areas and there might be systematic differences between unmeasured risk factors for inflammation between individuals living in urban and non-urban areas. We aimed to control for confounding by socio-economic differences between groups exposed to different concentrations of air pollutants in cities by controlling for family income.

Misclassification of exposure should be non-differential in this study, which might have resulted in biased parameter estimates toward the null. For daily average $PM_{2.5}$ and NO_2, we used concentrations measured at the nearest monitor from the participants' homes. The actual ambient concentrations at the homes of participants would differ from these measures. Although fixed-site monitors are usually centrally located to measure pollutant concentrations across a city, accurate measurements may vary because some pollutants are spatially more heterogeneous than others (Brauer, Hystad, & Poplawski, 2011; Özkaynak, Baxter, Dionisio, & Burke, 2013). We also did not take into account the amount of time spent at home or exposures that might have occurred indoors or at other locations. Studies have also shown that the composition of particulate matter varies across regions, such as rural and urban (Chow et al., 2006; Kundu & Stone, 2014) and between seasons (Minguillón, Querol, Baltensperger, & Prévôt, 2012; Plummer, Ham, Kleeman, Wexler, & Pinkerton, 2012). However, there is also some evidence to suggest that traffic-related air pollution may have a stronger inflammatory effect than air pollution from other sources (Hennig et al., 2014). The geographical and seasonal variations of pollutants could potentially influence the inflammatory responses after air pollution exposure (Schneider et al., 2008). We did not investigate effect modification by geographic region or season in the associations between $PM_{2.5}$, NO_2, and biomarkers of inflammation. In addition, we note that the NAPS program gradually replaced $PM_{2.5}$ monitors based on newer technology between 2007 and 2013. The newer monitors measure the semi-volatile $PM_{2.5}$ mass that was not captured by the older instruments. Our dataset was created based on data from 2007 to 2011, so we may have utilized readings from both older and newer monitors.

2.5. Tables and figures

Table 2.1. Characteristics of study participants aged 18-79 living in a Census Metropolitan Area or Census Agglomeration Area. Canadian Health Measures Survey 2007-2011.

	All	Using Statins	Not Using Statins
Sample (n)	6322	845	5477
Weighted (n)	20757437	2362581	18394856
ln(hsCRP) (mg/L plasma)			
Mean (SE)	0.16 (0.03)	0.47 (0.05)	0.12 (0.03)
Missing data–n (%)	809861 (4)	69800 (3)	740062 (4)
Fibrinogen (g/L plasma)			
Mean (SE)	2.99 (0.01)	3.31 (0.03)	2.95 (0.02)
Missing data-n (%)	576303 (3)	60048 (2.5)	516255 (3)
WBC (per 10^{-9}L blood)			
Mean (SE)	6.71 (0.04)	7.03 (0.09)	6.67 (0.05)
Missing data-n (%)	234120 (1)	28836 (1)	205284 (1.1)
Platelets (per 10^{-9}L blood)			
Mean (SE)	235.50 (1)	228.57 (3)	236.39 (1)
Missing data-n (%)	178422 (1)	23491 (1)	154930 (0.8)
Daily $PM_{2.5}$ ($\mu g/m^3$)			
Mean (SE)	6.61 (0.09)	6.60 (0.16)	6.61 (0.10)
Missing data-n (%)	5390022 (26)	744302 (32)	4645720 (25)
Annual $PM_{2.5}$ ($\mu g/m^3$)			
Mean (SE)	8.35 (0.02)	8.33 (0.05)	8.35 (0.03)
Missing data-n (%)	0 (0)	0 (0)	0 (0)
Daily NO_2 ($\mu g/m^3$)			
Mean (SE)	22.23 (0.41)	17.67 (0.72)	22.82 (0.45)
Missing data-n (%)	6043549 (29)	682089 (29)	5361460 (29)

	All	Using Statins	Not Using Statins
Annual NO_2 ($\mu g/m^3$)			
Mean (SE)	27.23 (0.25)	25.59 (0.64)	27.44 (0.27)
Missing data-n (%)	0 (0)	0 (0)	0 (0)
Expressed Racial Identity – n (%)			
White	16288291 (79)	2072941 (88)	14215350 (77)
Chinese	1059262 (5)	36242 (2)	1023020 (6)
South Asian	872164 (4)	105568 (5)	766596 (4)
First Nations	437326 (2)	38522 (2)	398804 (2)
Other	2100393 (10)	109307 (5)	1991086 (11)
Annual income (dollars) – n (%)			
< 25,000	2238484 (11)	324771 (14)	1913713 (10)
25,000 to 50,000	4487595 (22)	692777 (29)	3794819 (21)
50,000 to 75,000	4350438 (21)	506528 (21)	3843910 (21)
75,000 to 100,000	3642194 (18)	308384 (13)	3333810 (18)
100,000 to 200,000	5073640 (24)	448628 (19)	4625012 (25)
≥ 200,000	965085 (5)	81494 (3)	883592 (5)
Temperature (°C) – n (%)			
< 0	4709825 (23)	449022 (19)	4260803 (23)
0 to 17	12619512 (61)	1456032 (62)	11163480 (61)
≥ 18	3428100 (17)	457528 (19)	2970573 (16)

ln(hsCRP): natural log of C-reactive protein; WBC: White blood cells.

Table 2.2. Associations of biomarkers of inflammation (dependent variables) with annual residential ambient $PM_{2.5}$ ($\mu g/m^3$)* adjusted for race, income, and temperature in Canada. Canadian Health Measures Survey 2007-2011.

	All			Using statins			Not using statin			Effect Modification
	β	95% CI	2-sided p	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
ln(hsCRP) (mg/L plasma)	-0.01	(-0.06, 0.03)	0.617	-0.05	(-0.14, 0.04)	0.260	-0.01	(-0.06, 0.05)	0.810	<0.001
Weighted n	19947575			2292781			17654794			
Fibrinogen (mg/L plasma)	-0.03	(-0.09, 0.03)	0.339	-0.09	(-0.18, 0.00)	0.052	-0.02	(-0.09, 0.04)	0.457	<0.001
Weighted n	20181134			2302533			17878601			
WBC ($x10^9$/L blood)	-0.06	(-0.18, 0.05)	0.278	-0.04	(-0.19, 0.10)	0.545	-0.07	(-0.20, 0.06)	0.286	0.031
Weighted n	20523317			2333745			18189572			
Platelets ($x10^9$/L blood)	0.82	(-4.55, 6.18)	0.756	2.04	(-6.77, 10.85)	0.637	0.68	(-4.73, 6.10)	0.797	0.037
Weighted n	20579015			2339090			18239925			

CI: Confidence interval; hsCRP: C-reactive protein; WBC: White blood cell count.

* $PM_{2.5}$ is annual average at centroid of residential postal code calculated using land use regression models.

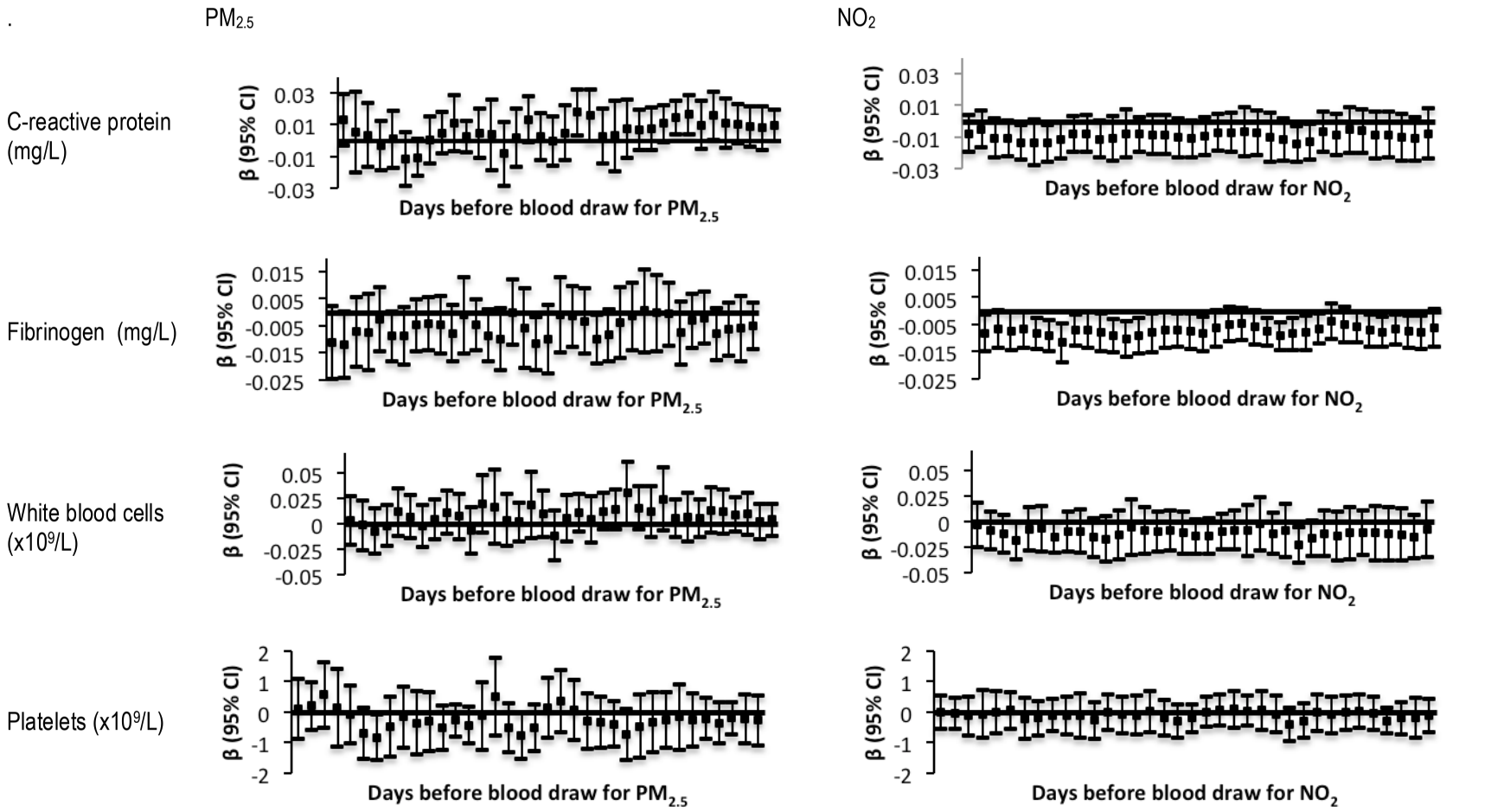
Table 2.3. Associations of biomarkers of inflammation (dependent variables) with annual residential ambient NO_2 (10 µg/m^3)* adjusted for race, income, and temperature in Canada. Canadian Health Measures Survey 2007-2011.

