UNIVERSITY OF MILAN

EPIGET - Epidemiology, Epigenetics and Toxicology Laboratory

Department of Clinical Sciences and Community Health

PhD in Biomedical Statistics

STATISTICAL METHODS TO ASSESS THE SUSCEPTIBILITY TO PARTICULATE MATTER AND HEALTH EFFECTS MEDIATED BY microRNAs CARRIED IN PLASMA EXTRACELLULAR VESICLES

Cicle XXVIII

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Ogniqualvolta una teoria ti sembra essere l'unica possibile, prendilo come un segno che non hai capito né la teoria né il problema che si intendeva risolvere.

Karl Popper

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ABSTRACT

Air pollution exposure is a major problem worldwide and has been linked to many diseases. PM_{10} is one of the components of air pollution and it includes a mixture of compounds. Several studies suggest that PM produces significant effects on respiratory and cardiovascular system, in relation to acute as well as chronic exposure. This process has been extensively studied, but to date it has not yet been fully understood. Ambient particles have been shown to produce a strong inflammatory reaction, and beside pro-inflammatory mediators, cell-derived membrane Extracellular Vesicles (EVs) are also released. EVs (particularly microvesicles) might be the ideal candidate to mediate the effects of air pollution, since potentially they could transfer miRNAs, after internalization within target cells through surface-expressed ligands, enabling intercellular communication in the body. Another gap in our current knowledge regarding PM-related health effects is the identification of susceptible subjects. Recent research findings pointed out obesity as a susceptibility factor to the adverse effects of PM exposure partly due to an increase in particle absorption. According these findings, our hypothesis is that, EVs might be the ideal candidate mechanism to mediate the effects of air pollution, since potentially they could be produced by the respiratory system, reach the systemic circulation and lead to the development of endothelial dysfunction. Moreover, EVs after internalization within target cells through surface-expressed ligands, may transfer miRNAs enabling intercellular communication in the body. Finally, obese individuals might represent one of the best population to investigate the effects of environmental air particles on several molecular mechanisms and, as a final objective, on cardiovascular and respiratory parameters. The main proposal of this research project is to develop the appropriate statistical methodology to address the following specific aims:

- **Aim 1**. Determine whether exposure to air particles and PM-associated metals can modify EVs in plasma in terms of miRNAs content.
- **Aim 2.** Determine whether the changes found in ECVs (Aim 1) are associated with respiratory, cardiac and inflammatory outcomes such as: single breath carbon monoxide diffusing capacity DLcoRapp, Forced expiratory volume in the 1st second FEV1, Forced Vital Capacity FVC, Heart Rate, Sistolic Blood Pressure SBP, Diastolic Blood Pressure DBP, C-Reactive Protein CRP, and Fibrinogen.
- **Aim 3.** Investigate the potential role of miRNAs as mediators of the effect of PM_{10} exposure on respiratory, cardiac and inflammatory outcomes listed in Aim2.

We used a cross-sectional study investigating the effects of particulate air pollution on a population of susceptible overweight/obese subjects, recruited in Lombardy Region, Italy. The population study will include 2000 overweighed/obese (BMI between 25 and 29.9 is considered overweight and an adult who has a BMI of 30 or higher is considered obese subjects, recruited at the Center for Obesity and Weight Control (Department of Environmental and Occupational Health, University of Milan and IRCCS Fondazione Ca'Granda – Ospedale Maggiore Policlinico). We will follow a two-stage, split sample study design. The first (discovery) stage involves genome-wide miRNA expression profiling, by means of OpenArray technology, among 1000 of the aforementioned 2000 participants (the first 1000 subjects consecutively recruited at the Center for Obesity and Weight Control). The second (replication) stage involves a replication analysis of the top 10 miRNAs that resulted from the first stage. At December 31, 2013 (first stage) we recruited 1303 subjects, 87% of whom living in the province of Milan. At April 2015 we recruited a total of 1786 evaluable subjects. Due to technical problems the replication data were not available for statistical analysis at the time of the layout of the thesis.
Different normalization strategies on miRNAs expression data were evaluated and compared in different set of miRNAs: Endogenous U6, Global Mean and Mean of 4 more stable miRNAs. The performance of the different normalization strategies was assessed by: (1) evaluating their ability to reduce the experimental induced (technical) variation, (2) determining their power to extract true biological variation. We showed for large scale miRNA expression profiling Global Mean normalization strategy outperforms the other normalization strategy in terms of:

- ✓ better reduction of technical variation:
  - lower % of miRNAs differentially expressed before and after FDR adjustment
  - lower Fold change range;
- ✓ more accurate appreciation of biological changes.
  - higher % of miRNAs differentially expressed before and after FDR adjustment;
  - higher Fold Change range;

PM_{10} exposure assessment is based on daily PM_{10} concentration estimates by the FARM model (the flexible air quality regional model), a three-dimensional Eulerian grid model for dispersion, transformation and deposition of particulates, capable to simulate PM_{10} concentration. By means of ArchGis software the residential address of each subject was georeferenced and the resulting map was superimposed on the map of FARM Model. In this this way to each subject was attributed: (a) the estimated daily exposure of the cell containing their residential address; (b) the exposure of the cell containing the address of the Center for Obesity and Work; (c) the daily

average exposure for Milan, calculated as the average of the 22 cells that falls into the city boundaries.

Since in each run of OpenArray were simultaneous analysed up to 4 OpenArray plates, identified by a barcode, for a total of 12 samples (3 per plate) it was possible identify an hierarchical data structure with three levels: sample level (level-1), barcode level (level-2) and run level (level-3). In order to verify the association between miRNAs expression and PM_{10} we developed a three-levels hierarchical linear model (HLM) using the MIXED procedure in SAS.

The following list of first 10 top miRNAs were identified: miR_106a_002169, miR_152_000475, miR_181a_2__002317, miR_218_000521, miR_27b_000409, miR_30d_000420, miR_652_002352, miR_92a_000431, miR_25_000403, miR_375_000564.

Simple mediation models were applied in order to investigate the role of miRNAs expression as potential mediator on the effect of PM_{10} on respiratory, cardiac and inflammatory outcomes such as: single breath carbon monoxide diffusing capacity DLco, Forced expiratory volume in the 1st second FEV1, Forced Vital Capacity FVC, Heart Rate, Sistolic Blood Pressure SBP, Diastolic Blood Pressure DBP, C-Reactive Protein CRP, and Fibrinogen. 95% BC bootstrap Confidence intervals for Indirect effect were estimated.

Finally, Multiple Parallel mediation models were applied in order to investigate the role of a set of miRNAs expression identified by means of simple mediation models as potential set of parallel mediator on the effect of PM_{10} on respiratory, cardiac and inflammatory outcomes.

A significant indirect effect of PM_{10} on:

- DLcoRapp, was found through the following mediators: mir_106a_002169, mir_152_000475, mir_218_000521 expression;
- FEV1Rapp was found through the following mediators: mir_27b_000409 mir_30d_000420 mir_92a_000431 mir_181a_2_002317 mir_218_000521 expression;
- FVCRapp was found through the following mediators: mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression;
- Heart Rate was found through the following mediator: mir_218_000521 expression;
- Sistolic Blood Pressure was found through the following mediator: mir_92a_000431 expression;
- *CRP* was found through the following mediator: mir_106a_002169 and mir_652_002352 expression.
- Fibrinogeno was found through the following mediator: mir_375_000564 expression.

Finally, the total indirect effect of PM_{10} exposure:

- on DLcoRapp obtained summed the indirect effects across all mediators: mir_106a_002169, mir_152_000475, and mir_218_000521 expression is statistically different from zero;
- on FEV1Rapp obtained summed the indirect effects across all mediators: mir_27b_000409 mir_30d_000420 mir_92a_000431 mir_181a_2_002317 mir_218_000521 expression is statistically different from zero;
- on FVCRapp obtained summed the indirect effects across all mediators mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression is statistically different from zero;
- on *CRP* obtained summed the indirect effects across all mediators mir_106a_002169 and mir_652_002352 expression is statistically different from zero.

1. INTRODUCTION

1.1 Air pollution

Air pollution exposure is a major problem worldwide and has been linked to many diseases. Air pollution consists of gas and particle contaminants that are present in the atmosphere. Gaseous pollutants include SO2 , NOx , ozone, carbon monoxide (CO), volatile organic compounds (VOCs), certain toxic air pollutants, and some gaseous forms of metals. Particle pollution ($PM_{2.5}$ and PM_{10}) includes a mixture of compounds. The majority of these compounds can be grouped into five categories: sulfate, nitrate, elemental (black) carbon, organic carbon, and crustal material (*Table 1).*

Some pollutants are released directly into the atmosphere. These include gases, such as SO_2, and some particles, such as crustal material and elemental carbon. Other pollutants are formed in the air. Ground-level ozone forms when emissions of NOx and VOCs react in the presence of sunlight. Similarly, some particles are formed from other directly emitted pollutants. For example, particle sulfates result from SO_2 and ammonia (NH3) gases reacting in the atmosphere. Commonly, emissions come from large stationary fuel combustion sources (such as electric utilities and industrial boilers), industrial and other processes (such as metal smelters, petroleum refineries, cement kilns, manufacturing facilities, and solvent utilization), and mobile sources including highway vehicles and non-road sources (such as recreational and construction equipment, marine vessels, aircraft, and locomotives). Sources emit different combinations of pollutants. For example, electric utilities release SO_2 , NOx , and Particles Fossil fuel combustion is the primary source contributing to CO_2 emissions. Major sources of fossil fuel combustion include electricity generation, transportation (including personal and heavy-duty vehicles), industrial processes, residential, and commercial [1].

Table 1: Health, Environmental, and Climate Effects of Air Pollution Components. *U.S Environmental Protection Agency's Report 2010*

Health, Environmental, and Climate Effects of Air Pollution		
Pollutant	**Health Effects**	**Environmental and Climate Effects**
Ozone (O_3)	Decreases lung function and causes respiratory symptoms, such as coughing and shortness of breath; aggravates asthma and other lung diseases leading to increased medication use, hospital admissions, emergency department (ED) visits, and premature mortality.	Damages vegetation by visibly injuring leaves, reducing photosynthesis, impairing reproduction and growth, and decreasing crop yields. Ozone damage to plants may alter ecosystem structure, reduce biodiversity, and decrease plant uptake of CO_2. Ozone is also a greenhouse gas that contributes to the warming of the atmosphere.
Particulate Matter (PM)	Short-term exposures can aggravate heart or lung diseases leading to symptoms, increased medication use, hospital admissions, ED visits, and premature mortality; long-term exposures can lead to the development of heart or lung disease and premature mortality.	Impairs visibility, adversely affects ecosystem processes, and damages and/or soils structures and property. Variable climate impacts depending on particle type. Most particles are reflective and lead to net cooling, while some (especially black carbon) absorb energy and lead to warming. Other impacts include changing the timing and location of traditional rainfall patterns.
Lead (Pb)	Damages the developing nervous system, resulting in IQ loss and impacts on learning, memory, and behavior in children. Cardiovascular and renal effects in adults and early effects related to anemia.	Harms plants and wildlife, accumulates in soils, and adversely impacts both terrestrial and aquatic systems.
Oxides of Sulfur (SOx)	Aggravate asthma, leading to wheezing, chest tightness and shortness of breath, increased medication use, hospital admissions, and ED visits; very high levels can cause respiratory symptoms in people without lung disease.	Contributes to the acidification of soil and surface water and mercury methylation in wetland areas. Causes injury to vegetation and local species losses in aquatic and terrestrial systems. Contributes to particle formation with associated environmental effects. Sulfate particles contribute to the cooling of the atmosphere.
Oxides of Nitrogen (NOx)	Aggravate lung diseases leading to respiratory symptoms, hospital admissions, and ED visits; increase susceptibility to respiratory infection.	Contributes to the acidification and nutrient enrichment (eutrophication, nitrogen saturation) of soil and surface water. Leads to biodiversity losses. Impacts levels of ozone, particles, and methane with associated environmental and climate effects.
Carbon Monoxide (CO)	Reduces the amount of oxygen reaching the body's organs and tissues; aggravates heart disease, resulting in chest pain and other symptoms leading to hospital admissions and ED visits.	Contributes to the formation of CO_2 and ozone, greenhouse gases that warm the atmosphere.
Ammonia (NH_3)	Contributes to particle formation with associated health effects.	Contributes to eutrophication of surface water and nitrate contamination of ground water. Contributes to the formation of nitrate and

1.2 Particulate Matter

PM is a mixture of suspended particles that vary in chemical composition and size. Particulate matter varies in number, size, shape, surface area, chemical composition and characteristics, and can be of both natural and anthropological origin. The size distribution of urban ambient particle pollution is usually characterized by aerodynamic diameter (AED) which is defined as the diameter of a sphere of unit density (lg/cm^3) that has the same inertial properties in the gas as the particle of interest. The size distribution of suspended particles in the atmosphere is tri-modal and includes coarse particles, line particles, and ultrafine particles. Coarse particles (PM2,5-10: often defined as those with an aerodynamic diameter > 2.5μm) are primarily produced by natural mechanisms such as grinding and wind, and derive mainly from soil and other crustal materials. Fine particles ($PM_{2.5}$: diameter < 2.5 μm) are derived chiefly from combustion processes. In the urban environment these process are associated with transportation, manufacturing, and power generation. Ultrafine particles are often defined as particles less than or equal to 0,1 μm.

Particles can either be directly emitted into the air (primary PM) or be formed in the atmosphere from gaseous precursors such as sulfur dioxide, oxides of nitrogen, ammonia and non-methane volatile organic compounds (secondary particles). Primary PM and the precursor gases can have both man-made (anthropogenic) and natural (non-anthropogenic) sources. Anthropogenic sources include combustion engines (both diesel and petrol), solid-fuel (coal, lignite, heavy oil and biomass) combustion for energy production in households and industry, other industrial activities (building, mining, manufacture of cement, ceramic and bricks, and smelting), and erosion of the pavement by road traffic and abrasion of brakes and tires. Agriculture is the main source of ammonium. Secondary particles are formed in the air through chemical reactions of gaseous pollutants. They are products of atmospheric transformation of nitrogen oxides (mainly emitted by traffic and some industrial processes) and sulfur dioxide resulting from the combustion of sulfur-containing fuels. Secondary particles are mostly found in fine PM.

PM_{10} and $PM_{2.5}$ include inhalable particles that are small enough to penetrate the thoracic region of the respiratory system. The health effects of inhalable PM are well documented. They are due to exposure over both the short term (hours, days) and long term (months, years) and include:

- respiratory and cardiovascular morbidity, such as aggravation of asthma, respiratory symptoms and an increase in hospital admissions;
- mortality from cardiovascular and respiratory diseases and from lung cancer.

There is good evidence of the effects of short-term exposure to PM_{10} on respiratory health, but for mortality, and especially as a consequence of long-term exposure, $PM_{2.5}$ is a stronger risk factor than the coarse part of PM_{10} (particles in the 2.5–10 μm range). All-cause daily mortality

is estimated to increase by 0.2–0.6% per 10 μg/m3 of PM_{10} [2, 3].Long-term exposure to $PM_{2.5}$ is associated with an increase in the long-term risk of cardiopulmonary mortality by 6–13% per 10 μg/m3 of $PM_{2.5}$ [4-6]. Susceptible groups with pre-existing lung or heart disease, as well as elderly people and children, are particularly vulnerable. For example, exposure to PM affects lung development in children, including reversible deficits in lung function as well as chronically reduced lung growth rate and a deficit in long term lung function [7]. There is no evidence of a safe level of exposure or a threshold below which no adverse health effects occur. The exposure is ubiquitous and involuntary, increasing the significance of this determinant of health. At present, at the population level, there is not enough evidence to identify differences in the effects of particles with different chemical compositions or emanating from various sources. It should be noted, however, that the evidence for the hazardous nature of combustion-related PM (from both mobile and stationary sources) is more consistent than that for PM from other sources [8]. The black carbon part of $PM_{2.5}$, which results from incomplete combustion, has attracted the attention of the air quality community owing to the evidence for its contribution to detrimental effects on health as well as on climate. Many components of PM attached to black carbon are currently seen as responsible for health effects, for instance organics such as PAHs that are known carcinogens and directly toxic to the cells, as well as metals and inorganic salts.

1.3 PM and Cardiovascular Health Effects

According to the WHO, diseases of heart and cardiovascular system are the largest single cause of death (accounting for about 3.7 million deaths) in the European Union. Cardiovascular diseases account for the largest number of premature deaths before 75 years of age [9]. Numerous health studies have shown acute [10-15] and chronic [16-18] particulate air pollution exposures to be associated with early death, particularly from cardiovascular and respiratory disease [10, 11, 19]. Metals, which are constituents of particulate air pollution, are also associated with CVD [19-38]. Epidemiological and animal studies have suggested many potential mechanisms by which particles may impact health. Airway or parenchymal inflammatory responses to particulate matter (PM) have been hypothesized to be the inciting events of a cascade of pathophysiologic changes in autonomic cardiac, systemic inflammation, and hemostatic activities. All these processes may ultimately lead to the acute cardiac events associated with PM exposure [39]. However, the relative importance of each potential pathways and the steps along these pathways are not well understood, particularly as to how they relate to specific particle components and sources, for which biological pathways are likely to differ. One of the most important gaps in our current knowledge regarding PM-related health effects is the

identification of susceptible subjects [40]. Recent research findings pointed out obesity as a susceptibility factor to the adverse effects of PM exposure partly due to an increase in particle absorption [40, 41]. A positive correlation between exhaled nitric oxide, a marker of pulmonary inflammation, and Body Mass Index (BMI) have been shown in healthy adults [42]. BMI was associated with a graded increase in the estimated total lung dose of deposited fine particles in an inhalation study of healthy children (6–13 years of age) [43]. In a panel study of 44 senior citizens, vascular inflammatory response (measured by C-reactive protein) to ambient levels of $PM_{2.5}$ (particulate matter with aerodynamic diameter ≤2.5μm) averaged over 1–7 days was greater in obese (BMI ≥ 30 kg/m2) than in non-obese subjects [44]. Moreover, a differential autonomic cardiac response (measured as heart rate variability) to metal particulates have been observed between obese and non-obese individuals [40]. These findings suggest that obese individuals might represent a candidate population to investigate PM mediated cardiovascular effects and related pathogenetic mechanisms. The mechanisms linking PM exposure and cardiovascular disease have not yet been fully elucidated. It has been proposed that inhaled fine particulate matter translocates directly into systemic circulation through the pulmonary capillary bed, promoting atherothrombosis by breaching endothelial integrity and inciting a local inflammatory reaction [45].