	All			Using statins			Not using statin			Effect Modification
	β	95% CI	2-sided p	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
ln(hsCRP) (mg/L plasma)	-0.05	(-0.11, 0.02)	0.132	-0.02	(-0.11, 0.07)	0.702	-0.05	(-0.11, 0.02)	0.131	0.001
Weighted n	19947575			2292781			17654794			
Fibrinogen (g/L plasma)	-0.04	(-0.08, 0.00)	0.040	-0.04	(-0.12, 0.04)	0.280	-0.04	(-0.07, 0.00)	0.052	<0.001
Weighted n	20181134			2302533			17878601			
WBC (x10^9/L blood)	-0.11	(-0.21, 0.00)	0.043	0.06	(-0.08, 0.20)	0.414	-0.13	(-0.24, -0.01)	0.034	0.003
Weighted n	20523317			2333745			18189572			
Platelets (x10^9/L blood)	-0.45	(-5.27, 4.38)	0.850	5.25	(-1.97, 12.47)	0.147	-1.30	(-6.09, 3.49)	0.580	0.352
Weighted n	20579015			2339090			18239925			

CI: Confidence interval; hsCRP: C-reactive protein; WBC: White blood cell count.

*NO_2 is annual average at centroid of residential postal code calculated using land use regression models.

Figure 2.1. Adjusted* linear regression β (95% confidence interval) of biomarkers of inflammation by same- and previous-days average residential ambient $PM_{2.5}$ ($1\ \mu g/m^3$) and NO_2 ($10\ \mu g/m^3$). Canadian Health Measures Survey 2007-2011



* Adjusted for expressed race, income and temperature.

Table 2.4. Associations of fibrinogen with daily average residential ambient NO_2 ($10\ \mu g/m^3$) adjusted for race, income, and temperature in Canada and stratified by Statin usage. Canadian Health Measures Survey 2007-2011.

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	-0.01	-0.03, -0.00	0.035	-0.01	-0.01, -0.00	0.038	0.001
1	-0.01	-0.02, -0.00	0.035	-0.01	-0.01, 0.00	0.104	<0.001
2	-0.01	-0.02, -0.00	0.050	-0.01	-0.01, 0.00	0.051	<0.001
3	-0.01	-0.02, -0.00	0.028	-0.01	-0.01, 0.00	0.108	0.001
4	-0.01	-0.02, 0.00	0.089	-0.01	-0.01, -0.00	0.011	<0.001
5	-0.01	-0.03, 0.00	0.143	-0.01	-0.01, -0.00	0.006	0.001
6	-0.01	-0.03, -0.00	0.026	-0.01	-0.02, -0.00	0.005	<0.001
7	-0.01	-0.02, -0.00	0.049	-0.01	-0.01, 0.00	0.052	<0.001
8	-0.01	-0.02, 0.00	0.147	-0.01	-0.01, 0.00	0.075	0.001
9	-0.02	-0.03, -0.00	0.015	-0.01	-0.01, 0.00	0.078	<0.001
10	-0.01	-0.03, 0.00	0.051	-0.01	-0.01, -0.00	0.023	<0.001
11	-0.02	-0.03, -0.00	0.042	-0.01	-0.01, -0.00	0.011	<0.001
12	-0.01	-0.03, 0.00	0.072	-0.01	-0.01, -0.00	0.020	<0.001
13	-0.01	-0.03, 0.00	0.055	-0.01	-0.01, 0.00	0.061	<0.001
14	-0.01	-0.02, 0.00	0.088	-0.01	-0.01, 0.00	0.089	<0.001
15	-0.01	-0.02, 0.01	0.201	-0.01	-0.01, 0.00	0.063	<0.001
16	-0.01	-0.02, 0.01	0.385	-0.01	-0.01, -0.00	0.034	<0.001
17	-0.01	-0.02, 0.00	0.156	-0.01	-0.01, -0.00	0.022	<0.001
18	-0.01	-0.02, 0.01	0.314	-0.01	-0.01, 0.00	0.092	<0.001
19	-0.01	-0.02, 0.01	0.281	-0.00	-0.01, 0.00	0.193	<0.001
20	-0.01	-0.02, 0.01	0.319	-0.00	-0.01, 0.00	0.172	<0.001
21	-0.01	-0.02, 0.00	0.088	-0.00	-0.01, 0.00	0.105	<0.001
22	-0.01	-0.02, 0.01	0.380	-0.01	-0.01, -0.00	0.009	<0.001
23	-0.01	-0.02, -0.00	0.041	-0.01	-0.01, -0.00	0.003	<0.001
24	-0.02	-0.03, -0.00	0.009	-0.01	-0.01, -0.00	0.028	<0.001
25	-0.02	-0.03, -0.00	0.016	-0.01	-0.01, 0.00	0.055	<0.001
26	-0.01	-0.03, 0.00	0.120	-0.01	-0.01, 0.00	0.066	<0.001
27	0.00	-0.02, 0.01	0.651	-0.00	-0.01, 0.00	0.420	<0.001
28	-0.01	-0.02, 0.00	0.153	-0.00	-0.01, 0.00	0.269	<0.001

29	-0.01	-0.02, -0.00	0.043	-0.00	-0.01, 0.00	0.108	<0.001
30	-0.01	-0.02, -0.00	0.006	-0.01	-0.01, 0.00	0.068	<0.001
31	-0.01	-0.02, -0.00	0.021	-0.01	-0.01, -0.00	0.026	<0.001
32	-0.01	-0.02, 0.00	0.095	-0.01	-0.01, 0.00	0.058	<0.001
33	-0.01	-0.02, 0.01	0.190	-0.01	-0.01, -0.00	0.032	<0.001
34	-0.02	-0.03, -0.00	0.021	-0.01	-0.01, 0.00	0.054	<0.001
35	-0.01	-0.02, -0.00	0.037	-0.00	-0.01, 0.00	0.204	<0.001

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Chapter 3.

Final Discussion and Conclusions

3.1. Summary

This cross-sectional study using linked data between the Canadian Health Measures Survey (CHMS), the national land use regression model dataset and data from the National Air Pollutants Surveillance (NAPS), brings additional epidemiological knowledge on the effects of daily and annual air pollution exposure and systemic inflammation in Canada. Furthermore, it provides new information on the influence of Statins on these effects.

This research had the aim of investigating the effects of fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2) on four biomarkers of inflammation in a sample of adults who were representative of the Canadian population. Our first *a priori* hypothesis was that, in cities, daily and annual exposures to $PM_{2.5}$ and NO_2 would be associated with increases in biomarkers of inflammation. In addition, I hypothesized that using HMG-CoA reductase inhibitors, or Statins, would reduce the inflammatory effects in users when exposed to $PM_{2.5}$ and/or NO_2.

The paper (Chapter 2) reported that, contrary to our hypothesis, we found that annual and daily NO_2 was inversely associated with fibrinogen. The results of the regression models on the association between annual NO_2 and fibrinogen showed that for an increase of 10 $\mu g/m^3$ in annual NO_2, there was an association between -0.077 to -0.002 g/L plasma fibrinogen 19 times out of 20. My findings also showed that, for daily NO_2, 75% (27 of 36 models) of the models using average NO_2 and levels of fibrinogen on the day of the blood draw and the previous 35 days, showed an inverse association ($p<0.05$).

Finally, after stratification of all my models by Statins usage, despite the fact that the results of effect modification analyses showed that some of the associations between biomarkers and pollutants were found significantly different ($p<0.05$), the magnitudes of the associations were very small, which means that although statistically significant, the differences are not clinically significant.

3.2. Research Contributions

Research reporting on the association between air pollution and mortality in major cities across the country has been common (Crouse, Goldberg, & Ross, 2009; Gan et al., 2010; Gilbert & Goldberg, 2005; Stieb, Judek, & Burnett, 2002; Villeneuve et al., 2003) however, Canadian research looking at air pollution exposure and systemic inflammation is sparse (Allen et al., 2011; Kajbafzadeh et al., 2015).

In this study, I used a model based on the causal diagram that I created, based on a priori knowledge, and on assumptions of the relationships (causal and temporal) between the variables. My findings on the inverse association between NO_2 and fibrinogen were found to be consistent with other studies. Although these results may be puzzling when trying to explain the molecular mechanism behind this association, it may perhaps bring forward a new perspective on the influence of NO_2 on fibrinogen not as a biomarker of inflammation but maybe on its role in coagulation.

Finally, this study also looked at the possible protective effects of Statins after exposure to $PM_{2.5}$ and NO_2. Following a review by Li, Rittenhouse-Olson, Scheider, & Mu (2012) on the effect of particulate matter on CRP, it speculated that the lack of finding an association in a population of adults with chronic inflammatory conditions may have been due to the use of anti-inflammatory medications. Similarly, the study conducted by Hertel et al. (2010) had found that Statins users did not show an increase in CRP when modeling an interaction for the 28-day mean exposure of $PM_{2.5}$. Instead, as discussed previously, despite the fact that my findings showed statistical significance while modeling effect modification by Statins usage, the difference in effects between statin users and non-users are small and unlikely to be clinically important.

3.3. Limitations and Future Work

Using administrative data gave us access to a large sample size, and clear protocols and procedures throughout the study. Methods to convince participants of the importance of taking part in the study were implemented to help reduce systematic bias. Despite those measures, response rate for the CHMS cycles 1 and 2 were still low which may have consequently induced a non-response bias. It is important to note that the CHMS response rate is based on multiple calculations, not just a simple multiplication of the response rates, for the obtainment of a combined response rate at the different levels: dwellings, households, questionnaires and MEC.

In addition, this cross-sectional design gave us access to only one measurement for each biomarker. Having only one measurement prevented us from observing the possible baseline inflammatory level of each respondent. For example, the respondent may have exercised before the MEC appointment, which could have led to a change in their CRP level or he/she may have had the beginning of a dental infection, which could have led to an increase in their WBC count. Using this biomarker measure implied the possibility of information bias leading to non-differential misclassification, which could possibly lead to a bias toward the null. The accuracy of the respondents' inflammatory level due to air pollution would have been strengthened with more than one phlebotomy collection, because a few measurements of each biomarker over several days would have allowed us to model the temporality of inflammation relative to air pollution to better assess the inflammatory response that was attributable to the exposure.

Also, I hypothesised that using the respondents' home addresses instead of the clinic addresses to quantify the respondents' daily exposure would allow for a more accurate assessment of exposure to $PM_{2.5}$ and NO_2. The daily air pollution dataset was created using the closest monitor to the residential postal code however the daily mobility of the respondents was not taken in consideration. Adding work location would have improved the exposure assessment (Setton et al., 2010). Omitting this information, as well as indoor exposure and time spent outdoors, may have potentially introduced measurement error in air pollutant exposure due to the daily mobility of the respondents (Setton et al., 2010; Zou, Wilson, Zhan, & Zeng, 2009).

Moreover, different kinds of particulate matter monitors are used by the National Air Pollutants Surveillance (NAPS). Although all the monitors use mass concentrations to assess the level of $PM_{2.5}$ in the air, they do not do this with equal accuracy. Specifically, the tapered element oscillating microbalance (TEOM) has been found to underestimate concentration of fine particulate matter because of nitrate evaporation which leads to reporting differences in the $PM_{2.5}$ levels (Environment Canada, 2013; as cited in Hystad et al., 2011). In addition to this difference between monitors, the NAPS program gradually replaced $PM_{2.5}$ monitors, based on newer technology, between 2007 and 2013. The newer monitors measure the semi-volatile $PM_{2.5}$ mass that was not captured by the older instruments (Environment Canada, 2014). My dataset was created based on data from 2007 to 2011, which means that I may have utilized readings gathered from old and new monitors. To avoid measurement error, in the case where multiple monitors were present at one location, I should have selected the TEOM monitors when possible, despite their limitations, as they are used in greater number throughout the country. The way I created my dataset may have produced differential misclassification of exposure, which could have created information bias toward, or away from, the null.

Alternatively, some authors (Becker et al., 2005; Wu et al., 2012) argued that evaluating the true impact of $PM_{2.5}$ exposure on systemic inflammation may be better done by analysing the chemical compositions of the particulate matter (PM) which was not possible in my study. The toxicity of PM is not necessarily related to its mass concentration, but instead, to its size where the smallest particles are found to have the highest toxicity (de Kok, Driece, Hogervorst, & Briedé, 2006; Valavanidis, Fiotakis, & Vlachogianni, 2008). Moreover, PM's chemical composition has been found to vary both in time and space across the cities (Chow et al., 2006; Levy, Mihele, Lu, Narayan, & Brook, 2014); this implies that the chemical composition that the participants are exposed to across the country will be quite different. There is now some evidence suggesting that particulate matter from traffic exposure may be more toxic than particulate from other sources (Brook et al., 2010; Hennig et al., 2014; Kajbafzadeh et al., 2015). Furthermore, the CHMS was not designed to allow region-specific analyses due to the small number of collection sites. Although I can investigate the national average associations between $PM_{2.5}$ and inflammatory markers, the associations might

be elevated in some areas compared to others due to different composition of particles. Due to averaging at the national level, it is thus possible that regional associations between $PM_{2.5}$ and inflammatory markers will be missed by this analysis, as well as the variability in the associations in different regions in the country.

As mentioned previously, this study was based on the linkage of three different datasets. The dataset for the daily air pollution concentration was created based on the date and time of the respondents' visit to the mobile examination centre (MEC). In contrast, the national land use regression model dataset was created based on the air pollution concentrations from the year 2006 while the CHMS surveys were conducted between 2007 and 2011. Thus discrepancy in air pollution concentration levels between the year of the creation of the national land use regression model dataset, and the administration of the CHMS two cycles may have introduce differential misclassification in the exposure measurement, which possibly would lead to a bias away from, or toward, the null.

With regard to temperature, studies have found that change in temperature may induce fluctuations in air pollution concentration, as well as variation in biomarker levels (Halonen, Zanobetti, Sparrow, Vokonas, & Schwartz, 2010; Levy et al., 2014; Lewis, De Young, Ferrare, & Allen Chu, 2010; Plummer et al., 2012; Schäuble et al., 2012; WHO, 2003). However, only the temperature of the day of the blood draw was a part of my model. Recent studies have shown that using a few days average, or different moving averages, would be a better indicator of the effect of temperature on the biomarker levels (Halonen et al., 2010; Schäuble et al., 2012; Wilker, Yeh, & Wellenius, 2012).

As discussed in chapter 2, the findings on the association between NO_2 and fibrinogen show conflicting trends between studies, however the inverse association found in my study has been seen in other studies. Both short-term and long-term studies (Hajat et al., 2015; Steinvil et al., 2008) have reported a reduction between 1.16% and 3.88% in fibrinogen after NO_2 exposure. The associations found in my study were for most of the NO_2 concentrations from the day of the blood draw to 35 days previous to the blood draw. My study also found an association with annual average NO_2 from 1 to 5 years prior to the blood draw. Knowing that NO_2 has hourly and daily

variations, and that its concentrations vary with distance to source (WHO, 2005), one could question the root of the cumulative adverse health effects on chronic exposure, or on repeated NO_2 exposure over days to weeks. A probable explanation may reside in the interplay of chronic and repeated acute exposures.

To learn more about the interplay between NO_2 and inflammation, controlled clinical studies looking at NO_2 exposure and biomarkers of inflammation may help understanding this relationship. Most clinical exposure studies on NO_2 exposure relate to lung function. This interest may be due to the fact that although 70 to 90% of NO_2 is inhaled, a significant portion of this is removed in the nasopharynx, and it is only when oral respiration becomes more predominant, for example during exercise, that NO_2 travels deeper in the respiratory tract (WHO, 2005). These studies also use concentration levels that are much higher that normal daily exposures. Similar to our case, because the goal is not to induce changes in pulmonary function, studying changes in biomarker levels could be enhanced by using concentrations closer to daily averages, and by using different lengths of time of exposure and different time differentials between exposure and response.

My study focused on systemic inflammation, and fibrinogen, being an acute phase reactant, was used as a biomarker of inflammation. As discussed in chapter 1, fibrinogen also plays a role in coagulation. A decrease in fibrinogen level may signify, an increased turnover of fibrinogen due to an increase consumption of fibrinogen. Following these results, it may be hypothesised that a decrease concentration in fibrinogen following NO_2 exposure may lead to a decrease in fibrin formation however the clinical significance is not clear.

As a result, the association between air pollution exposure and systemic inflammation seems to be based on the intricacy of different relationships – air pollution, temperature, and biomarkers. The existence of different time frame between the effects of temperature and air pollution on biomarkers levels, as well as the effects of temperature on air pollution levels in addition to the changes in the biomarkers level due to these influences, complicates the understanding of these relationships. In this study, while trying to establish a pathway between air pollution exposure and atherosclerosis or

diabetes, where both conditions are known to have the potential to lead to cardiovascular disease or death, I postulated that this pathway was operating through systemic inflammation. Yet, to be able to understand this pathway, the difficulty resides in learning about, and understanding, the different time frames.

In summary, the association between air pollution exposure and inflammation needs to be studied and modeled by incorporating different time frames between air pollutants levels and temperature change. In addition, more research is needed to look into finding which biomarkers of inflammation could be more specific to the exposures, if any. Likewise, the understanding of the interaction between different biomarkers would bring forward the predictive power of their combinations. Furthermore, the assessment of exposure needs to be modeled by keeping in mind the interaction – on either multiplicative or additive scales - between pollutants which may affect the impact of exposure on the health outcomes (Mauderly & Samet, 2009; WHO, 2003) Multicollinearity is also a concern when modeling multiple pollutants together. Finally, the emergence of the understanding of the Statins' pleiotrophy and the influence of Statins in the relationships between air pollution and inflammation adds another layer of interest in future studies.

In conclusion, I believe that research on air pollution exposure and systemic inflammation is of great importance due to the detrimental downstream health effects. Even in cities with low pollutants levels, previous research found that increased air pollutant concentrations were associated with adverse effects on health and that "…it is important to understand how long-term, short-term, and very short-term (subdaily over the course of several hours) exposure to air pollution affects disease mechanisms and particularly disease progression and reversibility" (Giles et al., 2011). By addressing these knowledge gaps we may move forward and perhaps support the hypothesis that air pollution exposure impacts the prevalence of atherosclerosis and diabetes through systemic inflammation.

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Appendix A.

Original Abstracts for Manuscript Chapter 2

Background: Air pollution exposure might induce systemic inflammation, but previous research results were inconsistent. Statins reduce biomarkers of inflammation so might protect against air pollution-induced inflammation.

Objectives: We investigated associations between fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2) with four biomarkers of systemic inflammation (high sensitivity C-reactive protein (hsCRP), fibrinogen, white blood cells (WBC), and platelets). We also investigated whether Statin usage modified the associations between air pollutants and biomarkers of inflammation.