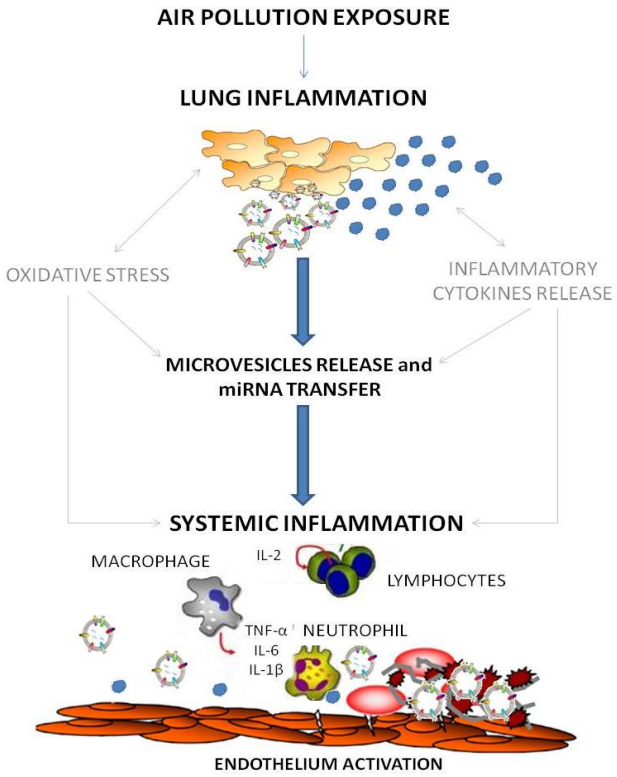


Figure 1: Proposed mechanism for air pollution effects on exosomes release and cell-to-cell communication.

However, just a very small fraction of these fine and ultrafine particles accumulate in extra pulmonary organs such as the liver and the spleen, [46] and currently there is no final evidence that fine particles physically enter and deposit in blood vessels. An alternative hypothesis is that ambient particles produce a strong inflammatory reaction in the lungs followed by the release of proinflammatory mediators that are able to reach the systemic circulation *(Figure 1)* [47, 48]. In spite of more than two decades of mechanistic research, at front of high degree of consistency of the epidemiology findings showing increased cardiovascular risk, the evidence on intermediate mechanisms remains moderate or weak [49]. In addition, inflammatory and oxidative responses have little specificity and can be activated by a multitude of trigger, thus limiting our capability to correlate them to air pollution exposure as well as to provide the groundwork for the development of targeted biomarkers and prevention strategies. Beside the release of proinflammatory mediators, cell-derived membrane vesicles are also released, representing another new mode of intercellular communication that has recently become the subject of increasing interest [48].

1.4 Extracellular Vescicles (EVs) and microRNAs in PM-related cardiovascular effects

Intercellular communication is an essential hallmark of multicellular organisms and can be mediated through direct cell–cell contact or transfer of secreted molecules. In the last two decades, a third mechanism for intercellular communication has emerged that involves intercellular transfer of extracellular vesicles (EVs). EVs might be the ideal candidate to mediate the effects of air pollution, since potentially they could be produced by the respiratory system, [50, 51] reach the systemic circulation [52] and lead to the development of endothelial disfunction [53]. EVs are spherical structures limited by a lipid bilayer that can be generated by cells and secreted into the extracellular space and are likely composed of both exosomes (EXs) and microvesicles (MVs). There are various types of secreted membrane microvesicles that have distinct structural and biochemical properties depending on their intracellular site of origin, and these features probably affect their function. Microparticles originating from platelets, endothelial cells and monocytes have been most extensively studied [54]. Platelet microparticles were originally studied because of their procoagulant activity [54] and recent studies have investigated their involvement in the pathophysiology of vascular disorders [54]. They could also participate in a defensive shedding of complement attack complexes [55] or in deployment of immunomodulating activities [56]. The term exosome is used to identify a particular subgroup of vesicles, ranging from 40 to 100 nanometers (nm), released as a consequence of multivesicular endosome (a membrane-bound intracellular vesicle, containing EXs) fusion with the plasma

membrane [57], whereas the term microvesicle is used for those EVs, larger than 100 nm, that are shed from the plasma membrane. EVs are released from cell membranes by triggers such as endotoxin encounter, hypoxia or oxidative stress conditions, cytokines release, thrombin production [58] and could be one of the means used by tissues to adapt to these stimuli [59]. In particular, DNA damaging conditions have been related to activation of the p53 that leads to increased exosomes secretion [60]. EV membranes are enriched in molecules characteristic of their parent cell and express adhesion molecules on their surface (i.e., ICAM1), which could favor their capture by recipient cells. The fate of exosomes after binding the surface of recipient cells is not known but recent evidence suggests that they might fuse with recipient cell membranes and deliver their content directly into the cytoplasm of the recipient cells. It has been suggested that EVs, after internalization in the target cells through surface-expressed ligands, may transfer microRNAs (miRNAs) [61, 62] allowing intercellular and inter-organ communication in the body [62]. Moreover, miRNA expression in circulating exosomes has been detected also in plasma of normal subjects and a predictive role of peripheral blood miRNA signatures in human disease has been also hypothesized. MiRNAs are small, endogenous, single stranded noncoding RNAs of 20-22 nucleotides [63] that post-transcriptionally regulate gene expression by either triggering mRNA cleavage or repressing translation [64]. One single miRNA can regulate hundreds of mRNAs in interrelated gene pathways and a single mRNA can be targeted by several different miRNAs [65]. Moreover, miRNA expression in circulating EVs has been detected also in plasma of normal subjects and a predictive role of peripheral blood miRNA signatures in human diseases has been also hypothesized [62]. Changes in the expression of several miRNAs have been implicated in disease mechanisms that may be related to PM exposure such as oxidative stress [66] and regulation of inflammation [67]. Recently, our group showed in foundry workers of an electric-steel plant facility that air particles, particularly those rich in lead and cadmium, are able to modify miRNAs expression in blood [68].

2. AIM AND HYPOTHESES

According these findings, our hypothesis is that obese individuals might represent one of the best population to investigate the effects of environmental air particles on several molecular mechanisms and, as a final objective, on cardiovascular and respiratory parameters. EVs might be the ideal candidate mechanism to mediate the effects of air pollution, since potentially they could be produced by the respiratory system, reach the systemic circulation and lead to the development of endothelial dysfunction. Moreover, EVs after internalization within target cells through surface-expressed ligands, may transfer miRNAs enabling intercellular communication in the body. The main proposal of this research project is to develop the appropriate statistical methodology to address the following specific aims:

- **Aim 1**. Determine whether exposure to air particles and PM-associated metals can modify EVs in plasma in terms of miRNAs content.
- **Aim 2.** Determine whether the changes found in ECVs (Aim 1) are associated with respiratory, cardiac and inflammatory outcomes such as: single breath carbon monoxide diffusing capacity DLco, Forced expiratory volume in the 1st second FEV1, Forced Vital Capacity FVC, Heart Rate, Sistolic Blood Pressure SBP, Diastolic Blood Pressure DBP, C-Reactive Protein CRP, and Fibrinogen.
- **Aim 3.** Investigate the potential role of miRNAs as mediators of the effect of PM_{10} exposure on respiratory, cardiac and inflammatory outcomes listed in Aim2.

3. METHODS

3.1 Study design

The SPHERE study is a cross-sectional study investigating the effects of particulate air pollution on a population of susceptible overweight/obese subjects, recruited in Lombardy Region, Italy. Lombardy is situated in the Northern part of Italy and is divided physically into three parts from north to south: Alpine and pre-Alpine mountains, foothills and a zone of alluvial plains sloping to the Po river. The region covers an area of 23.864 km^2 with a population of about 10 million people [69] and consists of 12 provinces, among which Milan is the regional capital. The Milan metropolitan area is home to 7 million inhabitants with 1,3 million residing in the core municipality [70].

3.2 Study Population

The population study will include 2000 overweighed/obese (BMI between 25 and 29.9 is considered overweight and an adult who has a BMI of 30 or higher is considered obese subjects, recruited at the Center for Obesity and Weight Control (Department of Environmental and Occupational Health, University of Milan and IRCCS Fondazione Ca'Granda – Ospedale Maggiore Policlinico). This study population has been chosen because some evidence shows that obesity may bring greater susceptibility [71, 72] to the adverse cardiovascular effects of PM exposure. In addition, It has been shown that obese people are more susceptible to the effect of inflammation in the short-term exposure to PM, presenting higher levels of C-reactive protein and interleukin-6, compared with normal-weight subjects, at the same exposure of PM level [44]. The recruitment period started in September 2010 and will continue until the end of 2015. The eligibility criteria for participants are as follows: 1) older than 18 years at enrollment; 2) obese/overweight according to the following definition: overweight is defined as a BMI between 25 and 30 kg/cm², obesity is defined as a BMI of 30 kg/cm² or more; 4) domiciled in Lombardy at the time of the recruitment; 5) agreement to sign an informed consent and donate a blood sample. Exclusion criteria include: experienced previously diagnosed cancer, heart disease or stroke in the last year or other chronic diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, depression, bipolar disorder, schizophrenia and epilepsy.

Epidemiological and Clinical Data collection

At recruitment, each study subject is asked to:

- fill in a lifestyle and a diet questionnaire,

- donate a 15 ml blood sample (for molecular tests),
- provide a 50 ml urine sample (for metal internal dose assessment),
- provide a lock of hair cut next to the root in the occipital area of the head (for metal internal dose assessment)

As part of the routine protocol, for each subject presenting at the Center, extensive physical examination, spirometry, ECG are performed and biochemical tests are also collected, including Emocrome, Fibrinogen, C-reactive protein, Total cholesterol, HDL, LDL, Triglyceride, Serum creatinine, AST, ALT, Gamma-Glutamyltransferase, Glucose, Homocysteine, TSH, Glycated haemoglobin, Postprandial glycaemia, Insulin level, 2-hour post glucose insulin level, Urinary pH, Uric acid.

Lifestyle questionnaire

The lifestyle questionnaire collects information on socio-demographic data, residential area (complete address, characteristics of the house, and traffic), education, past and present health status of the subjects and their first-degree relatives, medications in the last year, employment history and for employed subjects address of the plant of their current work, smoking history, passive smoking at home and at workplace, physical activity levels and sedentary behavior commuting time and transport mode.

Diet questionnaire

The questionnaire on eating habits included questions on the number of servings from each food in a usual week or month. Several different types of food were investigated, including: legumes, vegetables, fruits, nuts, red and white meat, fish, eggs, dairy products, cereals, snacks, oil and butter, alcoholic beverage, thea and coffee. Number of servings from each food were translated into usual daily micronutrients intake weighting for serving size, age class and gender. Both questionnaires were checked for completeness at the time of data collection in order to ensure high quality data.

Lung and cardiac function

Pulmonary functions are measured, at the same day of blood drawing, with an electronic flow volume spirometer V-max 22 with Autobox (SensorMedics), according to European Respiratory Society/American Thoracic Society guidelines (ERS/ATS 2005) [73]. Tests are performed on patients in the sitting position, and are repeated until at least three reproducible forced expiratory curves have been obtained. Lung function parameters are: forced expiratory volume in one second (FEV1),; forced vital capacity (FVC); best peak expiratory flow (PEF); forced expiratory flows at 25%, 50%, and 75% of FVC (FEF25, FEF50, FEF75); mid-expiratory flow (FEF25–

75) derived from the best maneuver (defined as the one with the highest sum of FEV1 + FVC). The single breath carbon monoxide diffusing capacity (DLco) is also measured [74]. All parameters are expressed as a percentage of the predicted normal values, [75] and adjusted for sex, age, height. A resting ECG and rhythm strip is also recorded and blood pressure is measured with the participant supine, after 5 minutes of rest.

3.3 Two-stage, split sample study design

We will follow a two-stage, split sample study design. We foresee to collect at least 600 subjects/year. The first (discovery) stage involves genome-wide miRNA expression profiling among 1000 of the aforementioned 2000 participants (the first 1000 subjects consecutively recruited at the Center for Obesity and Weight Control). The second (replication) stage involves a replication analysis of the top 10 miRNAs that resulted from the first stage.

First stage

At December 31, 2013 we recruited 1303 subjects, 87% of whom living in the province of Milan. Evaluable patients were 1250. Mean BMI of our study population is 33.5 Kg/cm^2 (±5.5): nearly 27.8% are overweight, 38.6% are obese, and 33.6% severe obese. The percentage of obese individuals in Lombardy is about 10.3% of the total adult population [76]. The participation rate in the years 2010-2013 was 90%. The study population is composed by 74% of female, mean aged 52 years.

Second Stage

At April 2015 we recruited a total of 1786 evaluable subjects.

Due to technical problems the replication data were not available at the time for statistical analysis at the time of the layout of the thesis. Statistical analisys results were performed only on data coming from First Stage.

3.4 Laboratory Methods

Stage 1 will involve using the OpenArray technology (Applied Bioscience) that allows to run 2,688 TaqMan® qPCR reactions in parallel. In Stage 2, we will replicate the results obtained on the discovery set by standard real time PCR on an Applied Biosystems 7900HT Real-Time PCR System. Laboratory methods can be summarized as follows:

- ***Isolations ECVs:*** Plasma was thawed on ice and subjected to 3 consecutive centrifuged, this passage has been useful to eliminate dead cells and debris. The supernatant was

subjected to ultracentrifuge and then it was discarded and the pellet remaining were stored at -20 ° C.

- ***miRNAs extraction:*** miRNAs were extracted using the Qiagen Kit miRNReasy Mini Kit and RNeasyMinEluteClean Up Kit.
- ***High throughput miRNAs:*** high throughput analysis is the set of scientific analysis able to perform tests on a large number of data in a limited time due to machinery and automated instrumentation. For a complete analysis of all miRNAs we performed a reverse transcription, followed by a reaction of preamplification and then we evaluated the expression of miRNAs through a Real-Time PCR.
- ***Reverse transcription:*** this technique of reverse transcription allows to produce cDNA starting from RNA and for this reason is a technique that is exploited to study gene expression.
- ***Reaction of preamplification:*** knowing that the amount of miRNAs contained in the microvesicles extracted from 1.5 mL of plasma is very low, before the Real-time PCR must perform a reaction of preamplification.
- ***Real Time PCR****:* The product of the reaction of preamplification was transferred from the plate 96 to 384 , performing an ottuplicato. Finally, using the Robot AccuFillTM System (Life Technologies) was set up the Open Array Plate formed by 48 subarray with 64 holes, for a total of 3072 holes of 300 μm in diameter and 300 μm of depth *(Figure 2).* Each hole has an outer hydrophobic , while inside it has a hydrophilic coating (the reagents are kept inside by means of surface tension).

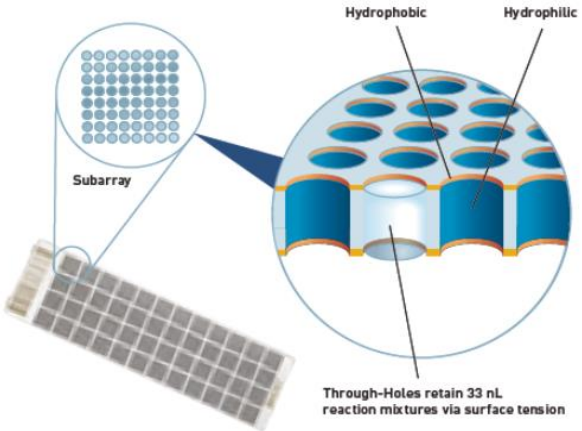


Figure 2: OpenaArray Plate.

In this system, each real time PCR reaction has a final volume of 33 nanoliters. After loading the sample, the plate was covered with adhesive Lid and filled with fluid immersion.

The plate thus prepared was loaded in QuantStudioTM 12K Flex OpenArray (Life technologies) which allows the simultaneous analysis of up to 4 OpenArray plates, for a total of 12 samples (3 per plate). The Real-Time PCR , also ca called quantitative PCR or real-time quantitative. PCR (RTQ - PCR), is a method of amplification (polymerase chain reaction or PCR) and simultaneous

quantification of samples for DNA and cDNA. The fluorescence is generated during PCR due to several possible chemical reactions. (Figure 3). The main chemical are based on the binding of fluorescent dyes that intercalate in the double helix of DNA , such as SYBR Green, or the hybridisation of specific probes , such as TaqMan probes. In this experiment it were used TaqMan probes.The use of probes revealing fluorescence is one of the most reliable and accurate method because the TaqMan probes are designed so as to pair up to specific target sequences.

A typical cycle of OpenArrayflex normal PCR and includes 3 phases:

1. Heat denaturation of template DNA (94-99 °C);
2. Annealing to the sequence of the oligonucleotides (30-65 °C);
3. Extension by the DNA polymerase from the heat-resistant primers (65-72 °C).

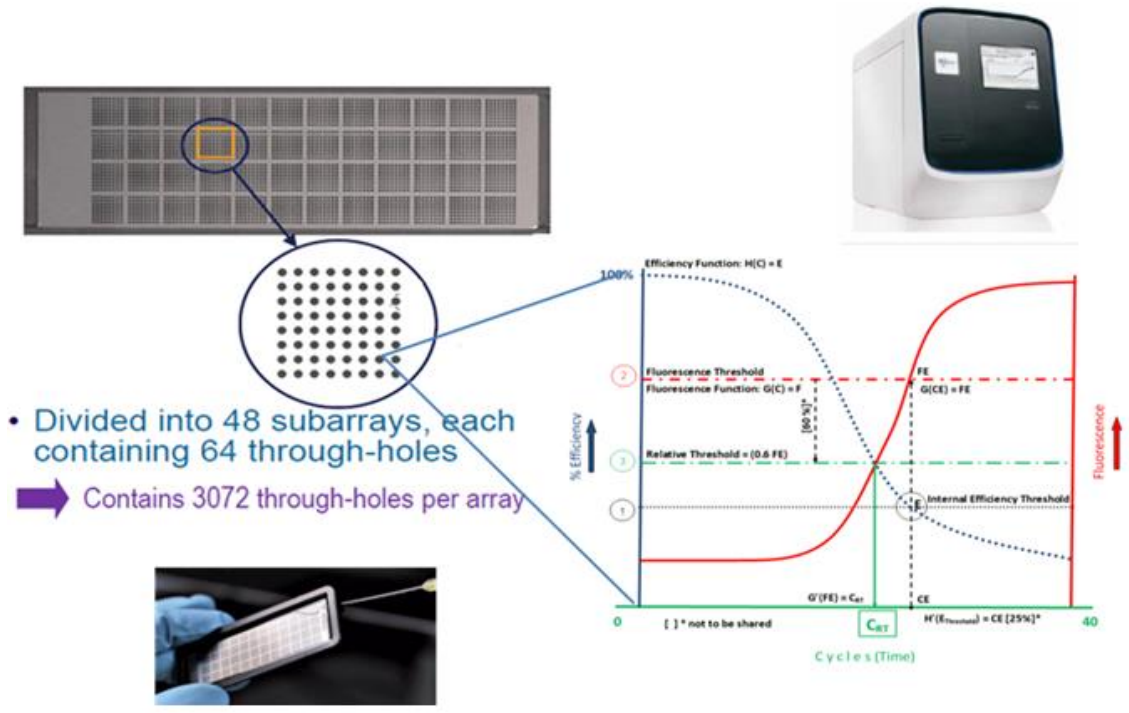


Figure 3: QuantStudioTM 12K OpenArray and OpenaArray flex Real Time PCR cycle.