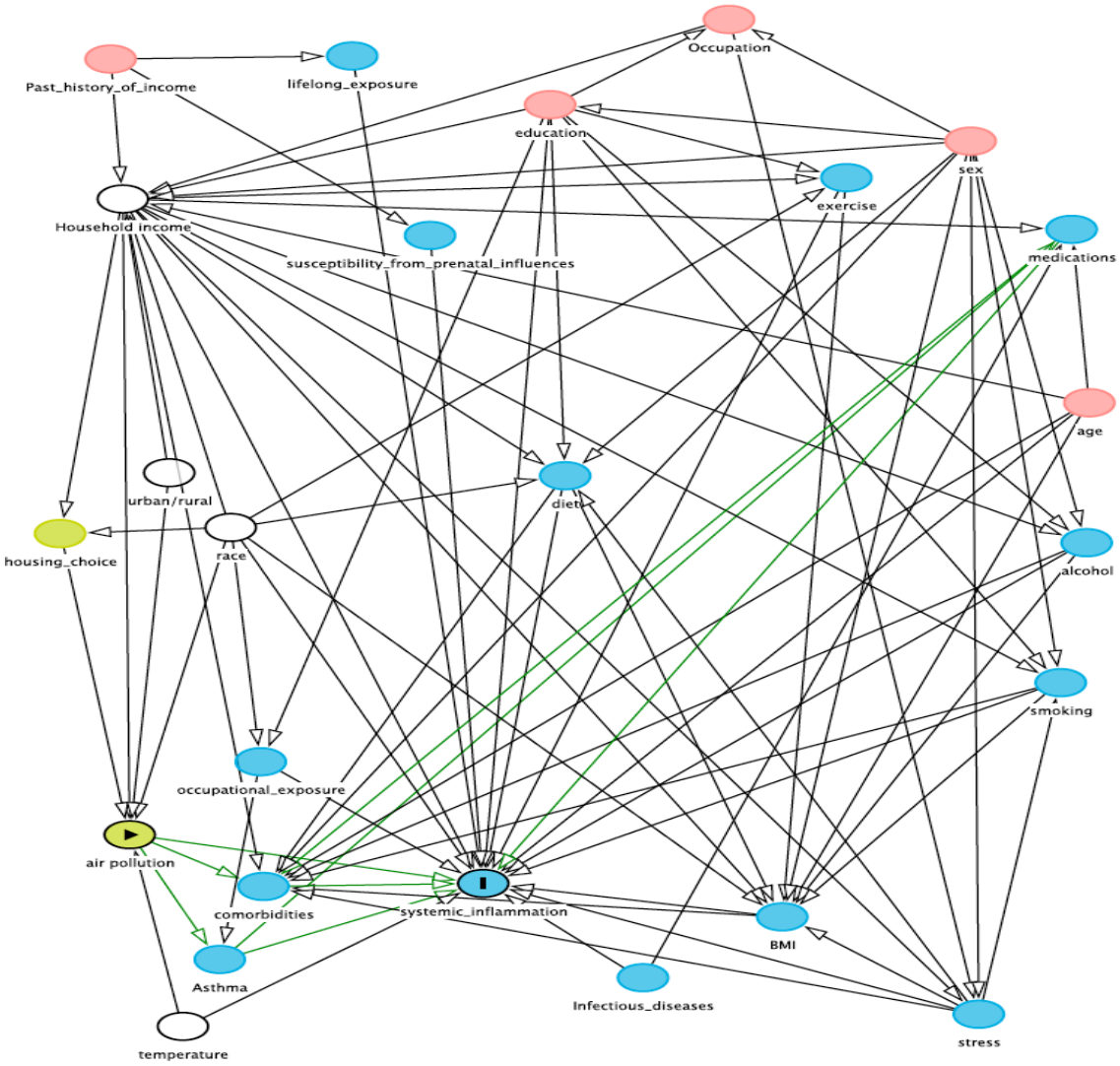
Methods: We linked residential daily and annual average ambient air pollution data to the Canadian Health Measures Survey (2007-2011), a nationally-representative cross-sectional survey. Our sample included 6,322 Canadian adults aged 18-79 and living in cities. We used weighted linear regression to model the associations between air pollutants and inflammatory biomarkers and investigated effect modification by Statin usage.

Results: Contrary to our hypothesis, daily NO_2 was inversely associated ($p<0.05$) with fibrinogen in 27 of 36 models using average NO_2 on the day of blood draw and the previous 35 days. Annual average NO_2 was also inversely associated ($p<0.05$) with fibrinogen. Inverse associations were slightly stronger among those taking statins (effect measure modification on additive scale $p<0.05$), but differences were not meaningfully large.

Conclusions: Annual and daily average NO_2 were inversely associated with fibrinogen in this sample, which is consistent with several previous studies. Our results neither support the hypothesis that $PM_{2.5}$ or NO_2 induce inflammation nor that Statins protect against this.

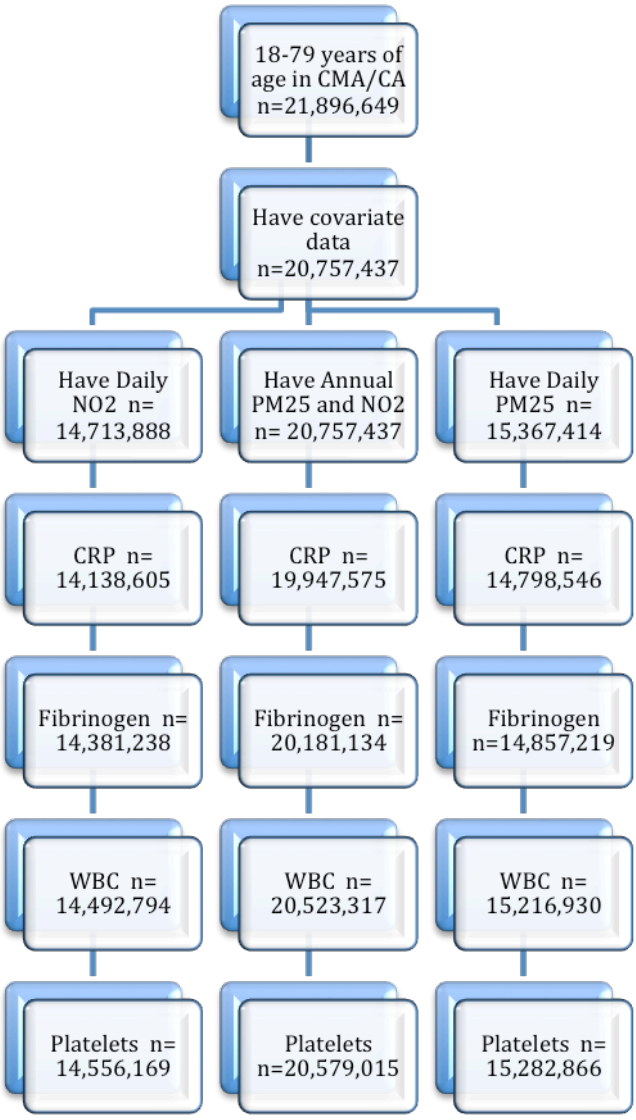
Appendix B.

Directed Acyclic Graph



Appendix C.

Sample Selection



Appendix D.

Daily Models

Associations of daily average residential ambient $PM_{2.5}$ and NO_2 with four biomarkers adjusted for race, income, and temperature in Canada

Table D1. Association between lnCRP and daily $PM_{2.5}$

Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p
0	0.01	0.00, 0.03	0.106
1	0.01	-0.02, 0.03	0.661
2	0.00	-0.02, 0.02	0.721
3	0.00	-0.02, 0.01	0.692
4	0.00	-0.02, 0.02	0.912
5	-0.01	-0.03, 0.01	0.172
6	-0.01	-0.02, 0.00	0.068
7	0.00	-0.01, 0.02	0.944
8	0.01	-0.01, 0.02	0.440
9	0.01	-0.01, 0.03	0.197
10	0.00	-0.01, 0.01	0.605
11	0.00	-0.01, 0.02	0.532
12	0.00	-0.02, 0.03	0.732
13	-0.01	-0.03, 0.01	0.401
14	0.00	-0.02, 0.02	0.825
15	0.01	0.00, 0.03	0.070
16	0.00	-0.01, 0.02	0.737
17	0.00	-0.02, 0.02	0.948
18	0.01	-0.01,0.02	0.547
19	0.02	0.00, 0.03	0.020
20	0.02	0.00, 0.03	0.052
21	0.00	-0.01, 0.02	0.760
22	0.00	-0.02, 0.03	0.775
23	0.01	-0.01, 0.03	0.410
24	0.01	-0.01, 0.02	0.278
25	0.01	-0.01, 0.02	0.272
26	0.01	0.00, 0.02	0.069
27	0.01	0.00, 0.03	0.011
28	0.02	0.00, 0.03	0.010
29	0.01	-0.01, 0.03	0.193
30	0.02	0.00, 0.03	0.041
31	0.01	0.00, 0.03	0.150

Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p
32	0.01	0.00, 0.02	0.088
33	0.01	0.00, 0.02	0.173
34	0.01	-0.01, 0.02	0.239
35	0.01	0.00, 0.02	0.053

Table D2. Association between fibrinogen and daily $PM_{2.5}$

Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p
0	-0.01	-0.02, 0.00	0.101
1	-0.01	-0.02, 0.00	0.058
2	-0.01	-0.02, 0.01	0.267
3	-0.01	-0.02, 0.01	0.300
4	0.00	-0.01, 0.01	0.666
5	-0.01	-0.02, 0.00	0.063
6	-0.01	-0.02, 0.00	0.107
7	0.00	-0.01, 0.01	0.328
8	0.00	-0.01, 0.01	0.386
9	0.00	-0.02, 0.01	0.395
10	-0.01	-0.02, 0.00	0.144
11	0.00	-0.02, 0.01	0.895
12	0.00	-0.01, 0.00	0.317
13	-0.01	-0.02, 0.00	0.086
14	-0.01	-0.02, 0.00	0.088
15	0.00	-0.01, 0.01	0.982
16	-0.01	-0.02, 0.01	0.438
17	-0.01	-0.02, 0.00	0.026
18	-0.01	-0.02, 0.00	0.125
19	0.00	-0.01, 0.01	0.909
20	0.00	-0.01, 0.01	0.814
21	0.00	-0.02, 0.01	0.596
22	-0.01	-0.02, 0.00	0.036
23	-0.01	-0.02, 0.00	0.082
24	0.00	-0.02, 0.01	0.568
25	0.00	-0.01, 0.01	0.816
26	0.00	-0.01, 0.02	0.927
27	0.00	-0.01, 0.01	0.974
28	0.00	-0.01, 0.01	0.927
29	-0.01	-0.02, 0.00	0.198
30	0.00	-0.01, 0.01	0.539
31	0.00	-0.01, 0.01	0.681
32	-0.01	-0.02, 0.00	0.106
33	-0.01	-0.02, 0.00	0.208
34	-0.01	-0.02, 0.01	0.321
35	0.00	-0.01, 0.00	0.265

Table D3. Association between WBC and daily $PM_{2.5}$

Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p
0	0.00	-0.02, 0.03	0.761
1	0.00	-0.03, 0.02	0.925
2	-0.01	-0.03, 0.02	0.517
3	0.00	-0.02, 0.02	0.877
4	0.01	-0.01, 0.04	0.299
5	0.01	-0.01, 0.03	0.508
6	0.00	-0.02, 0.02	0.855
7	0.00	-0.02, 0.02	0.634
8	0.01	-0.01, 0.02	0.278
9	0.01	-0.02, 0.02	0.502
10	-0.01	-0.03, 0.03	0.590
11	0.02	-0.01, 0.05	0.171
12	0.02	-0.02, 0.05	0.343
13	0.00	-0.02, 0.03	0.765
14	0.00	-0.02, 0.02	0.837
15	0.02	-0.01, 0.05	0.253
16	0.01	-0.01, 0.03	0.385
17	-0.01	-0.04, 0.01	0.349
18	0.01	-0.02, 0.03	0.614
19	0.01	-0.01, 0.03	0.236
20	0.00	-0.02, 0.03	0.703
21	0.01	-0.01, 0.03	0.168
22	0.01	0.01, 0.03	0.145
23	0.03	-0.00, 0.06	0.053
24	0.02	-0.01, 0.04	0.143
25	0.01	-0.01, 0.04	0.324
26	0.02	-0.01, 0.06	0.117
27	0.01	-0.01, 0.02	0.523
28	0.01	-0.02, 0.03	0.575
29	0.01	-0.01, 0.02	0.505
30	0.01	-0.01, 0.04	0.221
31	0.01	-0.01, 0.03	0.271
32	0.01	-0.01, 0.03	0.264
33	0.01	-0.01, 0.03	0.324
34	0.00	-0.02, 0.02	0.797
35	0.00	-0.01, 0.02	0.581

Table D4. Association between platelets and daily $PM_{2.5}$

Days before blood draw ($PM_{2.5}$)	Platelets β	95% CI	2-sided p
0	0.11	-0.89, 1.11	0.823
1	0.20	-0.57, 0.98	0.595
2	0.58	-0.49, 1.65	0.276
3	0.15	-1.13, 1.43	0.808
4	-0.07	-1.03, 0.89	0.881
5	-0.69	-1.52, 0.14	0.100
6	-0.82	-1.57, -0.08	0.032
7	-0.47	-1.44, 0.51	0.334
8	-0.16	-1.18, 0.85	0.744
9	-0.35	-1.40, 0.70	0.499
10	-0.30	-1.26, 0.66	0.530
11	-0.50	-1.23, 0.24	0.175
12	-0.27	-0.79, 0.26	0.310
13	-0.43	-1.03, 0.18	0.157
14	-0.12	-1.23, 0.99	0.827
15	0.52	-0.75, 1.78	0.406
16	-0.51	-1.32, 0.30	0.206
17	-0.77	-1.52, -0.02	0.044
18	-0.51	-1.28, 0.25	0.179
19	0.15	-0.83, 1.14	0.750
20	0.37	-0.65, 1.40	0.458
21	0.08	-0.90, 1.06	0.865
22	-0.29	-1.19, 0.62	0.518
23	-0.32	-1.16, 0.52	0.437
24	-0.40	-1.14, 0.35	0.285
25	-0.73	-1.56, 0.09	0.080
26	-0.46	-1.49, 0.57	0.364
27	-0.34	-1.32, 0.65	0.485
28	-0.25	-1.16, 0.65	0.570
29	-0.16	-1.23, 0.91	0.757
30	-0.26	-1.14, 0.62	0.543
31	-0.21	-0.88, 0.46	0.527
32	-0.35	-1.03, 0.34	0.306
33	-0.19	-0.73, 0.34	0.464
34	-0.20	-1.00, 0.59	0.601
35	-0.26	-1.09, 0.56	0.513

Table D5. Association between lnCRP and daily NO_2

Days before blood draw (NO_2)	β	95% CI	2-sided p
0	-0.01	-0.02, 0.00	0.175
1	0.00	-0.02, 0.01	0.387
2	-0.01	-0.02, 0.00	0.084
3	-0.01	-0.02, 0.00	0.060
4	-0.01	-0.02, 0.00	0.021
5	-0.01	-0.03, 0.00	0.068
6	-0.01	-0.03, 0.00	0.022
7	-0.01	-0.02, 0.00	0.045
8	-0.01	-0.02, 0.00	0.169
9	-0.01	-0.02, 0.00	0.183
10	-0.01	-0.02, 0.00	0.052
11	-0.01	-0.03, 0.00	0.134
12	-0.01	-0.02, 0.01	0.300
13	-0.01	-0.02, 0.00	0.138
14	-0.01	-0.02, 0.00	0.170
15	-0.01	-0.02, 0.00	0.165
16	-0.01	-0.02, 0.00	0.089
17	-0.01	-0.02, 0.00	0.063
18	-0.01	-0.02, 0.00	0.113
19	-0.01	-0.02, 0.00	0.215
20	-0.01	-0.02, 0.01	0.243
21	-0.01	-0.02, 0.01	0.396
22	-0.01	-0.02, 0.01	0.286
23	-0.01	-0.03, 0.01	0.185
24	-0.01	-0.02, 0.00	0.080
25	-0.01	-0.03, 0.00	0.014
26	-0.01	-0.02, 0.00	0.029
27	-0.01	-0.02, 0.01	0.291
28	-0.01	-0.02, 0.00	0.185
29	-0.01	-0.02, 0.01	0.447
30	-0.01	-0.02, 0.01	0.367
31	-0.01	-0.02, 0.01	0.226
32	-0.01	-0.02, 0.01	0.235
33	-0.01	-0.02, 0.00	0.177
34	-0.01	-0.02, 0.00	0.106
35	-0.01	-0.02, 0.01	0.318

Table D6. Association between fibrinogen and daily NO_2

Days before blood draw (NO_2)	β	95% CI	2-sided p
0	-0.01	-0.01, 0.00	0.019
1	-0.01	-0.01, 0.00	0.058
2	-0.01	-0.01, 0.00	0.034
3	-0.01	-0.01, 0.00	0.054
4	-0.01	-0.01, 0.00	0.006
5	-0.01	-0.01, 0.00	0.003
6	-0.01	-0.02, 0.00	0.002
7	-0.01	-0.01, 0.00	0.022
8	-0.01	-0.01, 0.00	0.034
9	-0.01	-0.01, 0.00	0.028
10	-0.01	-0.02, 0.00	0.007
11	-0.01	-0.02, 0.00	0.004
12	-0.01	-0.02, 0.00	0.009
13	-0.01	-0.02, 0.00	0.032
14	-0.01	-0.01, 0.00	0.049
15	-0.01	-0.01, 0.00	0.027
16	-0.01	-0.01, 0.00	0.030
17	-0.01	-0.01, 0.00	0.013
18	-0.01	-0.01, 0.00	0.068
19	0.00	-0.01, 0.00	0.124
20	0.00	-0.01, 0.00	0.114
21	-0.01	-0.01, 0.00	0.059
22	-0.01	-0.01, 0.00	0.005
23	-0.01	-0.01, 0.00	0.001
24	-0.01	-0.01, 0.00	0.009
25	-0.01	-0.01, 0.00	0.015
26	-0.01	-0.01, 0.00	0.026
27	0.00	-0.01, 0.00	0.265
28	-0.01	-0.01, 0.00	0.121
29	-0.01	-0.01, 0.00	0.039
30	-0.01	-0.01, 0.00	0.022
31	-0.01	-0.01, 0.00	0.008
32	-0.01	-0.01, 0.00	0.021
33	-0.01	-0.01, 0.00	0.017
34	-0.01	-0.01, 0.00	0.014
35	-0.01	-0.01, 0.00	0.072

Table D7. Association between WBC and daily NO_2

Days before blood draw (NO_2)	β	95% CI	2-sided p
0	0.00	-0.03, 0.02	0.758
1	-0.01	-0.03, 0.01	0.355
2	-0.01	-0.03, 0.01	0.210
3	-0.02	-0.04, 0.00	0.050
4	-0.01	-0.0, 0.001	0.510
5	-0.01	-0.03, 0.02	0.539
6	-0.02	-0.03, 0.00	0.044
7	-0.01	-0.03, 0.01	0.330
8	-0.01	-0.03, 0.01	0.373
9	-0.02	-0.04, 0.00	0.111
10	-0.02	-0.04, 0.01	0.126
11	-0.01	-0.04, 0.01	0.270
12	-0.01	-0.03, 0.02	0.709
13	-0.01	-0.03, 0.01	0.477
14	-0.01	-0.03, 0.01	0.324
15	-0.01	-0.03, 0.01	0.396
16	-0.01	-0.03, 0.01	0.316
17	-0.01	-0.03, 0.00	0.097
18	-0.01	-0.03, 0.00	0.116
19	-0.01	-0.03, 0.01	0.287
20	-0.01	-0.03, 0.01	0.390
21	-0.01	-0.03, 0.02	0.484
22	0.00	-0.03, 0.02	0.890
23	-0.01	-0.03, 0.01	0.256
24	-0.01	-0.03, 0.02	0.503
25	-0.02	-0.04, -0.01	0.013
26	-0.02	-0.03, 0.00	0.066
27	-0.01	-0.03, 0.01	0.312
28	-0.01	-0.04, 0.01	0.227
29	-0.01	-0.04, 0.01	0.380
30	-0.01	-0.03, 0.01	0.325
31	-0.01	-0.04, 0.02	0.396
32	-0.01	-0.04, 0.01	0.358
33	-0.01	-0.04, 0.01	0.322
34	-0.01	-0.04, 0.01	0.164
35	-0.01	-0.04, 0.02	0.563