The real time PCR is quantitative, as the data is collected during the exponential growth phase, when the quantity of the product of the reaction is directly proportional to the initial amount of nucleic acid. The quantification of the miRNA is based on the fluorescence detected at each reaction cycle : every cycle, the cDNA molecules doubled, reaching a plateau when the reactants

are exhausted and the operation of the enzyme decreases drastically. After finishing the reaction, the instrument software provides results in terms of: Related Cycle Threshold (CRT): the number of PCR cycles at which the efficiency of the reaction Real-Time PCR miRNA target is maximal. The higher the CRT , the lower the expression of miRNA. In order to evaluate the goodness of the reaction a parameter called Ampscore was used. A good amplified has a Ampscore exceeding 1.24. This means that the curve representing its amplification , as a result of the Real - Time PCR, has the characteristic of an exponential growth curve. With this information, determine the Ampscore can also help to discriminate false positives and false negatives.

3.5 Air pollution exposure assessment

Exposure is defined using a multifaceted approach, which integrates information coming from PM_{10} assessment with personal dose of metals in urine and hair. In particular, PM_{10} is assigned to each subjects following two approaches: I. use of daily PM_{10} concentration series from air quality monitors; II. use of daily PM_{10} concentration estimates by the FARM model (the flexible air quality regional model), a three-dimensional Eulerian grid model for dispersion, transformation and deposition of particulates, capable to simulate PM_{10} concentration using a 4 km–dispersion grid [15].

1. *Air Monitoring Stations data from ARPA Lombardy:* we collected recordings of daily PM_{10} data by 81 fixed monitoring stations selected by the Regional Environmental Protection Agency (ARPA Lombardy) from the approximately 150 monitors of the Regional Air Monitoring Network throughout Lombardy region, (Figure 4) on the basis of their reliability determined by standardized quality control procedures and correlation with in-situ measurements, of continuity of recording and of the ability to represent local background air pollution. Three stations are located in the city of Milan ("Verziere", "Pascal-Città Studi" and "via Senato"). We used daily concentration measured by single monitors in the study area to characterize PM_{10} exposure, at the date of recruitment and until 365 days before, for each subject. Thus, we are able to estimate both short- and long-term exposure to the pollutant investigated. As air monitoring station recordings for the area of interest are available from 2001, older exposures might also be estimated. Moreover, as patients were not recruited all at once, but during the first visit to the Center for Obesity and Work, PM_{10} exposure was not tied to a specific date, but randomly assigned during the work week, ensuring a great variability of exposure distributions.

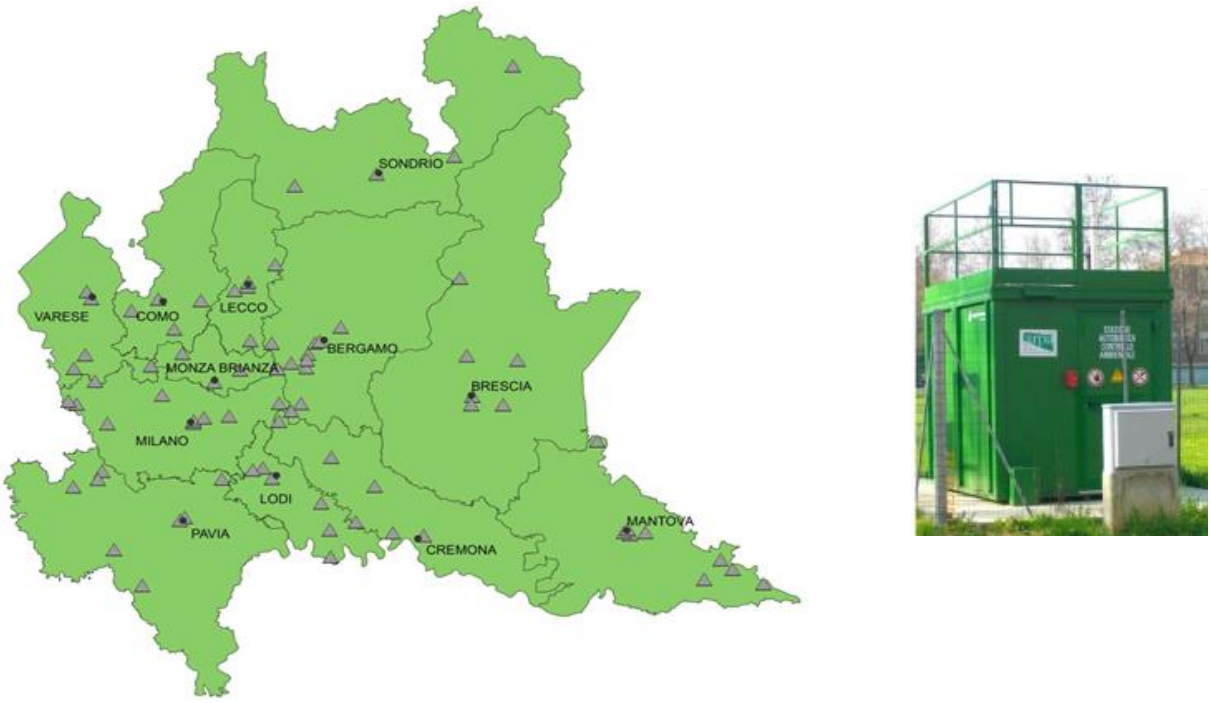


Figure 4: PM_{10} monitoring stations in Lombardy region

Data on meteorological variables were obtained from the monitoring stations of the Regional Environmental Protection Agency measuring respectively temperature (233 monitors) and relative humidity (163 monitors). (Figure 5) The apparent temperature were calculated using the following formula:

$$T_{app} = -2.653 + 0.994 * TEMP + 0.0153 * DEW^2$$

where the drew point DEW is defined as:

$$DEW = \left(\frac{U}{100}^{\frac{1}{8}}\right) * \left(112 + (0.9 * TEMP)\right) + 0.1 * TEMP - 112$$

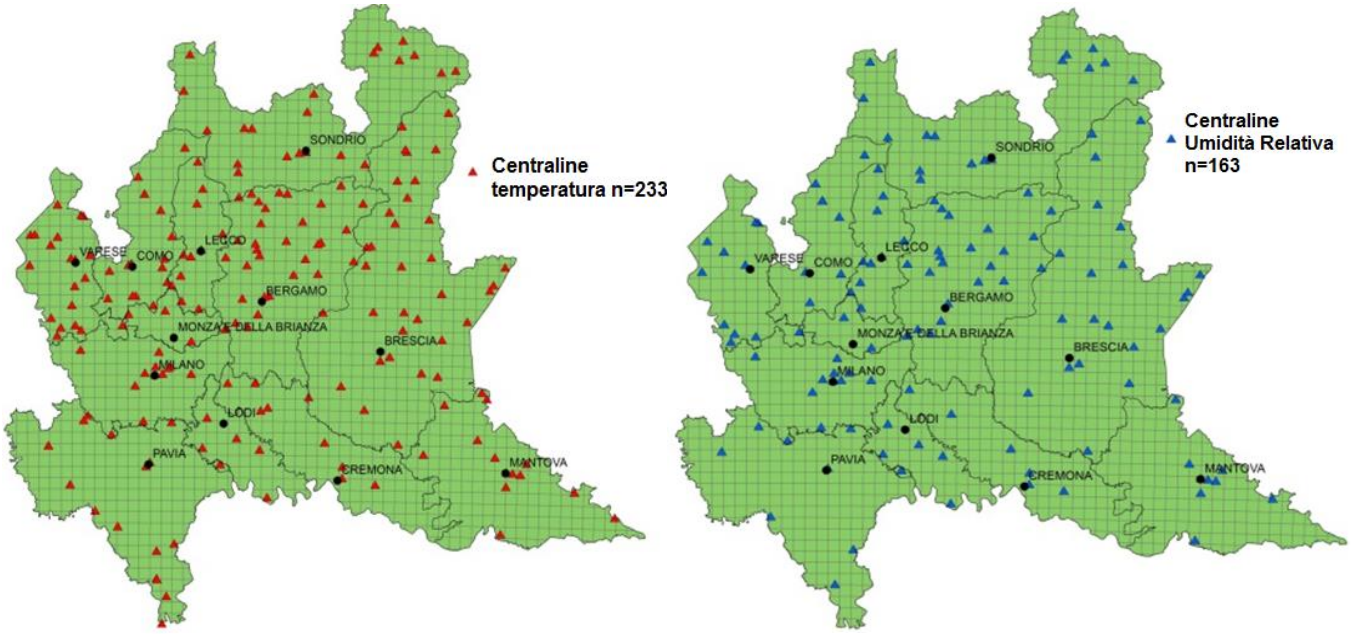


Figure 5: Temperature and Humidity monitoring stations in Lombardy region.

2. *FARM model (*flexible air quality regional model*) by ARPA Lombady:* Estimated daily average concentrations of PM_{10} for the years 2007-2012 based on the FARM model were obtained from ARPA Lombardy. The FARM model is a chemical transport model able to treat the main processes of chemical and physical nature of formation and removal of pollutants, in addition to the transport and dispersion by the action of wind and atmospheric mixing. The model provide an estimate of PM_{10} concentration in the atmosphere, from a considerable number of data that can be grouped as follows: information needed to characterize in space and time the emissions in the area of interest, information on meteorological variables involved in the processes of transport and dispersion of pollutants in the air, concentrations of conditions present at the boundary of this area and the beginning of the simulation period. The input data are built from meteorological observations of weather and hydrological network of ARPA and from the processing of the results of the global meteorological model of the European Centre ECMWF (European Centre for Medium-Range Weather Forecasts). The initial and boundary conditions are obtained starting from the data of the ARPA network of air quality and the results of the model CHIMERE (http://www.lmd.polytechniques.fr/chimere/chimere.php) applied at continental scale within the France forecasting system Prev'air. Finally, data on measured and simulated concentrations are harmonized through interpolation techniques [65, 77].Data elaborated by modeling systems integrates the ones of the monitoring network and allow to know air quality state on an extensive way on the territory.

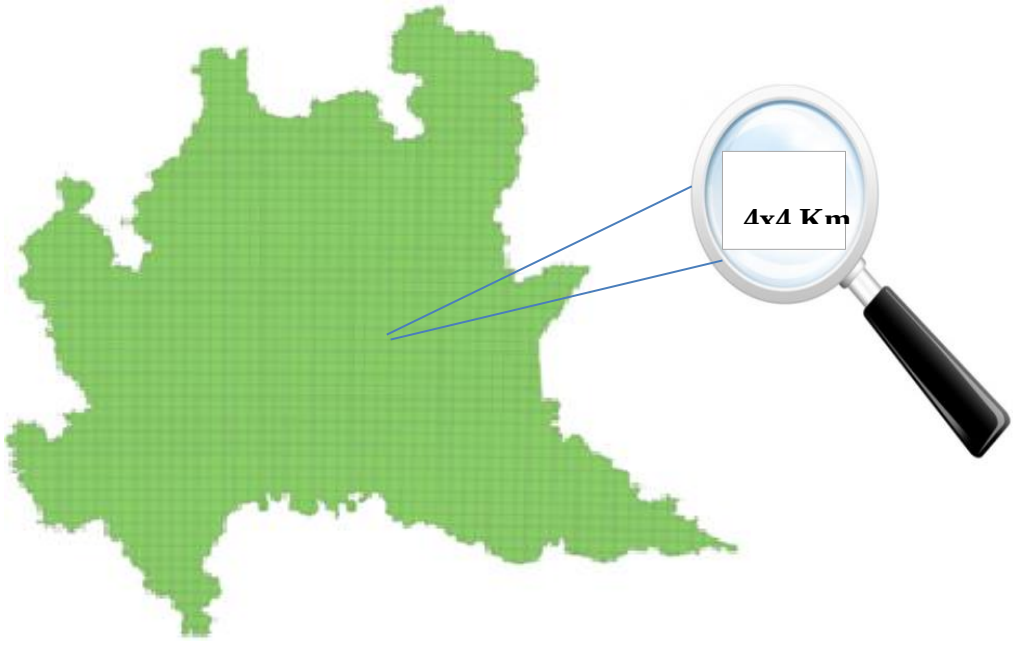


Figure 6: Grid 4x4Km from FARM model by ARPA Lombardy applied on the map of Lombardy Region.

FARM model allows to assess the population's risk of exposure to air pollution in regions where there are no direct observations. By this model the Lombardy region is divided into a grid of 1678 cells (4x4 km), each associated with daily PM10 concentration estimates. (Figure 6). Thus, the advantage of FARM dispersion modeling, on monitors, is that it allows to assess the population's risk of exposure to air pollution in regions where there are no direct observations. Data estimated from the models are available until 2012, since the data validation imply a lag time of nearly 6 months and will soon be available for further data analysis. Data estimated from the models are available until 2012, since the data validation imply a lag time of nearly 6 months and will soon be available for further data analysis. Given the time window of available data, will be possible to estimate both short-term (days) and long-term (months, years) exposure to the pollutants investigated at or before the index date (date of recruitment for each subject.

4. STATISTICAL ANALYSIS

Statistical analysis follows different strategies in the two different stages. We proceeded according the steps explained in the following paragraphs.

4.1 Ambient exposure attribution

Verification and possible correction of address of domicile / residence of subjects was performed as first step. Allocation of geographic coordinates to addresses of domicile / residence was performed using the GPSvisualizer software. ArchGIs was used for the attribution of individual ambient exposure to each subjects of the pilot study. In detail for the previous points I and II :

1. *Air Monitoring Stations data from ARPA Lombardy:* We geocoded the monitoring stations and the addresses of study subjects in order to assign them the daily PM_{10} concentration from: (1) the monitor at the lower distance to home address, defined "subject's residence"; (2) the nearest monitor to the Center for Obesity and Work ("Verziere"), defined "Policlinico"; (3) daily exposure for Milan created averaging the three available city monitors, defined as "average Milan"*(Figure 7)*.

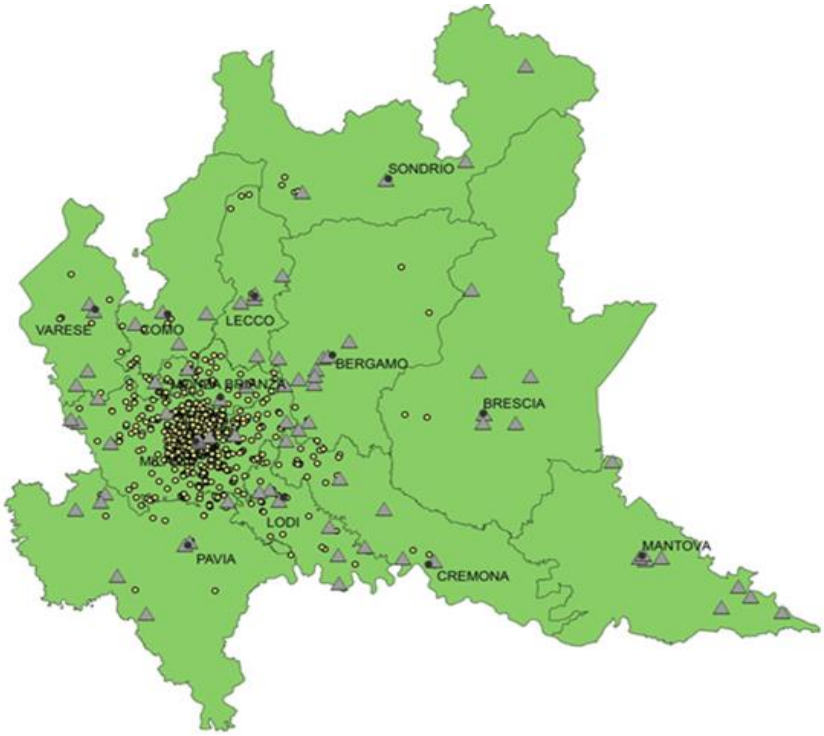


Figure 7: Attribution of PM10 from exposure Monitoring Stations ARPA to subjects .

Handling missing data in Air Monitoring Stations data series

In case of incomplete series, the missing data is estimated based on the values measured on the same day in other stations and the ratio between the annual average recorded in the station that

has the missing data and annual averages of the other stations *(63,64)*. The other stations were chosen among those closest and strongest correlation. The final series has been created averaging over monitors and imputing missing values under proportionality assumptions. Each station and pollutants had missing daily averages data. To fill the gaps for each pollutant A, first the q stations with yearly data coverage higher than 90% were selected ($1 \leq q < n$). Then a daily variability profile p_d was calculated as follows, based on the observation that relationships between observed concentrations of PM_{10} (expressed as Pearson correlation coefficient) at the different selected sites were always higher than 0.7:

$$p_d = \frac{\sum_{k=1}^{q} [A]_{d,k}}{\sum_{K=1}^{q} \beta_{d,k} * [A]_{y,k}}$$

Where:

$\beta_{d,k}$= 0 if $[A]_{d,k}$=missing

$\beta_{d,k}$= 1 if $[A]_{d,k}$=not missing

$[A]_{d,k}$ = daily average concentration of the pollutant A measured at the station k.

$[A]_{y,k}$= yearly average concentration of the pollutant A measured at the station k.

Then the data series for the q stations and the missing data days j were completed as follows:

$[A]_{j,k} = p_j * [A]_{y,k}$

To complete the time series of the n-q stations with yearly data coverage less than 90%, first an overall

pollutants daily average was calculated from the q stations:

$[A]_d = \frac{1}{q} * \sum_{k=1}^{q} [A]_{d,k}$

Thus a correlation line was built for the z (n-q) station with data coverage < 90%, starting from the i

daily average available:

$[A]_{i,z} = a * [A]_{i,d} + b$

The calculated slope (a) and intercept (b) was finally used to estimate the lost values j at each z station:

$[A]_{j,z} = \hat{a} * [A]_j + \hat{b}$

With such an approach it was possible to improve the data coverage to almost 100% for all the monitoring stations selected; only a few days were still missing for PM_{10} (no measurement available at all, 2 days out of 1460 days of observations).

2. *FARM model by ARPA Lombardy:* The residential address of each subject was georeferenced and the resulting map was superimposed on the map of FARM Model *(Figure 8).* In this this way to each subject was attributed: (a) the estimated daily exposure of the cell containing their residential address; (b) the exposure of the cell containing the address of the Center for Obesity and Work; (c) the daily average exposure for Milan, calculated as the average of the 22 cells that falls into the city boundaries.