Table D8. Association between platelets and daily NO_2

Days before blood draw (NO_2)	β	95% CI	2-sided p
0	0.00	-0.54, 0.55	0.987
1	-0.04	-0.55, 0.48	0.887
2	-0.12	-0.75, 0.52	0.707
3	-0.05	-0.82, 0.71	0.884
4	0.00	-0.68, 0.68	1.000
5	0.06	-0.55, 0.67	0.846
6	-0.21	-0.89, 0.46	0.520
7	-0.20	-0.77, 0.38	0.487
8	-0.10	-0.63, 0.42	0.687
9	-0.10	-0.71, 0.52	0.743
10	-0.10	-0.83, 0.63	0.786
11	-0.26	-0.86, 0.35	0.385
12	0.00	-0.59, 0.59	0.995
13	-0.09	-0.69, 0.52	0.763
14	-0.10	-0.73, 0.53	0.753
15	0.02	-0.64, 0.68	0.949
16	-0.19	-0.76, 0.38	0.505
17	-0.28	-0.84, 0.27	0.302
18	-0.20	-0.66, 0.26	0.385
19	0.01	-0.49, 0.50	0.973
20	0.07	-0.44, 0.58	0.775
21	0.09	-0.50, 0.68	0.747
22	0.05	-0.45, 0.54	0.842
23	0.06	-0.58, 0.70	0.840
24	-0.06	-0.66, 0.53	0.829
25	-0.39	-0.95, 0.16	0.156
26	-0.27	-0.85, 0.30	0.339
27	-0.01	-0.62, 0.60	0.970
28	-0.08	-0.69, 0.52	0.778
29	-0.01	-0.57, 0.55	0.966
30	0.05	-0.50, 0.59	0.860
31	-0.04	-0.58, 0.49	0.868
32	-0.27	-0.69, 0.14	0.188
33	-0.20	-0.72, 0.33	0.447
34	-0.18	-0.83, 0.47	0.581
35	-0.11	-0.66, 0.44	0.683

Appendix E.

Daily Models Stratified by Statin Usage

Associations of daily average residential ambient $PM_{2.5}$ and NO_2 with four biomarkers adjusted for race, income, and temperature in Canada and stratified by Statin usage

Table E1. Association between lnCRP and daily $PM_{2.5}$

	lnCRP Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	0.01	-0.01, 0.04	0.267	0.01	0.00, 0.03	0.122	0.001
1	0.00	-0.03, 0.03	0.977	0.01	-0.02, 0.04	0.640	0.002
2	-0.01	-0.03, 0.02	0.585	0.01	-0.02, 0.03	0.627	0.002
3	-0.01	-0.04, 0.01	0.246	0.00	-0.02, 0.02	0.907	0.001
4	-0.01	-0.04, 0.02	0.440	0.00	-0.02, 0.02	0.760	<0.001
5	-0.01	-0.04, 0.01	0.310	-0.01	-0.03, 0.01	0.244	0.002
6	0.00	-0.03, 0.03	0.960	-0.01	-0.02, 0.00	0.065	<0.001
7	-0.01	-0.04, 0.02	0.492	0.00	-0.01, 0.02	0.728	<0.001
8	-0.01	-0.03, 0.02	0.527	0.01	-0.01, 0.02	0.270	0.001
9	-0.01	-0.04, 0.03	0.717	0.01	0.00, 0.03	0.142	0.002
10	-0.02	-0.05, 0.00	0.037	0.01	0.00, 0.02	0.253	0.002
11	0.00	-0.03, 0.04	0.885	0.01	-0.01, 0.02	0.489	0.004
12	0.01	-0.01, 0.03	0.322	0.00	-0.02, 0.03	0.769	0.001
13	0.01	-0.01, 0.04	0.355	-0.01	-0.03, 0.01	0.370	0.001
14	0.00	-0.04, 0.03	0.827	0.00	-0.02, 0.02	0.765	<0.001
15	0.00	-0.03, 0.02	0.821	0.02	0.00, 0.03	0.052	0.001
16	-0.02	-0.05, 0.01	0.248	0.00	-0.01, 0.02	0.577	0.001
17	-0.01	-0.04, 0.02	0.694	0.00	-0.02, 0.02	0.972	<0.001
18	-0.01	-0.05, 0.03	0.707	0.01	-0.01, 0.02	0.446	<0.001
19	0.02	-0.01, 0.04	0.135	0.02	0.00, 0.03	0.028	<0.001
20	0.01	-0.02, 0.03	0.702	0.02	0.00, 0.3	0.041	0.001
21	0.00	-0.02, 0.02	0.994	0.00	-0.02, 0.02	0.724	0.001
22	-0.01	-0.04, 0.02	0.422	0.01	-0.02, 0.03	0.644	0.003
23	-0.02	-0.04, 0.01	0.199	0.01	-0.01, 0.02	0.289	0.002

	lnCRP Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
24	-0.01	-0.04, 0.02	0.592	0.01	0.00, 0.02	0.141	0.001
25	-0.02	-0.04, 0.01	0.119	0.01	0.00, 0.02	0.159	<0.001
26	0.00	-0.02, 0.02	0.781	0.01	0.00, 0.03	0.063	<0.001
27	0.01	-0.01, 0.03	0.508	0.02	0.00, 0.03	0.009	0.001
28	0.01	-0.01, 0.04	0.224	0.02	0.00, 0.03	0.013	0.001
29	0.01	-0.01, 0.04	0.209	0.01	-0.01, 0.03	0.241	0.002
30	0.00	-0.03, 0.03	0.997	0.02	0.00, 0.03	0.030	0.004
31	-0.01	-0.04, 0.02	0.351	0.01	0.00, 0.03	0.118	0.001
32	-0.02	-0.04, 0.01	0.204	0.01	0.00, 0.03	0.040	0.003
33	-0.01	-0.04, 0.02	0.456	0.01	0.00, 0.02	0.119	0.002
34	0.01	-0.02, 0.04	0.628	0.01	-0.01, 0.02	0.221	0.001
35	0.01	-0.02, 0.04	0.412	0.01	0.00, 0.02	0.131	0.003

Table E2. Association of fibrinogen and daily $PM_{2.5}$

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	-0.01	-0.04, 0.01	0.225	-0.01	-0.02, 0.00	0.112	0.014
1	-0.01	-0.03, 0.02	0.583	-0.01	-0.03, 0.00	0.048	0.002
2	0.00	-0.02, 0.01	0.621	-0.01	-0.02, 0.01	0.248	0.002
3	-0.01	-0.02, 0.01	0.317	-0.01	-0.02, 0.01	0.314	0.001
4	-0.01	-0.03, 0.01	0.177	0.00	-0.01, 0.01	0.837	0.005
5	-0.01	-0.03, 0.01	0.294	-0.01	-0.02, 0.00	0.078	0.005
6	-0.01	-0.04, 0.02	0.432	-0.01	-0.02, 0.00	0.148	0.029
7	-0.01	-0.04, 0.02	0.611	0.00	-0.01, 0.01	0.415	0.013
8	-0.01	-0.04, 0.02	0.424	0.00	-0.01, 0.01	0.570	0.019
9	0.00	-0.03, 0.02	0.708	0.00	-0.01, 0.01	0.456	<0.001
10	-0.01	-0.04, 0.01	0.233	-0.01	-0.02, 0.00	0.262	0.006
11	0.00	-0.02, 0.02	0.930	0.00	-0.01, 0.02	0.955	0.001
12	-0.01	-0.04, 0.02	0.543	0.00	-0.01, 0.01	0.460	0.056
13	-0.01	-0.03, 0.01	0.258	-0.01	-0.02, 0.00	0.132	0.006
14	0.00	-0.02, 0.01	0.644	-0.01	-0.02, 0.00	0.104	<0.001
15	0.00	-0.02, 0.02	0.980	0.00	-0.01, 0.01	0.887	0.001
16	0.00	-0.02, 0.03	0.965	-0.01	-0.02, 0.01	0.296	<0.001
17	-0.01	-0.03, 0.02	0.614	-0.01	-0.02, 0.00	0.012	<0.001
18	-0.01	-0.03, 0.02	0.611	-0.01	-0.02, 0.00	0.117	0.004
19	0.01	-0.01, 0.02	0.228	0.00	-0.02, 0.01	0.613	<0.001
20	0.00	-0.02, 0.02	0.795	0.00	-0.01, 0.01	0.552	0.009
21	-0.01	-0.03, 0.01	0.389	0.00	-0.01, 0.01	0.692	0.023
22	0.00	-0.02, 0.01	0.556	-0.01	-0.02, 0.00	0.024	<0.001
23	0.00	-0.02, 0.01	0.604	-0.01	-0.02, 0.00	0.078	<0.001
24	0.00	-0.02, 0.02	0.986	0.00	-0.02, 0.01	0.604	<0.001
25	-0.01	-0.03, 0.01	0.488	0.00	-0.01, 0.01	0.869	<0.001
26	0.00	-0.01, 0.01	0.886	0.00	-0.02, 0.02	0.943	<0.001
27	0.00	-0.02, 0.02	0.952	0.00	-0.01, 0.01	0.973	<0.001
28	0.00	-0.02, 0.02	0.925	0.00	-0.01, 0.01	0.918	<0.001
29	0.00	-0.01, 0.01	0.487	-0.01	-0.02, 0.00	0.167	<0.001
30	0.00	-0.01, 0.01	0.645	0.00	-0.01, 0.01	0.473	<0.001

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	0.00	-0.01, 0.01	0.944	0.00	-0.01, 0.01	0.627	<0.001
32	-0.01	-0.04, 0.01	0.300	-0.01	-0.02, 0.00	0.107	0.031
33	-0.01	-0.04, 0.01	0.372	-0.01	-0.02, 0.00	0.220	0.011
34	0.00	-0.02, 0.01	0.836	-0.01	-0.02, 0.00	0.263	<0.001
35	0.01	-0.01, 0.03	0.418	-0.01	-0.01, 0.00	0.130	<0.001

Table E3. Association between WBC and daily $PM_{2.5}$

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	-0.01	-0.06, 0.04	0.585	0.00	-0.02, 0.03	0.738	0.241
1	0.00	-0.05, 0.05	0.940	0.00	-0.03, 0.02	0.796	0.097
2	0.01	-0.05, 0.08	0.692	-0.01	-0.03, 0.01	0.429	0.130
3	0.01	-0.03, 0.06	0.629	0.00	-0.02, 0.02	0.762	0.141
4	0.01	-0.04, 0.06	0.590	0.01	-0.01, 0.04	0.312	0.173
5	0.01	-0.02, 0.04	0.552	0.01	-0.02, 0.03	0.574	0.120
6	0.02	-0.04, 0.08	0.428	0.00	-0.02, 0.02	0.761	0.127
7	0.02	-0.04, 0.08	0.484	0.00	-0.02, 0.02	0.924	0.075
8	0.01	-0.05, 0.07	0.731	0.01	-0.01, 0.03	0.271	0.160
9	-0.01	-0.07, 0.05	0.703	0.01	-0.01, 0.03	0.408	0.166
10	-0.02	-0.07, 0.03	0.448	0.00	-0.03, 0.02	0.754	0.223
11	0.04	-0.03, 0.11	0.305	0.02	-0.01, 0.05	0.202	0.088
12	0.07	0.01, 0.14	0.032	0.01	-0.03, 0.05	0.572	0.040
13	0.07	-0.02, 0.15	0.122	0.00	-0.03, 0.03	0.928	0.044
14	0.02	-0.03, 0.07	0.391	0.00	-0.02, 0.02	0.926	0.114
15	0.02	-0.02, 0.06	0.345	0.02	-0.01, 0.05	0.255	0.141
16	0.04	0.00, 0.08	0.039	0.01	-0.02, 0.03	0.679	0.044
17	0.04	-0.03, 0.10	0.260	-0.02	-0.04, 0.01	0.211	0.062
18	-0.01	-0.06, 0.05	0.781	0.01	-0.02, 0.03	0.577	0.227
19	0.00	-0.04, 0.04	0.932	0.01	-0.01, 0.03	0.334	0.104
20	0.00	-0.05, 0.05	0.999	0.00	-0.02, 0.03	0.757	0.190
21	0.07	0.02, 0.12	0.010	0.01	-0.01, 0.03	0.516	0.012
22	0.06	-0.03, 0.14	0.171	0.01	-0.01, 0.03	0.444	0.062
23	0.05	-0.03, 0.13	0.252	0.03	-0.01, 0.06	0.108	0.166
24	0.04	-0.02, 0.10	0.158	0.01	-0.01, 0.04	0.217	0.083
25	0.03	-0.02, 0.08	0.192	0.01	-0.02, 0.04	0.447	0.060
26	0.04	0.00, 0.09	0.065	0.02	-0.01, 0.06	0.230	0.067
27	0.03	-0.02, 0.07	0.250	0.00	-0.02, 0.02	0.700	0.015
28	0.02	-0.02, 0.06	0.355	0.00	-0.02, 0.03	0.761	0.034
29	0.00	-0.05, 0.06	0.896	0.01	-0.02, 0.03	0.547	0.305
30	0.00	-0.05, 0.04	0.909	0.02	-0.01, 0.04	0.231	0.262

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	-0.02	-0.06, 0.02	0.278	0.01	-0.01, 0.04	0.224	0.428
32	-0.01	-0.05, 0.03	0.497	0.01	-0.01, 0.03	0.176	0.486
33	0.02	-0.02, 0.05	0.318	0.01	-0.01, 0.03	0.378	0.067
34	0.04	0.00, 0.09	0.048	0.00	-0.02, 0.02	0.908	0.006
35	0.01	-0.04, 0.06	0.604	0.00	-0.01, 0.02	0.677	0.198

Table E4. Association between platelets and daily $PM_{2.5}$

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	-0.60	-2.14, 0.93	0.425	0.20	-0.86, 1.25	1.25	0.703
1	-0.08	-1.43, 1.26	0.897	0.27	-0.64, 1.17	1.17	0.551
2	0.85	-1.68, 3.38	0.494	0.54	-0.62, 1.71	1.71	0.348
3	0.87	-1.42, 3.17	0.440	0.08	-1.30, 1.46	1.46	0.907
4	1.05	-0.75, 2.84	0.240	-0.20	-1.20, 0.81	0.81	0.687
5	1.32	-0.52, 3.15	0.151	-0.92	-1.77, -0.06	-0.06	0.036
6	0.96	-2.03, 3.96	0.514	-1.01	-1.62, -0.40	-0.40	0.002
7	0.71	-2.29, 3.70	0.632	-0.64	-1.59, 0.32	0.32	0.183
8	1.30	-1.37, 3.97	0.326	-0.33	-1.26, 0.60	0.60	0.473
9	-0.41	-2.68, 1.86	0.713	-0.34	-1.39, 0.71	0.71	0.514
10	-0.40	-2.31, 1.51	0.669	-0.30	-1.32, 0.71	0.71	0.543
11	-0.60	-2.08, 0.88	0.408	-0.51	-1.32, 0.29	0.29	0.200
12	0.18	-1.53, 1.89	0.831	-0.34	-0.91, 0.23	0.23	0.234
13	-0.27	-2.20, 1.66	0.774	-0.48	-1.12, 0.16	0.16	0.133
14	-1.37	-2.78, 0.03	0.055	-0.02	-1.25, 1.21	1.21	0.975
15	-0.15	-1.67, 1.38	0.841	0.58	-0.75, 1.91	1.91	0.376
16	-0.17	-1.73, 1.38	0.819	-0.50	-1.37, 0.37	0.37	0.246
17	0.15	-1.34, 1.65	0.833	-0.82	-1.67, 0.03	0.03	0.058
18	-0.46	-1.36, 0.44	0.302	-0.49	-1.35, 0.37	0.37	0.249
19	0.94	-0.89, 2.77	0.300	0.05	-1.09, 1.19	1.19	0.924
20	2.20	0.57, 3.83	0.010	0.06	-0.92, 1.04	1.04	0.898
21	1.22	-0.86, 3.30	0.237	-0.08	-1.04, 0.88	0.88	0.865
22	-0.32	-1.49, 0.85	0.583	-0.27	-1.28, 0.74	0.74	0.585
23	-0.45	-1.78, 0.89	0.494	-0.31	-1.17, 0.54	0.54	0.459
24	-0.23	-1.73, 1.28	0.757	-0.42	-1.20, 0.35	0.35	0.271
25	-0.71	-1.94, 0.52	0.245	-0.74	-1.61, 0.14	0.14	0.096
26	-1.23	-2.38, -0.08	0.038	-0.36	-1.51, 0.78	0.78	0.521
27	-1.01	-2.02, -0.01	0.048	-0.28	-1.36, 0.80	0.80	0.600
28	-0.25	-2.59, 2.09	0.826	-0.26	-1.23, 0.71	0.71	0.587
29	1.01	-1.09, 3.11	0.331	-0.36	-1.38, 0.66	0.66	0.477
30	-0.08	-1.70, 1.55	0.925	-0.30	-1.20, 0.59	0.59	0.494

	Using statins			Not using statins			Effect modification
Days before blood draw ($PM_{2.5}$)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	-0.09	-1.86, 1.69	0.919	-0.22	-0.92, 0.49	0.49	0.530
32	-0.63	-1.98, 0.71	0.342	-0.32	-1.09, 0.45	0.45	0.396
33	0.61	-0.88, 2.10	0.405	-0.26	-0.82, 0.30	0.30	0.343
34	1.29	-0.88, 3.46	0.233	-0.36	-1.13, 0.41	0.41	0.350
35	0.44	-1.59, 2.46	0.660	-0.32	-1.13, 0.49	0.49	0.417

Table E5. Association between lnCRP and daily NO_2

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	0.00	-0.01, 0.02	0.725	-0.01	-0.02, 0.00	0.190	0.001
1	-0.01	-0.03, 0.02	0.596	0.00	-0.02, 0.01	0.469	0.002
2	0.00	-0.02, 0.01	0.680	-0.01	-0.02, 0.00	0.095	0.002
3	-0.01	-0.03, 0.00	0.151	-0.01	-0.02, 0.00	0.080	0.001
4	0.00	-0.02, 0.02	0.805	-0.01	-0.03, 0.00	0.026	<0.001
5	0.00	-0.02, 0.02	0.917	-0.01	-0.03, 0.00	0.084	0.002
6	-0.01	-0.03, 0.01	0.266	-0.01	-0.03, 0.00	0.028	<0.001
7	0.00	-0.02, 0.01	0.651	-0.01	-0.02, 0.00	0.048	<0.001
8	-0.01	-0.02, 0.01	0.502	-0.01	-0.02, 0.00	0.224	0.001
9	-0.01	-0.03, 0.00	0.137	-0.01	-0.02, 0.01	0.290	0.002
10	-0.01	-0.02, 0.01	0.345	-0.01	-0.02, 0.00	0.094	0.002
11	-0.01	-0.02, 0.01	0.327	-0.01	-0.03, 0.01	0.201	0.004
12	0.00	-0.02, 0.01	0.776	-0.01	-0.02, 0.01	0.358	0.001
13	0.00	-0.02, 0.02	0.865	-0.01	-0.02, 0.00	0.179	0.001
14	0.00	-0.02, 0.01	0.631	-0.01	-0.02, 0.00	0.200	<0.001
15	-0.01	-0.03, 0.01	0.511	-0.01	-0.02, 0.00	0.212	0.001
16	0.00	-0.02, 0.02	0.949	-0.01	-0.02, 0.00	0.102	0.001
17	0.00	-0.01, 0.02	0.570	-0.01	-0.02, 0.00	0.074	<0.001
18	0.01	-0.01, 0.02	0.513	-0.01	-0.02, 0.00	0.106	<0.001
19	0.00	-0.01, 0.02	0.537	-0.01	-0.02, 0.00	0.225	<0.001
20	0.00	-0.02, 0.01	0.966	-0.01	-0.02, 0.01	0.292	0.001
21	0.00	-0.02, 0.01	0.725	-0.01	-0.02, 0.01	0.459	0.001
22	-0.01	-0.03, 0.02	0.635	-0.01	-0.02, 0.01	0.377	0.003
23	-0.01	-0.03, 0.01	0.184	-0.01	-0.03, 0.01	0.273	0.002
24	-0.01	-0.03, 0.00	0.136	-0.01	-0.02, 0.00	0.142	0.001
25	-0.01	-0.03, 0.01	0.218	-0.01	-0.03, 0.00	0.022	<0.001
26	0.00	-0.02, 0.01	0.572	-0.01	-0.02, 0.00	0.042	<0.001
27	0.00	-0.02, 0.02	0.876	-0.01	-0.02, 0.01	0.327	0.001
28	0.00	-0.02, 0.02	0.941	-0.01	-0.02, 0.01	0.243	0.001
29	0.00	-0.02, 0.01	0.559	0.00	-0.02, 0.01	0.565	0.002
30	-0.01	-0.03, 0.00	0.141	0.00	-0.02, 0.01	0.572	0.004