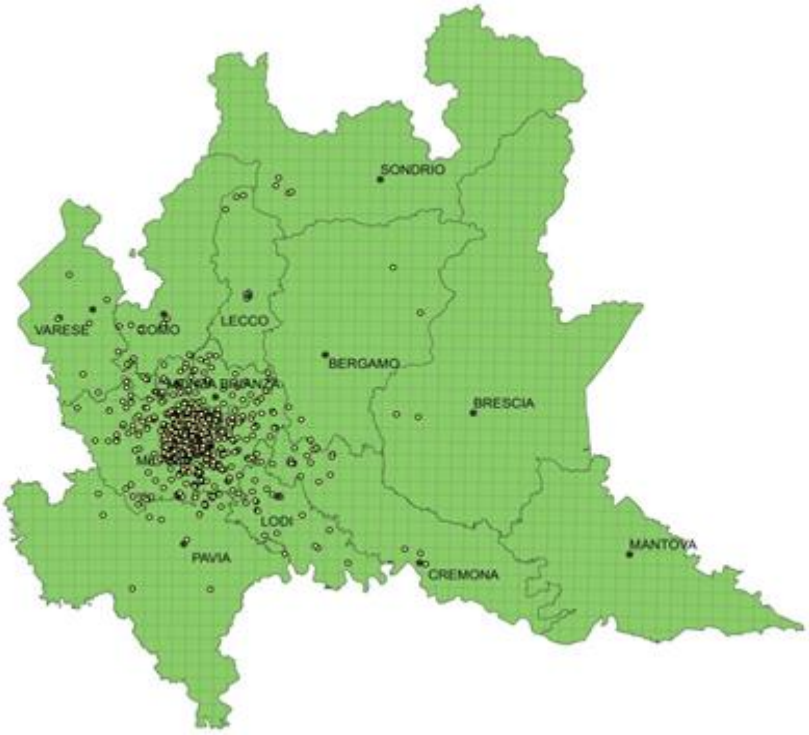


Figure 8: Attribution of PM_{10} exposure from FARM Model to subjects.

A map of predicted PM_{10} concentration for the study period 2010-2012 is shown in *Figure 9*. We assume that the day of the visit subjects are exposed to levels of PM_{10} measured by the "Policlinico" station, as the time elapsed in the Hospital to perform all the examinations (approximately five hours) is supposed to be sufficient to experience outcomes related to very short-term effect. About 57% of SPHERE subjects live in the city and an additional 28% work in Milan (even if they live outside the city), overall a 67% of subjects spent many hours a day in the city or travelling from workplace to residence. Thus, longer exposure effects are evaluated by both residential and Milan monitors using appropriate lag time from recruitment date, as sensitivity analysis.

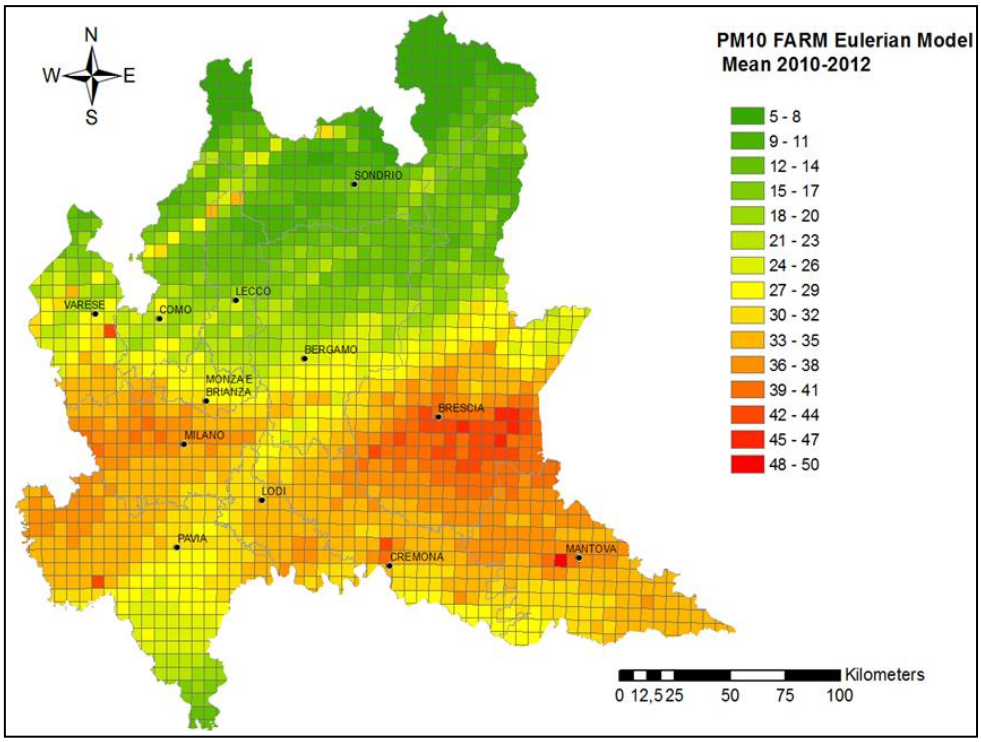


Figure 9: Prediction of PM_{10} concentration by FARM model (2010-2012)

However, very high correlation was observed among the three sources of exposition (RPoliclinico vs Average Milan=0.99; RSubjects' Residence vs Average Milan=0.94; RPoliclinico vs Subjects' Residence=0.99).With the intent of giving a map representation of PM_{10} from monitoring stations, we applied the Empirical Bayesian kriging (EBK) to expand the monitors PM_{10} point observation to the whole Lombardy territory. Empirical Bayesian kriging (EBK) is a geostatistical interpolation method that automates the most difficult aspects of building a valid kriging model. Other kriging methods in Geostatistical Analyst require you to manually adjust parameters in order to receive accurate results, but EBK automatically calculates these parameters through a process of subsetting and simulations. Empirical Bayesian kriging also differs from other kriging methods by accounting for the error introduced by estimating the underlying semivariogram [78, 79].

A map of observed PM_{10} mean concentration for the whole study period 2010-2013 is shown in *(Figure 10).*

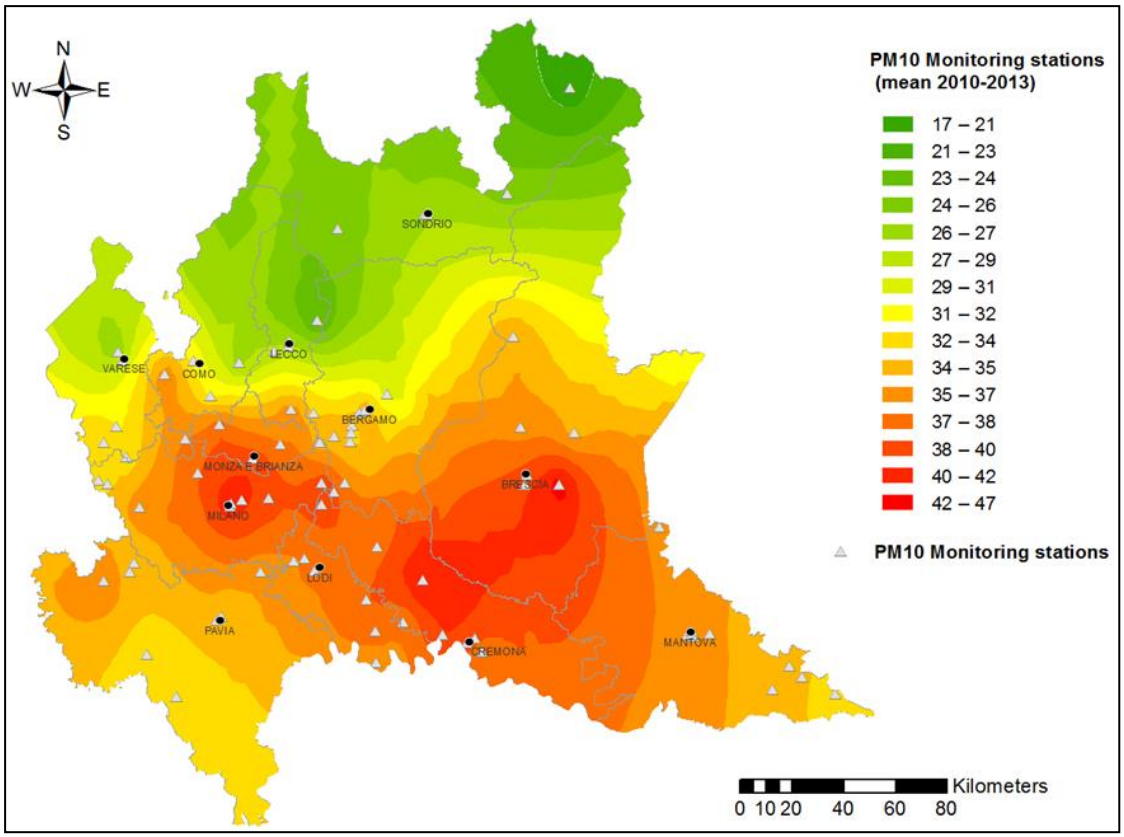


Figure 10: Prediction of PM_{10} concentration by Empirical Bayesian Kriging (2010-2013).

We assume that the day of the visit subjects are exposed to levels of PM_{10} measured by the "Policlinico" station, as the time elapsed in the Hospital to perform all the examinations (approximately five hours) is supposed to be sufficient to experience outcomes related to very short-term effect. The use of both monitoring stations and Eulerian model is forced for very recent data, when data estimated from FARM model are not available, while monitors data are.

4.2 miRNAs expression data analysis

Stage 1

The new technology used for real-time PCRs allowed to get a quantitative measurement in terms of Related Cycle Threshold (Crt) of the expression of the entire miRNome. Crt is the number of PCR cycles at which the efficiency of the reaction Real-Time PCR miRNA target is maximal. Thus the starting point was a dataset containing the Crt values of 733 human miRNAs for each subject of the first stage analysis (N=1250). First of all it was applied an automatic miRNAs selection, setting missing values and values with Crt >27 or Amp score >1.24 equal to the detection limit of CRT=28. In this way, miRNAs assumed not to be detected because having a Crt above the threshold of 28 was set at 28. The choice of Crt=28 (lower than the usual value of detection limit used in Real-time PCR) is due to the need of preamplification reaction, the large

number of miRNAs target and low volume of Real Time reaction. Afterwards, in order to try to remove the background noise, three different strategies of miRNAs selection were performed.

- SET1: Removing miRNAs completely not expressed (Crt=28) in all samples: 527 miRNAs remaining;
- SET2: Removing miRNAs do not expressed (Crt=28) in at least 30% of samples : 152 miRNAs remaining (keeping miRNAs expressed (Crt≠28) in at least 70% of samples);
- SET3: Removing miRNAs do not expressed (Crt=28) in at least 10% of samples : 105 miRNAs remaining (keeping miRNAs expressed (Crt≠28) in at least 90% of samples);

On each of these miRNAs sets three different normalization methods were applied. The normalization aimed at removing experiment-specific effects in order to maximize the true biological information contained in expression measures. No consensus exists about which procedure performs best [80, 81], the following normalization methods were applied*:*

- **Endogenous U6:**

DELTACrt= Crt- (Arithmetic Mean Ct of U6 across samples)

- **Global Mean:**

DELTACrt= Crt- (Arithmetic Mean Crt of each miRNAs across samples)

- **Mean of 4 more stable miRNAs:**

DELTACRT= CRT- (Arithmetic Mean Crt of 4 more stable miRNAs across samples). Finally, after calculating DELTACRT, relative quantification defined as: RQ=2^(-DELTACRT)[82] were calculated for each normalization methods applied. The performance of the different normalization strategies is assessed by [80, 81]: (1) evaluating their ability to reduce the experimental induced (technical) variation, (2) determining their power to extract true biological variation.

4.3 Association between miRNAs expression and PM_{10} exposure

Standard descriptive statistics were used to summarize data. In order to verify the association between miRNAs expression and PM_{10} we first fitted multiple linear regression models. We checked the assumptions of models by means of both graphical inspections and formal test which were particularly useful given the judge amount of miRNA to be tested.

We will validate the "iid" assumption of linear regression by examining the residuals of our final model and testing the heteroscedasticity by means of the White test, moreover the Durbin-Watson

statistic was used to test for first order correlation of error terms. We tested the normality assumption of errors using the normal probability plot and the Shapiro-Wilks statistic. Finally

plots of residuals versus predicted values and Lack of Fit tests were used to explore potential nonlinearity. Logarithmic transformation on miRNA expression was performed in order to improve the normality and linearity assumptions. The following adjusting variables were selected a priori, based on previous work investigating associations between miRNAs and particles in foundry workers [68]: age, body mass index, cigarette smoking (never, former, or current), and pack-years. We adjusted for percent of granulocytes (to control for possible shifts in leukocyte differential count), date, Seasonality (using sine and cosine) and apparent temperature. It was assumed that:

- **Exposure:** Is the daily PM_{10} exposure estimate (µg/m^^{3}) from Eulerian model for the 4x4 km cell containing the address of the Center for Obesity and Weight Control. The exposure lag period chosen for the analysis is of zero days (daily exposure of blood collection day). The hypothesis underlying this choice is that, the mechanism starting with PM_{10} inhalation, with subsequent inflammatory reaction in lungs, release of ECVs by the respiratory system in systemic circulation, and finally the potential transfer of miRNAs by ECVs, is a short term mechanism. Thus, ideally, the subject arrived at the Center for Obesity and Weight Controls inhaled the exposure estimated by Eulerian model in the 4x4 km cell containing the address of the Centre which triggers the above mechanisms.
- **Outcome:** Is the Relative quantification RQ of each miRNAs. log2 transformation was applied in order to satisfy the normality assumption of linear regression model.

Since in each run of OpenArray were simultaneous analysed up to 4 OpenArray plates, identified by a barcode, for a total of 12 samples (3 per plate) it was possible identify an hierarchical data structure with three levels: sample level (level-1), barcode level (level-2) and run level (level-3) *(Figure 11).*

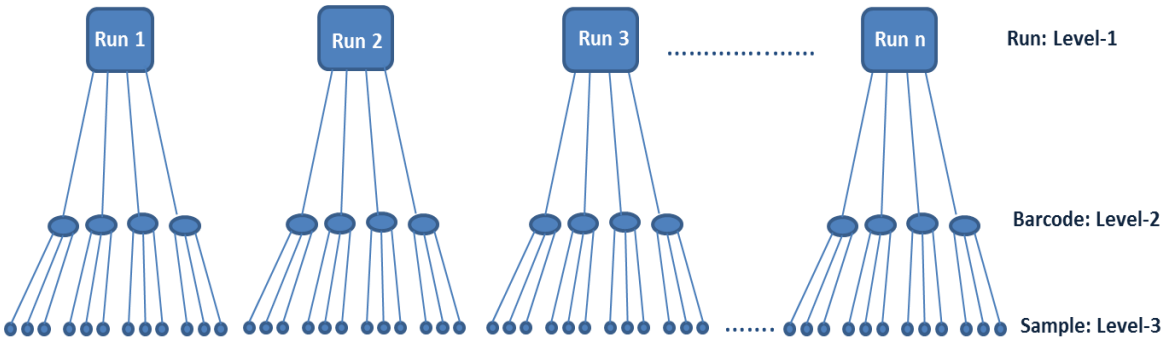


Figure 11: Three levels hierarchical data structure: run level (level-1), barcode level (level-2) and sample level (level-3). In each run of OpenArray were simultaneous analysed up to 4 OpenArray plates, identified by a barcode, for a total of 12 samples (3 per plate).

We developed a three-levels hierarchical linear model (HLM) [83, 84] using the MIXED procedure in SAS. The equations necessary for estimating three-levels mixed effect linear model are:

- ***Level-1 i samples :*** The level-1 model for three-level HLM, for sample i, barcode j, and run k, can be given as follows:

$$Y_{ijk} = \pi_{0jk} + \pi_{1jk} A_{1ijk} + e_{ijk} \qquad (Eq.1)$$

In *(Eq.1)*, Y_{ijk} is represented as the miRNAs expression of sample i associated with barcode j, and run k. Predictor is A_{1ijk} (say, *A*). The coefficient π_{0jk} is the intercept, π_{1jk} is the slope for *A*. Further, A_{1ijk} is a continuous predictor, and is grand-mean centered.

Grand mean centering was defined previously, under two-level HLM. The term e_{ijk} represents the random effect for sample *i*, barcode *j*, and run *k*, which is normally distributed with mean zero and variance σ^2 .

- ***Level-2 j barcode:*** The level-2 model is formulated by using level-1 intercept π_{0jk} and slopes π_{1jk} as outcomes. The level-2 equation, where X_{1jk}, is a continuous predictor and grand-mean centered, can be given as follows:

$$\pi_{0jk} = \beta_{00k} + \beta_{01k} X_{1jk} + r_{0jk} \qquad (Eq.2)$$
$$\pi_{1jk} = \beta_{10k} + \beta_{11k} X_{1jk} + r_{1jk}$$

*T*he parameters β_{00k}, β_{10k} are level-2 intercepts. Further, the coefficients β_{01k} , β_{11k}, are level-2 slopes. The terms r_{0jk}, r_{1jk}, and are random effects for barcode j, and run k.

- ***Level-3 k barcode:*** For run *k*, the level-3 model can be formulated as follows:

$$\beta_{00k} = \gamma_{000} + \gamma_{001} W_{1k} + u_{00k} \qquad (Eq.3)$$
$$\beta_{01k} = \gamma_{010} + \gamma_{011} W_{1k} + u_{01k}$$
$$\beta_{10k} = \gamma_{100} + \gamma_{101} W_{1k} + u_{10k}$$
$$\beta_{11k} = \gamma_{110} + \gamma_{111} W_{1k} + u_{11k}$$

In the level-three model, the level-2 intercepts and slopes are used as outcomes. The terms u_{00k}, u_{02k}, u_{10k}, u_{11k} in *(Eq.3)* are random effects associated with run *k*. The single-equation can be formulated as follows, by substituting *(Eq.3)* in *(Eq.2)*, and then substituting the newly produced *(Eq.2)* in *(Eq.1)*.

$$Y_{ijk} = \pi_{0jk} + \pi_{1jk} A_{1ijk} + e_{ijk}$$
$$Y_{ijk} = \beta_{00k} + \beta_{01k} X_{1jk} + r_{0jk} + \beta_{10k} A_{1ijk} + \beta_{11k} X_{1jk} A_{1ijk} + r_{1jk} A_{1ijk} + e_{ijk}$$
$$Y_{ijk} = \gamma_{000} + \gamma_{001} W_{1k} + \gamma_{010} X_{1jk} + \gamma_{011} W_{1k} X_{1jk} + \gamma_{100} A_{1ijk} + \gamma_{101} W_{1k} A_{1ijk} + \gamma_{110} X_{1jk} A_{1ijk} +$$
$$+ \gamma_{111} W_{1k} X_{1jk} A_{1ijk} + [u_{00k} + u_{01k} X_{1jk} + u_{10k} A_{1ijk} + u_{11k} X_{1jk} A_{1ijk} + r_{0jk} + r_{1jk} A_{1ijk} + e_{ijk}] \qquad (Eq.4)$$

In *(Eq.4)*, the parameter γ_{000} is interpreted as the predicted miRNAs expression for a reference sample associated with a reference barcode in a reference run (in this case, we assume all *Xs*, *Ws*, and *As* equal to zero). The terms γ_{001}, γ_{010}, γ_{100}, are the simple effects of individual sample, barcode, and run level predictors. The parameters γ_{011}, γ_{101}, γ_{110}, represent simple two-way interaction effects of the sample, barcode, and run level predictor. The terms γ_{111}, represent the three-way interaction effect on miRNAs expression due to sample, barcode, and run level predictors. Equation *(Eq.4)* also consists of residual terms associated with all three levels. The level-1 residual, e_{ijk}, is the unique effect of sample *i* on miRNA expression, associated with barcode j, and run k. Similarly, r_{0jk} is the unique effect of barcode *j* from run *k*, and u_{00k} is the unique run effect for *kth* run for a reference barcode and reference sample. The terms r's and u's are level-2 and level-3 residual terms respectively associated with slopes. The interaction terms of random effect and individual predictor or cross-level predictors are also present in the above model. For example, the term { r_{1jk} A_{1ijk} } is the interaction of the unique effect associated with sample A-slope, and { u_{11k} X_{1jk} A_{1ijk} } is the three-way interaction between X, A, and the residual term associated with the slope of X.

Assumptions

The following assumptions can be made for three-level HLM.

a) The error terms of each level-1 unit should have a mean of zero, and the error terms should be multivariate normally distributed. If, for example, we consider level-1 and level-2 units as sample and barcode, respectively, then the mean of the error within each barcode should be zero, and these error terms should be multivariate normally distributed.
b) It is assumed that the relationship between predictors and outcome variables, at all three levels, is linear.
c) Another assumption is the homogeneity of variance. That is, all barcodes should have equal variances in the sample.
d) Level-1 predictors are independent of the level-1 error term. In other words, the covariance between the level-1 predictors and the error term should equal zero.
e) Level-2 and level-3 error terms have a mean of zero and follow a multivariate normal distribution.
f) Level-2 predictors are independent of all level-2 error terms and level-3 predictors are independent of all level-3 error terms.
g) The level-1 error terms are independent of (uncorrelated to) level-2, and level-3 error terms in the model. That is, the correlation is zero between the level-1 error term and the

level-2 error term in the model for the level-1 intercept, or the error term in any of the equations used to estimate the slopes of level-1 variables.

The use of three-levels hierarchical linear models allowed to investigate other variability sources linked to the outcome. In particular we inspect the following research questions:

1) how much of the variability in miRNAs expression is attributable to barcodes and runs?
2) does the association between the level-1 predictor PM_{10} vary among barcode or run?

To answer to this questions we proceeded according the following model selection strategy:

- **Model 1 - Unconditional model:** For the three-level HLM, the unconditional model is formulated by using no predictors in the model and just random effect for the intercept to estimate the amount of variance in miRNA expression attributable to barcodes and runs. The resulting level-1, level-2 and level-3 unconditional models are:

  Level –1 Unconditional model:

  $$Y_{ijk} = \pi_{0jk} + e_{ijk} \quad (Eq.5)$$

  Level-2 Unconditiona

  $$\pi_{0jk} = \beta_{00k} + r_{0jk} \quad (Eq.6)$$
  $$\pi_{1jk} = \beta_{10k} + r_{1jk}$$

  Level-3 Unconditional model:

  $$\beta_{00k} = \gamma_{000} + u_{00k} \quad (Eq.7)$$
  $$\beta_{10k} = \gamma_{100} + u_{10k}$$

  SAS code

```
proc mixed data=dataset method=ml;
class barcode run;
model mirna=/solution;
random intercept/sub=run type=vc;
random intercept/sub=barcode(run) type=vc;run;
```

  This model allows to answer to the first research question, we used the three variance estimates to calculate the intra-class correlation coefficients ICCs for barcode and

  $$ICC_{barcode} = \frac{\sigma^2_{barcode}}{\sigma^2_{barcode}+\sigma^2_{run}+\sigma^2_{error}} \quad (Eq.8)$$

  $$ICC_{run} = \frac{\sigma^2_{run}}{\sigma^2_{barcode}+\sigma^2_{run}+\sigma^2_{error}} \quad (Eq.9)$$

  $ICC_{barcode}$ expresses the similarity (correlation between) of samples in the same barcode (within the same run); or alternatively informs us of how much of the total variation in

miRNAs expression exists between barcode. ICC_{run} expresses the similarityof barcode within the same run, ignoring within-class variation.

- **Model 2: Model 1 + level - 1 fixed effect**. We included the sample-level predictor PM_{10}

```
proc mixed data= dataset covtest method=ml;
class barcode run;
model mirna=PM10_policlinico /solution;
random intercept /sub=run type=vc;
random intercept /sub=barcode(run) type=vc;run;
```

Results indicate the relationship between level-1 predictor PM_{10} and the outcome miRNAs expression.

- **Model 3: Model2 + random slopes for level-1 predictor PM_{10}.** We expanded Model 2 specifying the PM_{10} predictor as random slope at both barcode and run level.

```
proc mixed data=dataset covtest method=ml;
class barcode run;
model mirna=PM10_policlinico /solution;
random intercept PM10_policlinico /sub=run type=vc;
random intercept PM10_policlinico /sub=barcode(run) type=vc; run;
```

This model allows to answer to the second research question: fixed effects results provide the same information as Model2, random slope results reveal if the relationships between the level-1 predictor PM_{10} and the outcome miRNAs expression vary between barcode and run. This analysis was conducted for exploratory purposes initially on the first top 10 miRNAs identified by a simple multivariable regression model and then applied to the three sets of miRNAs identified. The final model was adjusted for age, body mass index, cigarette smoking (never, former, or current), and pack-years. We adjusted for percent of granulocytes (to control for possible shifts in leukocyte differential count), date, Seasonality (using sine and cosine) and apparent temperature. The results were reported as Variation(%)=[2^(β*10) -1]x100 expresses the percentage variation in miRNAs expression associated with an increase of 10 (µg/m^3) in PM_{10}.

Finally, to control the expected proportion of incorrectly rejected null hypothesis in multiple comparisons, false discovery rate control (FDR) [85, 86] was applied. On the basis of FDR p-value significance (threshold of 0.20 or 0.10) and a set of top miRNAs were identified.

4.4 Mediation Analysis

4.4.1 The Simple Mediation Model

Mediation analysis is a statistical method used to help answer the question as to how some causal agent X transmits its effect on Y. What is the mechanism, be it emotional, cognitive, biological, or otherwise, by which X influences Y? The most basic of mediation models—the simple mediation model—is represented in conceptual diagram form in Figure 12. As can be seen, this model contains two consequent variables (M) and (Y) and two antecedent variables (X) and (M), with X causally influencing Y and M, and M causally influencing Y. A simple mediation model is any causal system in which at least one causal antecedent X variable is proposed as influencing an outcome Y through a single intervening variable M. In such a model, there are two distinct pathways by which a specific X variable is proposed as influencing *Y*. These pathways are found by tracing every way one can get from *X* to *Y* while never tracing in a direction opposite to the direction an arrow points. One pathway leads from *X* to *Y* without passing through *M* and is called the *direct effect* of *X* on *Y*. The second pathway from *X* to *Y* is the *indirect effect* of *X* on *Y* through *M*. It first passes from antecedent *X* to consequent *M* and then from antecedent *M* to consequent *Y*. The indirect effect represents how *Y* is influenced by *X* through a causal sequence in which *X* influences *M*, which in turn influences *Y*. In a mediation model, *M* is typically called a *mediator variable*. Once *X* exerts its effect on *M*, then *M*'s causal influence on *Y* produces variation in *Y*.

When thinking about whether a phenomenon or theory you are studying could be conceptualized as a mediation process, it is important to keep in mind that mediation is ultimately a causal explanation. It is assumed that the relationships in the system are causal, and, importantly, that *M* is causally located *between X* and *Y*. It must be assumed, if not also empirically substantiated, that *X* causes *M*, which in turn causes *Y*. *M* cannot possibly carry *X*'s effect on *Y* if *M* is not located causally between *X* and *Y*.

Estimation of the Direct, Indirect, and Total Effects of X

When empirically testing a causal process that involves a mediation n component, of primary interest is the estimation and interpretation of the direct and indirect effects along with inferential tests thereof. To derive these effects, one must also estimate the constituent components of the indirect effect, meaning the effect of X on M as well as the effect of M on Y, although the constituent components of the indirect effect are not of primary interest in modern mediation analysis. Many researchers often estimate the total effect of X on Y as well, although doing so is not required for the purpose of interpretation.

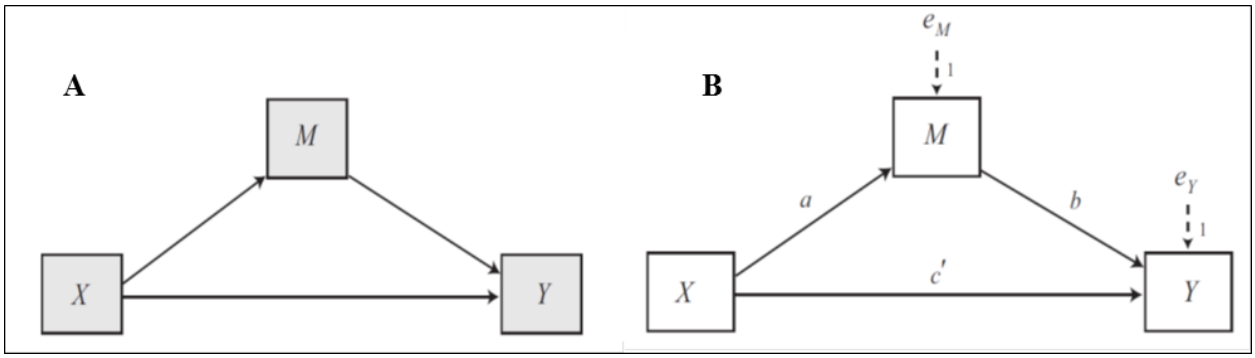


Figure 12 Conceptual (Panel A) and Statistical (Panel B) diagram of simple mediation model.

The simple mediation model represented in the form of a statistical diagram can be found in Figure 12 (Panel B). Notice that in comparing Panel A and B, there is little difference between the conceptual and statistical diagrams representing a simple mediation model. As there are two consequent variables in this diagram, two linear models are required, one for each consequent. This statistical diagram represents two equations:

$$M = i_1 + aX + e_M \qquad \textit{(Eq.10)}$$

$$Y = i_2 + c'X + bM + e_Y \qquad \textit{(Eq.11)}$$

where i_1 and i_2 are regression intercepts, e_M and e_Y are errors in the estimation of M and Y, respectively, and a, b, and c' are the regression coefficients given to the antecedent variables in the model in the estimation of the consequents. The coefficients of the model are treated as estimates of the putative causal influences of each variable in the system on others, and the analytical goal is to estimate these coefficients, piece them together, and interpret. These coefficients can be estimated by conducting two OLS regression analyses.

The Direct Effect of X on Y

In *(Eq.11)*, c' estimates the direct effect of *X* on *Y*. A generic interpretation of the direct effect is that two cases that differ by one unit on *X* but are equal on *M* are estimated to differ by c'units on *Y*. More formally,

$$c' = \left[\hat{Y} \mid (X = x, M = m)\right] - \left[\hat{Y} \mid (X = x - 1, M = m)\right] \qquad \textit{(Eq.12)}$$

where *m* is any value of *M*, | means *conditioned on* or *given*, and the hat over *Y* means *estimated* or *expected* from the model. In other words, for two cases with *M* = *m* but that differ by one unit on *X*, c' is the estimated value of *Y* for the case with *X* = *x* minus the estimated value of *Y* for the case with *X* = *x* − 1. As can be determined looking at equation 4.3, the sign of c'tells whether the case one unit higher on *X* is estimated to be higher(c'= +) or lower (c'= −) on *Y*. So a positive direct effect means that the case higher on *X* is estimated to be higher on *Y*, whereas a negative direct effect means that the case higher on *X* is estimated to be lower on *Y*. In the special case

where X is dichotomous, with the two values of X differing by a single unit (e.g., $X = 1$ and $X = 0$), $\hat{Y}$ can be interpreted as a group mean, so $c' = [\bar{Y}\,|(X = x, M = m)] - [\bar{Y}\,|(X = x - 1, M = m)]$, meaning c' estimates the difference between the two group means holding M constant. This is equivalent to what in analysis of covariance terms is called an *adjusted mean difference*.

The Indirect Effect of X on Y

Before defining the indirect effect, it is first necessary to discuss what a and b estimate. In this model, a quantifies how much two cases that differ by one unit on X are estimated to differ on M, with the sign determining whether the case higher on X is estimated to be higher (+) or lower (−) on M. That is, $a = \left[\widehat{M}\,|(X = x)\right] - \left[\widehat{M}\,|(X = x - 1)\right]$, when X is a dichotomous variable coded by a unit difference, a in (*Eq.10)* represents the difference between the two group means on M:

$$a = [M\,|(X = x)] - [M\,|\,(X = x - 1)]$$

The b coefficient from equation (*Eq.11)* has an interpretation analogous to c', except with M as the antecedent. Two cases that differ by one unit on M but that are equal on X are estimated to differ by b units on Y. As with a and c', the sign of b determines whether the case higher on M is estimated as higher (+) or lower (−) on Y:

$$b = \left[\hat{Y}\,|(M = m, X = x)\right] - \left[\hat{Y}\,|(M = m - 1, X = x)\right]$$

The indirect effect of X on Y through M is the product of a and b. The indirect effect tells us that two cases that differ by one unit on X are estimated to differ by ab units on Y as a result of the effect of X on M which, in turn, affects Y. The indirect effect will be positive (meaning the case higher on X is estimated to be higher on Y) if a and b are both positive or both negative, whereas it will be negative (meaning the case higher on X is estimated to be lower on Y) if either a or b, but not both, is negative. Although one can interpret the indirect effect without considering the signs of a and b, doing so can be dangerous, because the sign of ab is determined by two different configurations of the signs of a and b. A certain theory you are testing might predict ab to be positive because, according to the process the theory explains, a and b should both be positive. But what if, after estimation, a and b turned out to be negative? This would yield a positive indirect effect as predicted, yet this pattern of results for a and b is exactly opposite to what the theory predicts, and this should cast some doubt on whether the theory is adequately describing the process generating your data.

The Total Effect of X on Y

The direct and indirect effects perfectly partition how differences in *X* map on to differences in *Y*, the so-called *total effect* of *X*, denoted here as *c*. The total effect *c* quantifies how much two cases that differ by one unit on *X* are estimated to differ on *Y*. That is,

$$c = \left[\hat{Y} \mid (X = x)\right] - \left[\hat{Y} \mid (X = x - 1)\right]$$

In a simple mediation model, *c* can be derived by estimating *Y* from *X* alone:

$$Y = i3 + cX + eY \qquad \textit{(Eq.13)}$$

When *X* is a dichotomous variable coded by a single unit difference, *c* is the difference between the group means on *Y*: $c = [Y \mid (X = x)] - [Y \mid (X = x - 1)]$. Regardless of whether *X* is dichotomous, the total effect of *X* on *Y* is equal to the sum of the direct and indirect effects of *X*: $c = c' + ab$. This relationship can be rewritten as $ab = c - c'$, which provides another definition of the indirect effect. The indirect effect is the difference between the total effect of *X* on *Y* and the effect of *X* on *Y* controlling for *M*, the direct effect.

That the total effect of *X* is the sum of the direct and indirect effects can be illustrated by substituting *(Eq.11)* into equation *(Eq.12)*, thereby expressing *Y* as a function of only *X*:

$$Y = i_2 + b(i_1 + aX + e_M) + c'X + e_Y$$

which can be equivalently written as:

$$Y = (i_2 + bi_1) + (ab + c')X + (e_Y + be_M) \qquad \textit{(Eq.14)}$$

Although it may not look obvious, *(Eq.14)* is a simple linear function of *X*, just as *(Eq.13)*. In fact, equations *(Eq.13)*and *(Eq.14)*are identical if you make the following substitutions: $c = ab + c'$, $i3 = i2 + bi1$, and e_Y from equation *(Eq.13)* = $(e_Y + be_M)$ from equation *(Eq.14)*. So $ab + c'$ has the same interpretation as *c*. The sum of the direct and indirect effects quantifies how much two cases that differ by a unit on *X* are estimated to differ on *Y*.

4.4.2 Statistical Inferences

Inference about the Direct Effect of X on Y

The direct effect quantifies the estimated difference in Y between two cases that differ by one unit on *X* independent of *M*'s influence on *Y*. Inference for the direct effect of *X* on *Y* in a mediation analysis is typically undertaken using the standard method used for inference for any regression coefficient in a regression model. This involves testing a null hypothesis about ${}_Tc'$ against an alternative hypothesis or the construction of a confidence interval for ${}_Tc'$. Except in unusual circumstances, researchers focus on ascertaining whether a claim that ${}_Tc'$ is different from zero is justified based on the data available. If so, this supports the argument that *X* is

related to *Y* independent of the mechanism represented by *M*. If not, one can claim that there is no evidence of association between *X* and *Y* when the mechanism through *M* is accounted for. In other words, *X* does not affect *Y* independent of *M*'s effect on *Y*. In terms of a null hypothesis, this means testing $H0 : {}_{T}c' = 0$ against the alternative $Ha : {}_{T}c' \neq 0$. Framed in terms of a confidence interval, this involves determining whether an interval estimate for Tc' includes zero.