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	-0.01	-0.02, 0.01	0.290	-0.01	-0.02, 0.01	0.334	0.001
32	-0.01	-0.03, 0.01	0.424	-0.01	-0.02, 0.01	0.331	0.003
33	-0.01	-0.03, 0.01	0.267	-0.01	-0.03, 0.01	0.244	0.002
34	-0.01	-0.03, 0.01	0.244	-0.01	-0.02, 0.00	0.153	0.001
35	-0.01	-0.02, 0.01	0.379	-0.01	-0.02, 0.01	0.426	0.003

Table E6. Association between WBC and daily NO_2

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	0.03	0.00, 0.06	0.073	-0.01	-0.03, 0.02	0.619	0.019
1	0.01	-0.01, 0.04	0.358	-0.01	-0.03, 0.01	0.314	0.038
2	0.01	-0.01, 0.03	0.166	-0.01	-0.03, 0.01	0.162	0.012
3	0.01	-0.02, 0.04	0.630	-0.02	-0.04, 0.00	0.037	0.025
4	0.03	0.00, 0.05	0.038	-0.01	-0.03, 0.01	0.399	0.013
5	0.02	-0.01, 0.04	0.123	-0.01	-0.03, 0.02	0.477	0.027
6	0.00	-0.02, 0.03	0.748	-0.02	-0.03, 0.00	0.044	0.064
7	0.03	0.00, 0.05	0.047	-0.01	-0.04, 0.01	0.243	0.016
8	0.02	0.00, 0.05	0.066	-0.01	-0.04, 0.01	0.317	0.024
9	0.01	-0.01, 0.04	0.326	-0.02	-0.04, 0.00	0.105	0.011
10	0.00	-0.02, 0.03	0.715	-0.02	-0.04, 0.01	0.140	0.064
11	0.02	-0.01, 0.04	0.264	-0.02	-0.04, 0.01	0.283	0.032
12	0.03	0.00, 0.06	0.023	-0.01	-0.04, 0.03	0.634	0.034
13	0.03	0.00, 0.07	0.058	-0.01	-0.04, 0.02	0.420	0.024
14	0.02	-0.01, 0.04	0.238	-0.01	-0.03, 0.01	0.291	0.024
15	0.02	0.00, 0.04	0.090	-0.01	-0.03, 0.01	0.343	0.025
16	0.03	0.00, 0.06	0.035	-0.01	-0.04, 0.01	0.227	0.010
17	0.04	0.01, 0.08	0.008	-0.02	-0.04, 0.00	0.049	0.005
18	0.04	0.01, 0.06	0.004	-0.02	-0.04, 0.00	0.074	0.006
19	0.02	-0.01, 0.04	0.173	-0.01	-0.03, 0.01	0.249	0.012
20	0.01	-0.01, 0.03	0.263	-0.01	-0.03, 0.01	0.374	0.032
21	0.03	0.01, 0.06	0.012	-0.01	-0.04, 0.02	0.407	0.019
22	0.04	0.01, 0.07	0.009	0.00	-0.04, 0.03	0.751	0.016
23	0.02	0.00, 0.05	0.091	-0.01	-0.04, 0.01	0.222	0.017
24	0.03	0.00, 0.06	0.092	-0.01	-0.04, 0.02	0.452	0.022
25	0.02	-0.01, 0.05	0.274	-0.03	-0.04, -0.01	0.012	0.015
26	0.03	0.00, 0.06	0.030	-0.02	-0.04, 0.00	0.043	0.005
27	0.03	-0.01, 0.07	0.103	-0.01	-0.04, 0.01	0.243	0.023
28	0.01	-0.02, 0.04	0.493	-0.02	-0.04, 0.01	0.247	0.043
29	0.01	-0.02, 0.03	0.535	-0.01	-0.04, 0.02	0.388	0.051
30	0.00	-0.02, 0.02	0.864	-0.01	-0.04, 0.091	0.349	0.074

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	0.00	-0.03, 0.02	0.764	-0.01	-0.04, 0.02	0.437	0.131
32	0.01	-0.02, 0.03	0.455	-0.01	-0.04, 0.01	0.341	0.041
33	0.02	0.00, 0.05	0.058	-0.01	-0.04, 0.01	0.307	0.020
34	0.02	-0.01, 0.06	0.175	-0.02	-0.04, 0.01	0.161	0.024
35	0.02	-0.01, 0.05	0.143	-0.01	-0.04, 0.02	0.540	0.049

Table E7. Association between platelets and daily NO_2

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
0	1.27	0.15, 2.39	0.028	-0.15	-0.68, 0.39	0.571	0.822
1	0.74	-0.37, 1.86	0.183	-0.13	-0.62, 0.37	0.595	0.517
2	0.87	-0.63, 2.37	0.244	-0.23	-0.82, 0.36	0.423	0.688
3	0.64	-0.82, 2.10	0.373	-0.17	-0.94, 0.60	0.653	0.647
4	1.21	0.06, 2.35	0.039	-0.15	-0.83, 0.52	0.646	0.743
5	1.44	0.18, 2.70	0.027	-0.10	-0.67, 0.48	0.735	0.752
6	1.05	-0.14, 2.24	0.081	-0.36	-1.05, 0.32	0.284	0.736
7	1.19	-0.26, 2.63	0.103	-0.37	-0.92, 0.18	0.180	0.965
8	1.35	-0.15, 2.84	0.076	-0.28	-0.75, 0.20	0.242	0.977
9	0.89	-0.78, 2.56	0.284	-0.21	-0.78, 0.37	0.467	0.499
10	1.02	-0.37, 2.41	0.141	-0.22	-0.93, 0.48	0.524	0.552
11	0.78	-0.37, 1.94	0.176	-0.38	-0.96, 0.20	0.185	0.432
12	0.94	-0.14, 2.02	0.086	-0.12	-0.68, 0.45	0.673	0.526
13	0.57	-0.72, 1.85	0.374	-0.17	-0.77, 0.43	0.558	0.384
14	0.38	-0.79, 1.55	0.511	-0.18	-0.81, 0.46	0.576	0.273
15	0.83	-0.28, 1.94	0.135	-0.10	-0.74, 0.54	0.745	0.583
16	1.33	0.05, 2.61	0.042	-0.34	-0.86, 0.17	0.181	0.827
17	1.16	-0.17, 2.48	0.084	-0.42	-0.97, 0.12	0.118	0.676
18	1.14	0.30, 1.98	0.010	-0.32	-0.77, 0.13	0.157	0.553
19	0.80	-0.12, 1.71	0.085	-0.09	-0.59, 0.42	0.728	0.393
20	0.57	-0.32, 1.45	0.198	0.00	-0.52, 0.52	0.993	0.343
21	0.74	-0.18, 1.67	0.111	0.00	-0.59, 0.60	0.991	0.457
22	1.39	0.32, 2.45	0.013	-0.08	-0.55, 0.39	0.730	0.687
23	0.96	-0.23, 2.14	0.110	-0.04	-0.66, 0.59	0.898	0.419
24	0.70	-0.85, 2.25	0.363	-0.15	-0.71, 0.42	0.602	0.275
25	0.85	-0.43, 2.14	0.184	-0.53	-1.10, 0.03	0.064	0.512
26	0.70	-0.47, 1.88	0.228	-0.38	-0.95, 0.20	0.189	0.376
27	0.87	-0.43, 2.17	0.180	-0.12	-0.72, 0.49	0.695	0.479
28	0.57	-0.83, 1.98	0.410	-0.16	-0.76, 0.44	0.587	0.334
29	1.13	-0.22, 2.47	0.097	-0.15	-0.65, 0.34	0.525	0.761
30	0.47	-0.87, 1.81	0.479	-0.02	-0.52, 0.48	0.923	0.333

	Using statins			Not using statins			Effect modification
Days before blood draw (NO_2)	β	95% CI	2-sided p	β	95% CI	2-sided p	2-sided p
31	0.36	-0.75, 1.48	0.504	-0.11	-0.64, 0.43	0.681	0.195
32	0.09	-0.90, 1.07	0.858	-0.33	-0.74, 0.08	0.113	0.114
33	0.53	-0.37, 1.42	0.234	-0.26	-0.80, 0.28	0.324	0.237
34	0.83	-0.46, 2.12	0.198	-0.29	-0.93, 0.34	0.349	0.571
35	0.29	-0.84, 1.42	0.605	-0.17	-0.72, 0.37	0.520	0.253