Inference about the Indirect Effect of X on Y through M

The indirect effect quantifies how much two cases that differ by a unit on *X* are estimated to differ on *Y* as a result of *X*'s influence on *M*, which in turn influences *Y*. The indirect effect is relevant as to whether *X*'s effect on *Y* can be said to be transmitted through the mechanism represented by the $X \rightarrow M \rightarrow Y$ causal chain of events. As with the direct effect, investigators typically want to know whether the data allow for the claim that this estimated difference in *Y* attributable to this mechanism can be said to be different from zero. If so, one can claim *M* serves as a mediator of the effect of *X* on *Y*. As with inference about the direct effect, this inference can be formulated in terms of a null hypothesis test about ${}_{T}a_{T}b$ or by constructing an interval estimate. There are more than a dozen of available approaches [87-90] to statistical inference for the indirect effect, the most popular are:

- **The Normal Theory Approach (Sobel Test)**. Also called the *product of coef*fi*cients* approach to inference, the *delta method*, or the *Sobel test*, the normal theory approach is based on the same theory of inference used for inference about the direct effect, as well as other inferential tests widely used in the social sciences and described in elementary statistics books. The indirect effect *ab* is a sample-specific instantiation of ${}_{T}a_{T}b$, which is subject to sampling variance. With an estimate of the standard error of *ab* and assuming the sampling distribution of *ab* is normal, a *p*-value for *ab* can be derived given a specific null hypothesized value of ${}_{T}a_{T}b$, or an interval estimate can be generated. Before the normal theory approach can be implemented, an estimate of the standard error of *ab* is needed. There are a few such estimators circulating in the literature that have been used in mediation analysis [91-94]. The simplest is a function of *a* and *b* and their standard errors:

$$se_{ab} = \sqrt{a^2\, se_b^2 + b^2\, se_a^2} \qquad \textit{(Eq.15)}$$

  where se_a^2 and se_b^2 are the squared standard errors of a and b, respectively. A slightly more complex estimator includes an additional term:

$$se_{ab} = \sqrt{a^2\, se_b^2 + b^2\, se_a^2 + se_a^2\, se_b^2} \qquad \textit{(Eq.16)}$$

In practice, it typically makes little difference which estimator is used [92, 95]. *(Eq.15)* is sometimes called the "first-order" delta estimator of the standard error and *(Eq.16)* the "second-order" estimator.

With an estimate of the standard error of the indirect effect, the null hypothesis that ${}_Ta_Tb = 0$ can be tested against the alternative that ${}_Ta_Tb \neq 0$ by taking the ratio of *ab* to its standard error:

$$Z = \frac{ab}{se_{ab}}$$

and deriving the proportion of the standard normal distribution more extreme than ±*Z*.

Using confidence intervals over null hypothesis testing, the standard error of *ab* can be used to generate an interval estimate for ${}_Ta_Tb$ by assuming normality of the sampling distribution of *ab* and applying the following equation:

$$ab - Zci\%se_{ab} \leq TaTb \leq ab + Zci\%se_{ab} \qquad (Eq.17)$$

where *ci* is the confidence desired (e.g., 95) and *Zci*% is the value of the standard normal distribution above which (100 − *ci*)/2% percent of the distribution resides.

The normal theory approach is simple enough to conduct, and it can be conducted by hand fairly easily if one is careful using the output from any statistical software that estimates *a*, *b*, and their standard errors. Moreover the normal theory approach can be conducted even if one does not have the data used to estimate *a*, *b*, and their standard errors. Although most researchers would have the original data from their own studies, there could be some circumstances in which it is not available (time has passed; the data were destroyed, lost, or stored on an obsolete storage medium; etc.). In addition, one could apply this approach using the regression coefficients and standard errors provided in the tables or text of published studies conducted by someone else that include a mediation analysis but not a formal test of the indirect effect.

These benefits aside (ease of computation, not requiring the data), the normal theory approach suffers from two flaws that make it difficult to recommend. First, whether inference is based on a hypothesis test or the construction of a confidence interval, this method assumes that the sampling distribution of *ab* is normal. But it has been shown analytically and through simulation that the distribution is quite irregular in sample sizes that characterize most empirical studies [96, 97]. Because it is never possible to know for certain whether the sampling distribution is close enough to normal given the characteristics of one's problem to safely apply a method that assumes normality, it is desirable to use a test that does not require this assumption, if one is available. There are several inferential tests available that do not require this assumption and that better

respect the irregularity of the sampling distribution of *ab* than does the normal theory approach. Second, simulation research that has compared this approach to various competing inferential methods has shown that it is one of the lowest in power and generates confidence intervals that tend to be less accurate than some other methods described next [98]. If *X* does influence *Y* indirectly through *M*, the normal theory approach is relatively less likely to detect it than competing alternatives. So its relatively low power combined with the unrealistic normality assumption suggest to avoid the Sobel test when possible. A possible alternative is the calculation of Bootstrap Confidence Interval.

- **Bootstrap Confidence Intervals**. The downfall of the normal theory approach is the assumption it makes about the shape of the sampling distribution of the indirect effect over repeated sampling from the population. As a member of a class of procedures known as *resampling methods*, bootstrapping [99-103] is a versatile method that can be applied to many inferential problems a researcher might confront. It is especially useful when the behaviour of a statistic over repeated sampling is either not known, too complicated to derive, or highly context dependent. Regardless of the inferential problem, the essence of bootstrapping remains constant across applications. The original sample of size *n* is treated as a miniature representation of the population originally sampled. Observations in this sample are then "resampled" *with replacement*, and some statistic of interest is calculated in the new sample of size *n* constructed through this resampling process. Repeated over and over—thousands of times ideally—a representation of the sampling distribution of the statistic is constructed empirically, and this empirical representation is used for the inferential task at hand. In mediation analysis, bootstrapping is used to generate an empirically derived representation of the sampling distribution of the indirect effect, and this empirical representation is used for the construction of a confidence interval for ${}_{T}a_{T}b$. Unlike the normal theory approach, no assumption is made about the shape of the sampling distribution of *ab*. Bootstrap confidence intervals better respect the irregularity of the sampling distribution of *ab* and, as a result, yield inferences that are more likely to be accurate than when the normal theory approach is used. When used to test a hypothesis, the result is a test with higher power. There are six steps involved in the construction of a bootstrap confidence interval for ${}_{T}a_{T}b$:
    1. *Take a random sample of n cases from the original sample, sampling those cases with replacement, where n is the size of the original sample. This is called a bootstrap sample.*

2. *Estimate the indirect effect ab* in the bootstrap sample, where ab* is the product of a and b from (Eq.10) and (Eq.11).*
3. *Repeat (1) and (2) above a total of k times, where k is some large number, saving the value of ab* each time. Generally, k of at least a few thousand is preferred. More than 10,000 typically is not necessary, but in principle, the more the better.*
4. *Sort the k indirect effects ab estimated from steps (1), (2), and (3) from low to high.*
5. *For a ci% confidence interval, find the value of ab in this distribution of k estimates that defines the 0.5(100 − ci)th percentile of the distribution. This is the lower bound of a ci% confidence interval. It will be the value of ab in ordinal position 0.005k (100 − ci) of the sorted distribution.*
6. *Find the value of ab* in this distribution of k estimates that defines the [100 − 0.5(100 − ci)]th percentile of the distribution. This is the upper bound of a ci% confidence interval. It will be the value of ab in ordinal position k [1 − 0.005(100 − ci)] + 1 of the sorted distribution.*

To illustrate steps (1), (2), and (3) of this bootstrap sampling and estimation process, Table 2 provides a small-scale example. Suppose to have a sample of n = 10 cases in a study measured on variables X, M, and Y, and suppose to generate a bootstrap sampling distribution of the indirect effect of X on Y through M. Using the original data in the leftmost columns of the table, the obtained indirect effect is ab = 0.770. This is a point estimate of TaTb. A bootstrap confidence interval for TaTb is constructed by repeatedly taking a random sample of size n from the original sample, with replacement, and estimating the indirect effect in each resample.

Table 2: Bootstrap Estimates of a, b, and the Indirect Effect ab When Taking Two Bootstrap Samples from an Original Sample of Size n = 10 Original sample Bootstrap sample 1 Bootstrap sample 2

Original Sample				**Bootstrap sample1**				**Bootstrap sample2**			
Case	**X**	**M**	**Y**	**Case**	**X**	**M**	**Y**	**Case**	**X**	**M**	**Y**
1	4.1	3.4	2.1	1	3.8	6.3	3.2	1	4.5	2.5	4.3
2	4.2	5.4	2.2	2	4.2	5.4	2.2	2	4.3	8.6	5.3
3	3.3	3.2	4.6	3	4.5	2.5	4.3	3	4.1	3.4	2.1
4	4.3	8.6	5.3	4	3.3	3.2	4.6	4	3.0	5.3	6.5
5	3.0	5.3	6.5	5	4.1	3.4	2.1	5	4.8	2.1	4.8
6	4.5	2.5	4.3	6	4.9	2.9	3.3	6	4.5	3.3	5.1
7	3.8	6.3	3.2	7	4.5	3.3	5.1	7	4.2	5.4	2.2
8	4.9	2.9	3.3	8	4.3	8.6	5.3	8	4.9	2.9	3.3
9	4.5	3.3	5.1	9	4.8	2.1	4.8	9	3.3	3.2	4.6
10	4.8	2.1	4.8	10	3.0	5.3	6.5	10	3.8	6.3	3.2
a			*1.16*	*a*			*2.14*	*a*			1.16
b			*3.24*	*b*			*3.15*	*b*			3.18
ab			*3.76*	*ab**			*6.74*	*ab**			3.69

The middle columns of Table 2 contain one such bootstrap sample, which yields an indirect effect of ab = 0.526. The rightmost columns contain a second bootstrap sample with an indirect effect of ab= 1.039. As this process is repeated over and over, a distribution of ab is built which functions as an empirical proxy for the unknown sampling distribution of ab when taking a random sample of size n from the original population. Steps (5) and (6) are generic ways of describing how the endpoints of a confidence interval are constructed given k bootstrap estimates of the indirect effect. A specific example will help. If a ci = 95% confidence interval is desired, the lower and upper bounds of the interval are defined as the bootstrap values of ab that define the 2.5th and 97.5th percentiles in the distribution of k values of ab. Suppose k = 10, 000. In that case, after sorting the 10,000 values of ab obtained from repeated bootstrap sampling from low to high, the 2.5th and 97.5th percentiles of the distribution will be in ordinal positions 0.005(10, 000)(100−95) = 250 and (10, 000)[1−0.005(100−95)] + 1 = 9,751 in the sorted list, respectively. These are the lower and upper bounds of the 95% confidence interval for TaTb. Obviously, this is a computationally intensive process that requires a computer.

The use of bootstrap confidence as inferential approach it is not without its pitfalls and criticisms:

- first, in order to have much confidence in bootstrap-based inference, it is clearly important that one is able to muster some faith in the quality of one's sample as a reasonable representation of the population with respect to the distribution of the measured variables. Bootstrapping is founded on the notion that resampling with replacement from one's sample mimics the original sampling process. But if the sample does not adequately represent the population from which the sample was derived, then bootstrapping will produce results that are hard to trust. It is not required that the original sample be obtained randomly from the population, but merely that the distribution of the measured variables roughly mirrors the population distributions. Random sampling facilitates this representativeness, of course, but it isn't required;
- second, bootstrapping is particularly useful relative to the normal theory approach in smaller samples, because it is in smaller samples that the non-normality of the sampling distribution of ab is likely to be most severe, the large sample asymptotics of the normal theory approach are harder to trust, and the power advantages of bootstrapping are more pronounced. But if the original sample is

very small, in principle, there is a strong potential for one or two cases to distort a bootstrap analysis even more than they do a more traditional inferential procedure. If the original sample is very small, an unusual case or two are highly likely to appear in a bootstrap sample multiple times, and this can distort a bootstrap analysis;

- third, because bootstrap confidence intervals are based on random resampling of the data, the endpoints of the confidence interval are not fixed quantities. Rather, each time a bootstrap confidence interval is produced from the same data, a slightly different confidence interval will result, however the sampling variation from analysis to analysis can be made arbitrarily small simply by setting the number of bootstrap samples to an arbitrarily large number. This raises the question as to how many bootstrap samples is enough. It can be shown that the variation in the estimation of the limits of a confidence interval shrinks remarkably quickly as the number of bootstrap samples increases. Generally speaking, 5,000 to 10,000 bootstrap samples is sufficient in most applications. There is relatively little added value to increasing it above 10,000, as the gain in precision is fairly marginal beyond that.

  A bootstrap confidence interval calculated using the approach just described is called a *percentile bootstrap confidence interval*, because it is based entirely on values of *ab** that demarcate the upper and lower (100 − ci)/2% of the distribution of k bootstrap estimates of the indirect effect. It is also possible calculate *bias-corrected bootstrap confidence intervals (BC bootstrap confidence intervals)* are like percentile confidence intervals but the endpoints are adjusted as a function of the proportion of k values of *ab** that are less than ab, the point estimate of the indirect effect calculated in the original data. The endpoints will be adjusted upward or downward to varying degrees depending on that proportion. A variation on this variation, known as the bias-corrected and accelerated bootstrap confidence interval, makes an additional adjustment based on the skew of the distribution of k bootstrap estimates. The following steps [87, 104-106]; allow to generate a bias-corrected bootstrap confidence interval for the indirect effect:

1. *Follow steps (1) through (4) to generate k bootstrap estimates of the indirect effect, ab* as described before.*
2. *Calculate $Z(\tilde{p})$, the Z-score that cuts off the lower 100 $\tilde{p}$ % of the standard normal distribution from the rest of the distribution, and $\tilde{p}$ is the proportion of the k values of ab* that are less than ab calculated using the original data.*

3. *Calculate $Z_{low} = Z_{ci} + 2\,Z(\tilde{p})$ and $Z_{high} = -Z_{ci} + 2\,Z(\tilde{p})$), where Zci is the Z-score that cuts off the lower (100 − ci%)/2 percent of the standard normal distribution from the rest of the distribution. For instance, for a 95% confidence interval, $Z_{95} = -1.96$.*
4. *Calculate plow and phigh, the proportion of the standard normal distribution the left of Z_{low} and Z_{high}, respectively.*
5. *Find the value of ab* in the distribution of k estimates that defines the 100plow percentile of the distribution. This is the lower bound of a ci% bias-corrected bootstrap confidence interval, and will be the value of ab* in ordinal position (plow)k of the sorted distribution. If (plow)k is not an integer, round it down to the lowest integer.*
6. *Find the value of ab* in the distribution of k estimates that defines the 100phigh percentile of the distribution. This is the upper bound of a ci% bias-corrected bootstrap confidence interval, and will be the value of ab* in ordinal position (phigh)k of the sorted distribution. If (phigh)k is not an integer, round it up to the next highest integer.*

Observe that the upper and lower bounds of the 95% bootstrap confidence intervals calculated earlier are not equidistant from the point estimate. This is not due to the random resampling process but instead reflects the actual asymmetry of the sampling distribution of ab. Confidence intervals based on the normal theory approach to inference, by contrast, impose a symmetry constraint on this distance. The endpoints of a 95% confidence interval using equation 4.9 are necessarily 1.96 standard errors from the point estimate. The endpoints are symmetrical around the point estimate. Thus, percentile-based and BC bootstrap confidence intervals are called "asymmetric," whereas normal theory confidence intervals are "symmetric." Asymmetric approaches to interval estimation are preferred when the sampling distribution of the estimator is asymmetric and non-normal, as is the case for the sampling distribution of ab.

Bootstrapping is not the only approach to the construction of asymmetric confidence intervals. Although bootstrapping is recommended, it does have a few weaknesses, among them that it requires the original data (not usually a real problem typically), the endpoints of the confidence interval will vary from run to run (but not if you seed the random number generator yourself), and it isn't implemented in all software one might choose to use. An alternative to get around these problems: Monte Carlo confidence intervals. Monte Carlo confidence intervals [87, 98, 107] are simulation-based. This approach relies on the fact that though the distribution of ab is not normal, the sampling distributions of a and b tend to be nearly so. Furthermore, in simple mediation analysis using OLS

regression, a and b are independent across repeated sampling (i.e., their covariance is zero). Thus, an empirical approximation of the sampling distribution of ab can be generated by randomly sampling values of a and b from normally distributed populations with μ = a, σ = sea and μ = b, σ = seb, respectively, where a, b, sea, and seb are the OLS regression coefficients and standard errors from the mediation analysis. The sampled values of a and b are then multiplied together to produce ab*, and this process is repeated k times. Over the k replications, the upper and lower bounds of the confidence interval for ab can be generated using the procedure described in steps (4) through (6) for the construction of bootstrap confidence intervals. Monte Carlo method is almost as good as bootstrapping and better than the normal theory approach.

Inference about the Total Effect of X on Y

In a simple mediation model, the total effect of *X* on *Y* is the sum of the direct effect of *X* on *Y* and indirect effect of *X* on *Y* through *M*. Whereas there are many choices available for inferences about the indirect effect, inference for the total effect is simple and straightforward. Although the total effect is the sum of two pathways of influence, it can be estimated simply by regressing *Y* on *X*. The regression coefficient for *X* in that model, *c* in equation 4.4, is the total effect of *X*. Inference can be framed in terms of a null hypothesis test ($H0 : {}_{T}c = 0$ versus the alternative $Ha : {}_{T}c \neq 0$) or whether an interval estimate for ${}_{T}c$ includes zero.

4.4.3 The Parallel Multiple Mediator Model

In a parallel multiple mediator model, antecedent variable X is modelled as influencing consequent Y directly as well as indirectly through two or more mediators, with the condition that no mediator causally influences another. *Figure 13* depicts a parallel multiple mediator model with k mediators in conceptual form (Panel A) and in statistical diagram (Panel B). Observe that the parallel multiple mediator model looks much like a simple mediation model except that it includes more than one mediator. A defining feature of the parallel multiple mediator model that distinguishes it from an alternative multiple mediator model, for example the serial multiple mediator model, is the constraint that no mediator is modelled as influencing another mediator in the model. This constraint is apparent in *Figure 13* by the absence of any unidirectional arrows linking any mediator to any other mediator. This is not to say that the mediators are assumed to be independent. In fact, in most circumstances, the mediators are likely to be correlated. Even if they are not, there still may be some advantage to estimating a parallel multiple mediator model with k mediators rather than k simple mediation models.

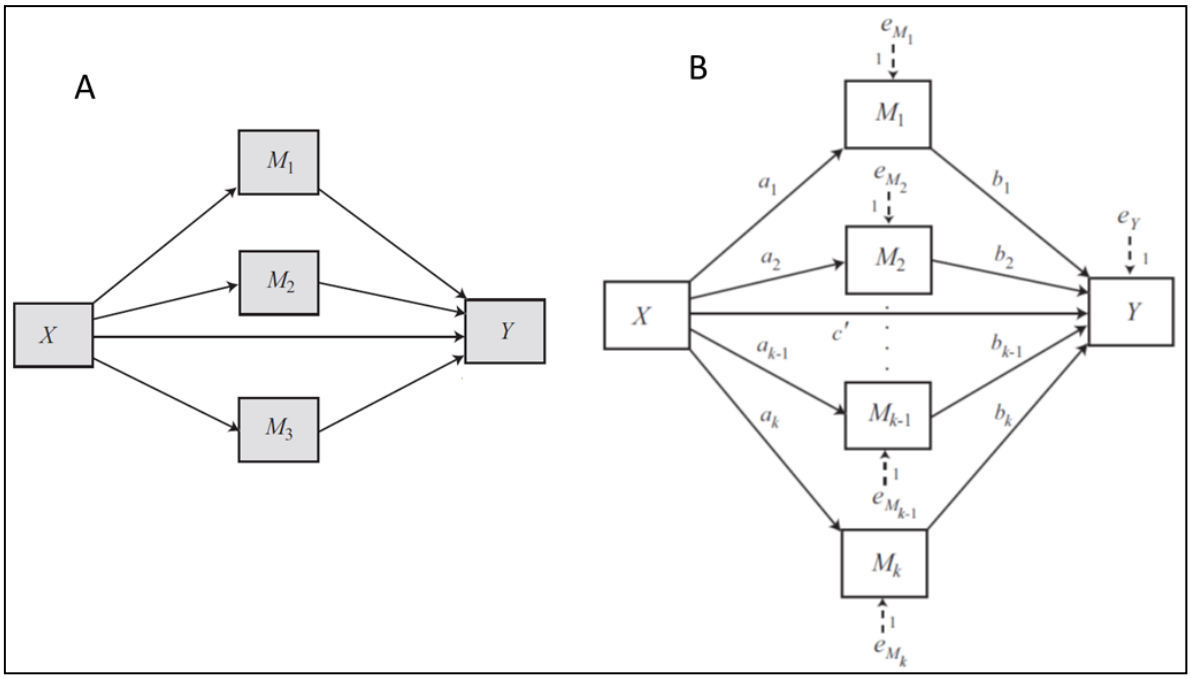


Figure 13 Conceptual (Panel A) and Statistical (Panel B) representing a parallel multiple mediator model with k mediators.

Doing so could result in a power boost for tests of indirect effects if each mediator is correlated with Y, and doing so affords the ability to compare the sizes of the indirect effects through different mediators. In principle, the number of mediators one can include in a parallel multiple mediator model is limited only by the number of cases in one's data file and the number of variables one has the foresight to measure as possible mediators. In practice, models with two mediators are most commonly estimated. But parallel multiple mediator models can be found with three, six, and even as many as seven mediators in a model simultaneously. As can be seen in *Figure 13*, a parallel multiple mediator model with k mediators has k + 1 consequent variables (one for each of the k mediators M and one for the outcome variable Y) and so requires k + 1 equations to estimate all the effects of X on Y. These equations are:

$$M_i = i_{Mi} + a_i X + e_{Mi} \text{ for all } i = 1 \text{ to } k \qquad \textit{(Eq.17)}$$

$$Y = i_Y + c'X + \sum_{i=1}^{k} b_i M_i + e_Y \qquad \textit{(Eq.18)}$$

In this set of equations, a_i estimates the effect of X on M_i , bi estimates the effect of M_i on Y controlling for X and the other $k - 1$ M variables, and c' estimates the effect of X on Y holding all k M variables constant. Consider a parallel multiple mediator with three proposed mediators. With k = 3 mediators, four equations are needed:

$$M_1 = i_{M1} + a_1 X + e_{M1} \qquad \textit{(Eq.19)}$$

$$M_2 = i_{M2} + a_2 X + e_{M2} \qquad \textit{(Eq.20)}$$

$$M_3 = i_{M3} + a_3 X + e_{M3} \qquad \textit{(Eq.21)}$$

$$Y = i_Y + c'X + b_1M_1 + b_2M_2 + b_3M_3 + e_{M3} \quad \textit{(Eq.22)}$$

In equations *(Eq.19)*, *(Eq.20)*, and *(Eq.21)*, a_1, a_2, and a_3 quantify the amount by which two cases that differ by one unit on X are estimated to differ on M_1 , M_2 , and M_3 , respectively. In equation *(Eq.22)*, b_1 estimates the amount by which two cases that differ by a unit on M_1 differ on Y holding M_2, M_3, and X constant. Similarly, b_2 estimates the amount by which two cases that differ by a unit on M_2 differ on Y holding M_1, M_3, and X constant, and b_3 estimates the amount by which two cases that differ by a unit on M_3 differ on Y holding M_1, M_2, and X constant. Finally, c′ estimates the amount by which two cases that differ by one unit on X differ on Y holding M_1, M_2, and M_3 constant. The interpretations of a_i and c' are not dependent on the scale of measurement of X. Whether X is a dichotomous variable or a continuum, the interpretation is the same. However, when X is a dichotomous variable with the two groups coded by a one unit difference, these can be interpreted as estimated mean differences. For instance, suppose the two groups are coded with $X = 0$ or $X = 1$. In that case:

$$a_i = [\overline{M_i}|(X = 1)] - [\overline{M_i}|(X = 0)] \text{ and } a_i = [\overline{Y*}|(X = 1)] - [\overline{Y*}|(X = 0)]$$

where $\overline{Y*}$ is an adjusted mean, with all mediators set to their sample means:

$$\overline{Y*} = i_Y + c'X + \sum_{i=1}^{k} b_i\overline{M}_i$$

Direct and Indirect Effects in a Parallel Multiple Mediator Model

In a parallel multiple mediator as in *Figure 13*, X is modeled to exert its effect on Y through $k + 1$ pathways. One pathway is direct, from X to Y without passing through any of the proposed mediators, and the other k pathways are indirect, each through a single mediator. In a multiple mediator model, the indirect effects are referred to as *specific indirect effects*. Thus, a model with k mediators has k specific indirect effects, one through M_1 ($X \rightarrow M_1 \rightarrow Y$), one through M_2 ($X \rightarrow M_2 \rightarrow Y$), and so forth, up through M_k ($X \rightarrow M_k \rightarrow Y$). As in a simple mediation model, the indirect effect of X on Y through a given mediator M_i is quantified as the product of paths linking X to Y through Mi. In a parallel multiple mediator model, only two paths link X to Y through M_i . The first of these paths is the effect of X to M_i , and the second is the path from M_i to Y. The regression coefficients corresponding to these paths, when multiplied together, yield the specific indirect effect of X on Y through M_i . So consider the three-mediator parallel multiple mediator model estimated with equations *(Eq.19)* through *(Eq.20)*. In this model, the specific indirect of X on Y through M_1 is a_1b_1, the specific indirect effect through M_2 is a_2b_2, and the specific indirect effect of X through M_3 is a_3b_3. Most generally, regardless of the number of mediators, the specific indirect effect of X on Y through M_i is estimated as

$a_i b_i$ from equations *(Eq.17)* and *(Eq.18)*. A specific indirect effect is interpreted just as in the simple mediation model, except with the addition of controlling for all other mediators in the model. Thus, the specific indirect effect of *X* on *Y* through M_i is the estimated amount by which two cases that differ by a unit on *X* are estimated to differ on *Y* as a result of the effect of *X* on M_i , which in turn affects *Y*, holding all other mediators constant. When added together, the specific indirect effects yield the *total indirect effect* of *X* on *Y* through all mediators in the model. In a model with *k* mediators:

$$Total\ indirect\ effect\ of\ X\ on\ Y = \sum_{i=1}^{k} a_i b_i$$

For example, in a parallel multiple mediator model with three mediators represented by equations *(Eq.19)* through *(Eq.22)*, the total indirect effect of *X* on Y is $a_1 b_1 + a_2 b_2 + a_3 b_3$. The direct effect of *X* quantifies how much two cases that differ by a unit on *X* are estimated to differ on *Y* independent of all mediators. As discussed earlier, this is c' in the model of *Y* from *X* and all mediators (e.g., equation *(Eq.22)* for the three-mediator model, or equation *(Eq.18)* more generally). As in the simple mediation model, the sum of the direct and indirect effects is the total effect of *X*. In a model with k mediators, from the coefficients in equations *(Eq.17)* and *(Eq.18)*.

$$c = c' + \sum_{i=1}^{k} a_i b_i \qquad (Eq.23)$$

where c is the total effect of *X*. The total effect can also be estimated by regressing *Y* on *X* alone For instance, in the three mediator model, $c = c' + a_1 b_1 + a_2 b_2 + a_3 b_3$. Isolation of the total indirect effect in equation *(Eq.23)* shows that the total indirect effect is equal to the difference between the total and the direct effects of *X*:

$$c - c' = \sum_{i=1}^{k} a_i b_i$$

Inference about the Direct Effect

As in the simple mediation model, inference about the direct effect of X on Y is straightforward. A test of the null hypothesis *H*0 : $_T c'$ = 0 versus the alternative *Ha* : $_T c' \neq 0$ is available in the output from any statistical package that can estimate *(Eq.18)* using OLS regression. Alternatively, a confidence interval can be constructed using equation $c' - t_{c\%}\, Se_{c'} \leq Tc' \leq c' - t_{c\%}\, Se_{c'}$.

Inference about Specific Indirect Effects

The normal theory approach for the indirect effect in a simple mediation model can be used for statistical inference about specific indirect effects in a parallel multiple mediator model without modification. For the specific indirect effect of X on Y through M_i , the first-order standard error estimator is:

$$Se_{a_i b_i} = \sqrt{a_i^2 Se_{b_i}^2 + b_i^2 Se_{a_i}^2}$$

where $Se_{b_i}^2$ and $Se_{a_i}^2$ are the squared standard errors of $a_i$and b_i. The second order estimator is:

$$Se_{a_i b_i} = \sqrt{a_i^2 Se_{b_i}^2 + b_i^2 Se_{a_i}^2 + Se_{b_i}^2 Se_{a_i}^2}$$

A test of the null hypothesis that ${}_T a_i {}_T b_i = 0$ is constructed by dividing $a_i b_i$ by the estimated standard error and deriving a *p*-value from the standard normal distribution. Alternatively, a c_i% confidence interval can be constructed as $a_i b_i - Zc_i\% Se_{a_i b_i} \leq {}_T a_i {}_T b_i \leq a_i b_i + Zc_i\% Se_{a_i b_i}$ where c_i is the confidence desired (e.g., 95) and $Zc_i\%$ is the value under the normal distribution that cuts off the upper $(100 - c_i)/2\%$ of the distribution from the rest.

However this approach is hard to trust. It makes the unrealistic assumption of normality of the sampling distribution of the specific indirect effect, and it is one of the more conservative tests available. Also in this case bootstrap confidence intervals are the better approach to inference when the original data are available for analysis. No assumptions about the shape of the sampling distribution of $a_i b_i$are made, and bootstrap confidence intervals tend to be more powerful than competing methods such as the normal theory approach [108]. Using the same procedure described before, a bootstrap confidence interval for a specific indirect effect is constructed by taking a random sample with replacement of size *n* from the sample, estimating each specific indirect effect $a_i b_i *$ in the resulting data, and repeating this resampling and estimation many times. With several thousand bootstrap estimates of each specific indirect effect, endpoints of the confidence interval are calculated using either the percentile or bias corrected method. If zero is outside of a c_i% confidence interval, then ${}_T a_i {}_T b_i$is declared different from zero with c_i% confidence, whereas if the confidence interval straddles zero, the conclusion is that there is insufficient evidence that *X* affects *Y* through M_i .

Pairwise Comparisons between Specific Indirect Effects

In a multiple mediator model, it is sometimes of interest to test whether one indirect effect is statistically different from another. If the indirect effect of *X* through mediator *i* (i.e., $a_i b_i$) is pertinent to the mechanism postulated by one theory and the indirect effect of *X* through mediator *j* (i.e., $a_j b_j$) quantifies the mechanism relevant to a second theory, an inference about whether ${}_T a_i {}_T b_i = {}_T a_j {}_T b_j$ affords a claim as to whether one mechanism accounts for more of the effect of *X* on *Y* than the other mechanism [109, 110],

with an important caveat described below. Although it might seem that such a comparison between specific indirect effects would be impossible if the mediators are measured on different metrics, it turns out this is not a problem at all. Remember that the specific indirect effect is interpreted as the amount by which two cases differing by a unit on *X* are estimated to differ on *Y* through the intervening variable independent of the other intervening variables. Notice that this interpretation does not include the metric of the intervening variable. Specific indirect effects are scaled entirely in terms of the metrics of *X* and *Y [68, 89, 111]*, so two specific indirect effects of the same antecedent on the same consequent can be meaningfully compared even if the mediator variables are measured on entirely different scales. Thus, standardization is not necessary to conduct an inferential test of the equality of specific indirect effects from *X* to *Y* in a multiple mediator model.

Two inferential approaches have been most widely discussed and disseminated in the literature. A normal theory approach is described by [68, 111] based on dividing $a_i b_i - a_j b_j$ by an estimate of its standard error. One estimator of the standard error of the difference is:

$$Se_{a_i b_i - a_j b_j} = \sqrt{b_i^2 Se_{a_i}^2 - 2 b_i b_j COV_{a_i a_j} + b_j^2 Se_{a_j}^2 + a_j^2 Se_{b_j}^2 - 2 a_i a_j COV b_i b_j + a_i^2 Se_{b_i}^2}$$

where $COV_{a_i a_j}$ is the covariance between a_i and a_j, and $COV b_i b_j$ is the covariance between b_i and b_j. [111] Offers a different standard error estimator that does not require the covariance between a_i and a_j by assuming it is zero, which is equivalent to constraining the correlation between the residuals in the models of M_i and M_j to be zero:

$$Se_{a_i b_i - a_j b_j} = \sqrt{b_i^2 Se_{a_i}^2 + b_j^2 Se_{a_j}^2 + a_j^2 Se_{b_j}^2 - 2 a_i a_j COV b_i b_j + a_i^2 Se_{b_i}^2}$$

The ratio of the difference to its standard error is then calculated and a *p*-value for a test of the null hypothesis that ${}_T a_i {}_T b_i = {}_T a_j {}_T b_j$ can be derived using the standard normal distribution. Alternatively, a 95% confidence interval for the difference can be computed as:

$$(a_i b_i - a_j b_j) \pm 1.96 Se_{a_i b_i - a_j b_j} \qquad \textit{(Eq.23)}$$

In this expression, 1.96 can be replaced with an appropriate critical *Z* from a table of normal probabilities for different confidence levels (e.g., 1.645 for 90% or 2.57 for 99% confidence).

Like all normal theory approaches discussed thus far, this method requires the assumption that the sampling distribution of the difference between specific indirect effects is normal. It turns out that this is a fairly reasonable assumption, but since an assumption can never be proven true, bootstrapping offers an alternative test without requiring this assumption. A bootstrap confidence interval is derived by estimating the difference between specific indirect effects over repeated bootstrap sampling and model estimation. Using the resulting empirical approximation of the sampling distribution of the difference between specific indirect effects, a confidence interval for the difference can be constructed using either the percentile method described earlier or through bias correction.

In a model with *k* mediators, will be conducted $k(k-1)/2$ pairwise comparisons, one for each possible difference between specific indirect effects. A confidence interval that does not contain zero provides

evidence that the two indirect effects are statistically different from each other, whereas a confidence interval that straddles zero supports the claim of no difference between the specific indirect effects.

It is tempting to treat this as a test of the difference in *strength* of the mechanisms at work linking *X* to *Y*, or that one indirect effect is larger than another in an absolute sense. However, such an interpretation is justified only if the point estimates for the two specific indirect effects being compared are of the same sign. Consider, for instance, the case where $a_ib_i = -0.30$ and $a_jb_j = 0.30$. A test of the difference between these specific indirect effects may lead to the claim that their difference is not zero, but this does not imply the mechanisms are of different strength or that one indirect effect is bigger. The point estimates suggest one mechanism results in a positive difference in *Y*, whereas the other yields a negative difference of equal magnitude. In an absolute sense, they are equal in size by the point estimates, yet statistically different by an inferential test which considers their sign. But one indirect effect is not *stronger* than the other. Nor can we say that X exerts a larger effect on Y through one of the mediators relative to the other.

Inference about the Total Indirect Effect

A multiple mediator model also contains a *total indirect effect*, defined as the sum of all specific indirect effects. It is possible to conduct an inferential test of the total indirect effect using either the normal theory approach or a bootstrap confidence interval. The normal theory approach requires an estimate of the standard error of the total indirect effect, but the formula for constructing it is quite complicated even in multiple mediator models with only two mediators. Given such complicated expressions is recommended to use a bootstrap confidence interval obtained as described before.

5. RESULTS

5.1 Study Population description at screening Stage.

Table 1 and Table 2 summarize continuous and categorical variables collected in the study at stage 1. A total of 1250 subjects, mainly women (74%), were enrolled (participation rate in the years 2010-2013 was 90%) with a mean age of 51.9±13.6 years and a mean BMI of 33.5±5.5 kg/m^2: 7.8% are overweight, 38.6% obese, and 33.6% severe obese (BMI ≥ 35 Kg/cm2). About 34% of subjects are former smokers and about 15% are current smokers with a median of 14.5 Pack/years. About 57% of SPHERE subjects live in the city and an additional 28% work in Milan (even if they live outside the city), overall a 67% of subjects spent many hours a day in the city or travelling from workplace to residence. Thus, longer exposure effects are evaluated by both residential and Milan monitors using appropriate lag time from recruitment date, as sensitivity analysis.

Table 3: Characteristics of study participants and data collected from the self-reported questionnaire at December 31, 2013.

Characteristics	**Categories**		**n=1250 (Stage 1)**
Sex	Male		330 (26.4%)
	Female		920 (73.6%)
Age	Years (mean±SD)		51.9±13.6
Education	Primary school or less		105 (8.4%)
	Secondary school		325 (26.0%)
	High school		493 (39.4%)
	University		188 (15.0%)
	Others		87 (7.0%)
	Missing		52 (4.2%)
Occupation	Employee		714 (57.1%)
	Unemployed		102 (8.2%)
	Pensioner		304 (24.3%)
	Housewife		93 (7.4%)
	Missing		37 (3.0%)
Ethnicity	White		1198(95.8%)
	Black		11 (0.9%)
	Asian		3 (0.3%)
	South America		38 (3.0%)
Year of enrollment		2010	129 (10.3%)
		2011	419 (33.5%)
		2012	385 (30.8%)
		2013	317 (25.4%)
Season of enrollment	Winter		320 (25.6%)
	Spring		313 (25.0%)
	Summer		190 (15.2%)
	Autumn		427 (34.2%)
Smoking	Never		599 (47.9%)
	Former		431 (34.5%)
	Current		190 (15.2%)

	Missing	30 (2.4%)
Cigarettes smoked* [cigarettes/day]	<= 5	53 (27.9%)
	5-10	53 (27.9%)
	10-15	33 (17.4%)
	15-20	37 (19.6%)
	20-40	13 (6.8%)
	Missing	1 (0.5%)
Pack/years (n=1153)	Median [Q1, Q3]	
	Among current and former smokers	14.5 [6.1-28.0]
	Including nonsmokers	0 [0-13.5]
Alcohol consumption	Yes	636 (50.9%)
	No	518 (41.4%)
	Missing	96 (7.7%)
Residence area	City	534 (42.7%)
	Peripheral area	331 (26.5%)
	Rural area	30 (2.4%)
	Village/small city	206 (16.5%)
	Missing	149 (11.9%)
Living area	Province of Milan (Excluding City of Milan)	379 (30.3%)
	City of Milan	713 (57.0%)
	Outside Milan	158 (12.7%)
Work area	Province of Milan (Excluding City of Milan)	94 (13.1%)
	City of Milan	339 (47.5%)
	Outside Milan	34 (4.8%)
	Missing	247 (34.6%)

Table 4: Characteristics of the study subjects and mean levels of the clinical measure investigated at December 31, 2013.

Characteristics	**N**	**Mean±SD or N(%)**
BMI, Kg/cm^2	1247	33.5±5.5
BMI categorical		
<30 Kg/cm^2		347 (27.8%)
30-35 Kg/cm^2		483 (38.6%)
≥35 Kg/cm^2		420 (33.6%)
Waist circumference, cm	1237	101.3±13.1
Blood pressure, mmHg	1247	
Sistolic		125.4±15.8
Diastolic		78.5±9.5
Above 140/90 mmHg		60 (4.8%)
Below 140/90 mmHg		1190 (95.2%)
Heart rate, bPM	1243	67.6±10.4
Uric acid	1163	5.2±1.4
Fibrinogen, mg/dl	1129	335±59
C-reactive protein	1160	0.3 [0.1-0.5]
Total cholesterol, mg/dl	1165	215.1±41
HDL		59.2±15.5

LDL		134.7±36.3
Triglyceride	1164	107 [77-145.5]
Serum creatinine, mg/dL	1165	0.8±0.3
AST, U/I	1159	19 [16-23]
ALT, U/I	1160	21 [16-30.5]
Gamma-Glutamyltransferase, U/L	1162	19 [13-30]
Glucose	1155	92 [86-101]
Homocysteine	1151	10.4 [8.6-12.7]
TSH	1163	1.7 [1.2-2.5]
Glycated hemoglobin, mmol/mol	1159	39 [36.6-43]
Postprandial glycaemia, mg/dl	1162	99 [90-112]
Insulin level	1158	12.3 [8.8-18]
2-hour post glucose insulin level	1155	46.4 [27.6-73]
Urinary pH	1144	5.6±0.7
Emocrome	1156	
White blood cells		6.8±1.7
Red blood cells		4.8±0.4
Hemoglobin		13.8±1.4
Hematocrit		40.7±3.4
Mean Corpuscolar Volume		85.1±6.4
Platelets		249.7±59

5.2 Comparison between FARM model and monitoring station PM_{10} levels.

Air quality modeling and ambient measurements are two different ways to estimate actual ambient concentrations of pollutants in the atmosphere. Both modeling and measurements have some degree of uncertainty associated with their estimates. The uncertainty of the FARM model results both from that of the model (due to the inability to perfectly describe the physical phenomena) and from that associated with the input data. Figure14 shows the box plots comparing PM_{10} concentrations of the two methods of exposure assessment by year: estimated PM_{10} concentrations are slightly lower than observed across investigated years. Table 3 reports a description of PM_{10} levels between 2010 and 2012 and by season, and selected weather variable distribution.

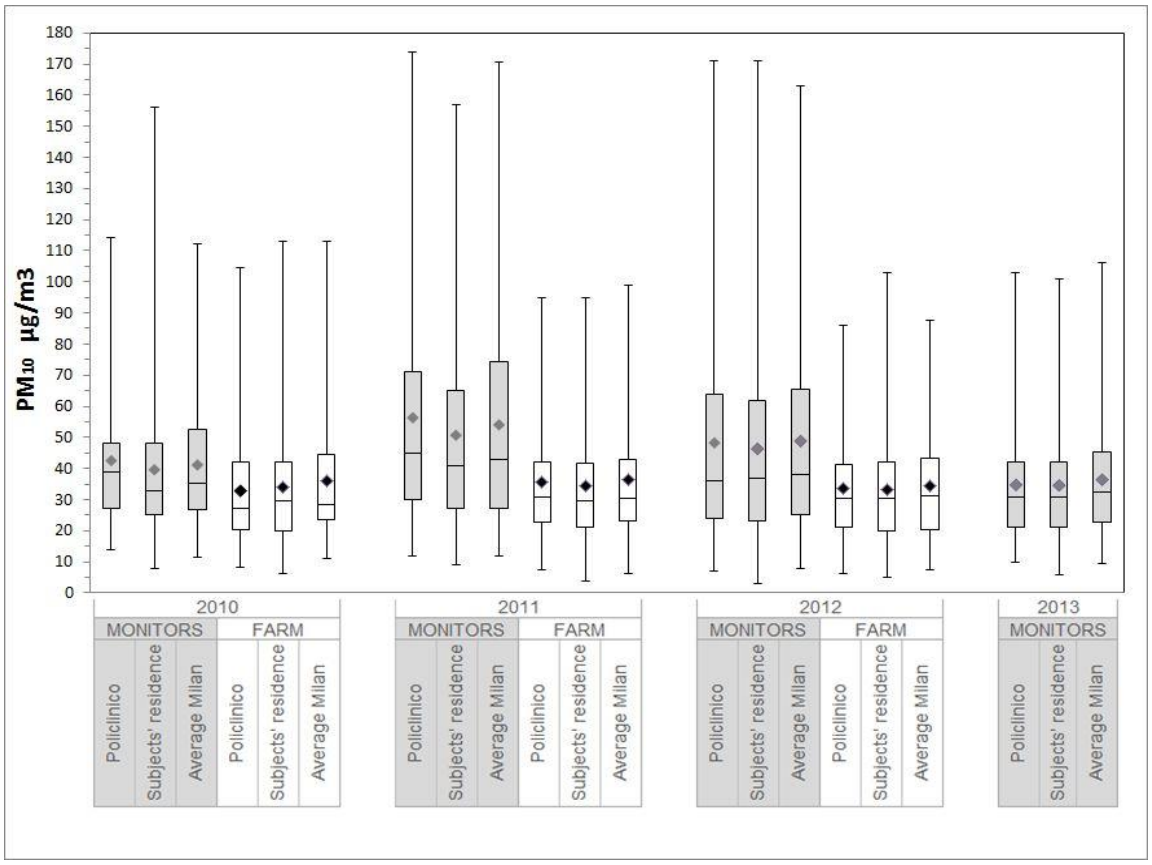


Figure 14: Mean PM10 level observed by monitors and estimated by FARM model by place and across years.

PM_{10} air concentrations depends, besides emissions, on weather conditions during the days, in particular by rainfall, atmospheric stability and wind. PM_{10} levels follows a seasonal trend, with critic periods concentrated in autumn and winter seasons, which are characterized by atmospheric stability, calm wind and absence of precipitations. PM_{10} air concentrations depends, besides emissions, on weather conditions during the days, in particular by rainfall, atmospheric stability and wind. Figure 15 shows the distributions of daily mean PM_{10} concentrations of all monitors and of all grid cells with a monitor falling into their boundary.

Table 5: PM_{10} profile and weather variables at the time of blood sampling for the entire period of the SPHERE Study and by seasons.

	Mean	**SD**	**Min**	**Q1**	**Median**	**Q3**	**Max**
PM $_{10}$ (µg/m^3)							
Monitory station (n=1250)							
Policlinico	47.0	30.5	7.0	26.0	38.0	59.0	174.0
Subjects' residence	44.2	28.3	3.0	24.0	36.0	56.0	171.0
Average Milan	46.7	29.5	7.7	25.7	37.7	60.0	170.7
FARM Model Estimate (n=931)							
Policlinico	34.5	18.0	6.2	21.6	29.8	41.8	104.7
Subjects' residence	33.8	18.3	4.0	20.4	29.7	42.1	113.1
Average Milan	35.6	18.7	6.4	22.8	30.4	43.1	113.0

Autumn/Winter							
Monitory station (n=747)							
Policlinico	58.8	33.5	7.0	33.0	52.0	76.0	174.0
Subjects' residence	55.0	30.8	3.0	33.0	48.0	72.0	171.0
Average Milan	58.6	31.8	11.3	34.3	51.3	76.7	170.7
FARMModel Estimate (n=569)							
Policlinico	40.1	19.4	7.4	26.6	35.9	52.8	104.7
Subjects' residence	39.5	19.6	4.9	25.8	35.6	51.4	113.1
Average Milan	41.7	20.0	6.4	27.2	37.0	55.3	113.0
Spring/Summer							
Monitory station (n=503)							
Policlinico	29.4	11.7	8.0	22.0	27.0	37.0	85.0
Subjects' residence	28.2	12.2	6.0	20.0	26.0	35.0	85.0
Average Milan	28.9	11.8	7.7	21.0	26.7	36.3	74.7
FARM Model Estimate (n=362)							
Policlinico	25.6	10.7	6.2	18.6	23.9	30.6	74.1
Subjects' residence	24.9	11.2	4.0	17.6	22.3	30.9	69.2
Average Milan	26.0	10.8	7.2	18.8	24.2	30.8	73.1
Weather variables (all seasons) (n=1250)							
Relative humidity	69.3	18.4	9.0	56.0	68.0	85.0	106.2
Temperature	12.6	7.8	-8.0	6.3	12.1	18.6	30.0
Apparent temperature	11.2	8.8	-9.0	3.8	10.6	18.0	31.9

SD: standard deviation, PM: particulate matter, Min: minimum, Q1: first quartile, Q3: third quartile, Max=maximum.

Monitoring stations available from 2010-2013.

FARM Model Estimate available from 2010-2012.

PM_{10} air concentrations depends, besides emissions, on weather conditions during the days, in particular by rainfall, atmospheric stability and wind. Figure 15 shows the distributions of daily mean PM_{10} concentrations of all monitors and of all grid cells with a monitor falling into their boundary. Darker area highlights winter months, characterized by major differences between the two methods of exposure assessment.

The winter months (October to February) are those where there is a more obvious difference between the two methods of exposure assessment; for the rest of the year the distributions are similar. In order to examine model performance of FARM model we used a standard performance metric: the mean fractional bias (MFB), which is a measure of the tendency of the model to over or under predict the observation from monitors. The MFB is defined as the normalized average difference between all model-observed pairs:

$$MFB = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{C_m - C_o}{C_o}\right)$$

and can vary between 200% and 200%. The model performance goal is met when the MFB is between ±30% [112]. (76,77). We obtain a MFB=-16% for the entire period of comparison and of MFB2010=-3%, MFB2011=-29%, MFB2012=-28%, by year.

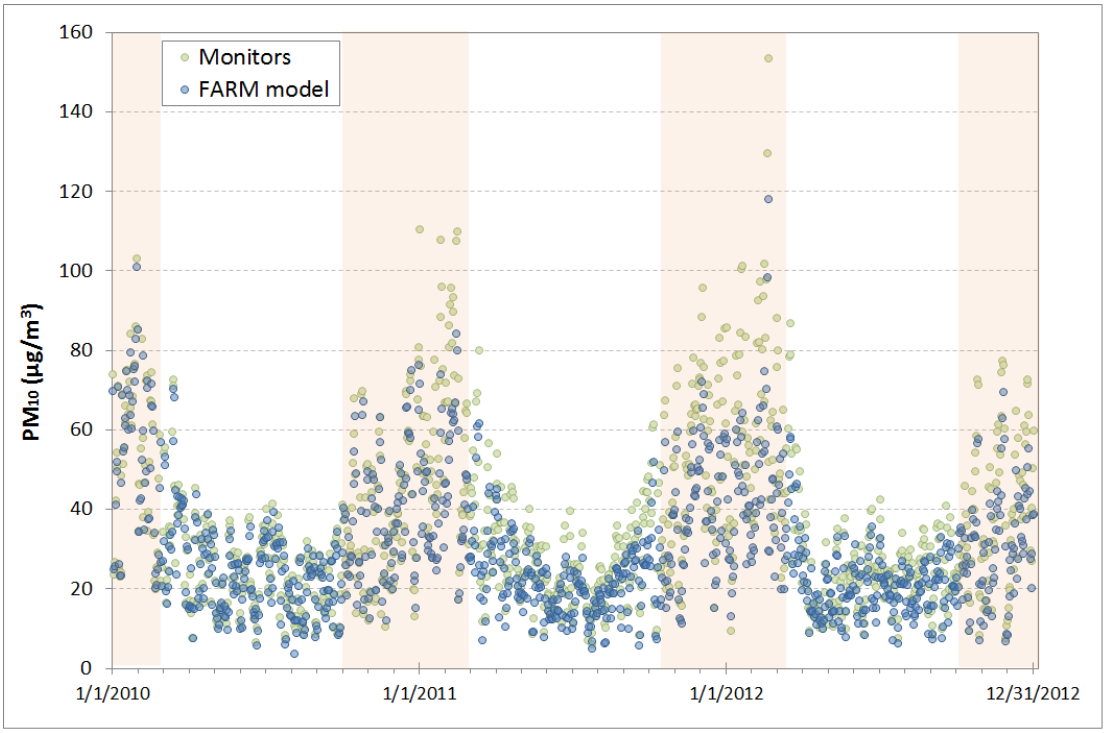


Figure 15: Estimated and observed daily mean PM_{10} concentrations (2010-2012).

These results suggest the fulfillment of the objective of performance, although with a tendency to underestimate the observed concentrations (negative values for the statistical index MFB). The differences between the two methods are consistent with what reported by ARPA Lombardy in the Annual Assessment Of Air Quality Modeling for years 2009-2011. (78).

Figure 16 shows mean concentration (2010-2012) measured by monitors and estimated by the FARM model in the corresponding cell. Each point is the PM_{10} mean concentration of a single monitor. Very high correlation was observed among the three sources of exposition (RPoliclinico vs Average Milan=0.99; RSubjects' Residence vs Average Milan=0.94; RPoliclinico vs Subjects' Residence=0.99). The cone dotted lines represents the range of quality data established by law [113] for the mean of the pollutant over the period, that is equal to ±50% [112, 114]. All the average concentrations fall into the quality range, except for three monitors, quite isolated and far from subjects' residences. The analysis was performed both by year and over the period 2010-2012 and comparable results were found. When comparing modeling results to observations, the measurements should not be considered the absolute truth. The differences

between the two methods of exposure assessment may be partly explained by the issue related to comparing a point measurement to a volumetric grid cell averaged modeled concentration.

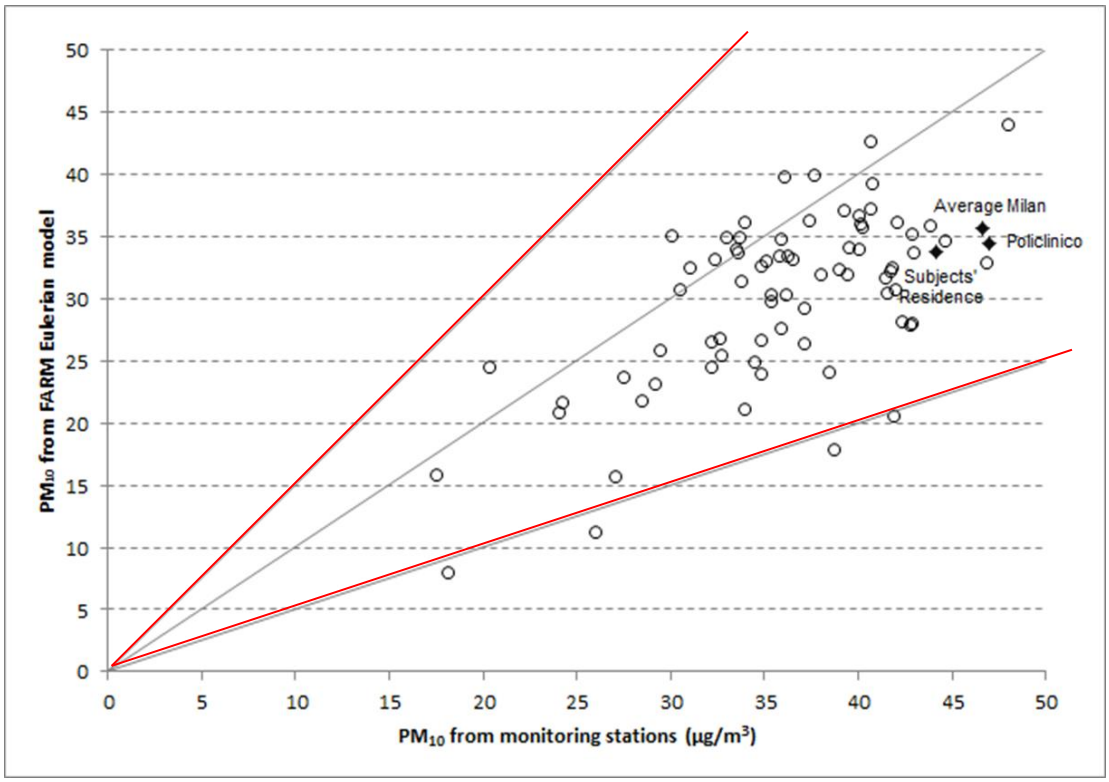


Figure 16: PM_{10} mean concentration (2010-2012) measured by monitors and estimated by the FARM model in the corresponding cell.

PM modeling use a 4 km on a side in the horizontal and between 10 to 10000 meters in the vertical. Since the modeling results represent an average concentration over the entire grid cell and the observations likely do not represent the average concentration over the same volume of air, it is not possible to exactly match modeled results to measurements[112].

We can summarize as follows the pros e cons of using PM_{10} exposure from FARM model air and from monitoring stations:

FARM model:

- ✓ provides an estimate of PM_{10} for a grid of 1678 cells 4x4Km; ;
- ✓ no missing values in PM_{10} series;
- ✓ is not easy to obtain (formal request to ARPA Lombardy);
- ✓ validation requires long times (7-8 months);
- ✓ high complexity of the model and the variables that feed it;

Monitoring Stations:

- ✓ easy availability from the site ARPA air quality;
- ✓ validation times from 3 to 6 months, but not validated series are also available;

- ✓ provides a punctual PM_{10} measure by 81 air monitoring stations;
- ✓ lengthy procedure of estimation of missing data that is not always possible when the number of missing is high.

In conclusion, individual air pollution exposure assessment is determined using two sources of information: actual monitor measurements for each and every day starting from January 1st, 1990 and a regional well validated modelling systems applied starting from 2007. This allows us to estimate both short-term (days) and long-term (months, years) exposure to the pollutants. Personal exposure of each study subject will be attributed based on their residential and work addresses and on questionnaire information on their time patterns (time at home, time at work, communing time, number of days at work, etc.). We decide to use FARM model data exposure for the analysis of the association between PM_{10} exposure and miRNAs expression.

5.3 Comparison between Normalization Strategies.

The performance of the different normalization strategies was assessed by [81]: (1) evaluating their ability to reduce the experimental induced (technical) variation, (2) determining their power to extract true biological variation. The point (1) was assessed calculating the standard deviation (SD) for each individual miRNAs across all samples upon applying different normalization procedures and plotting the cumulative distribution of the deltaCrt SD values (Figure 17).

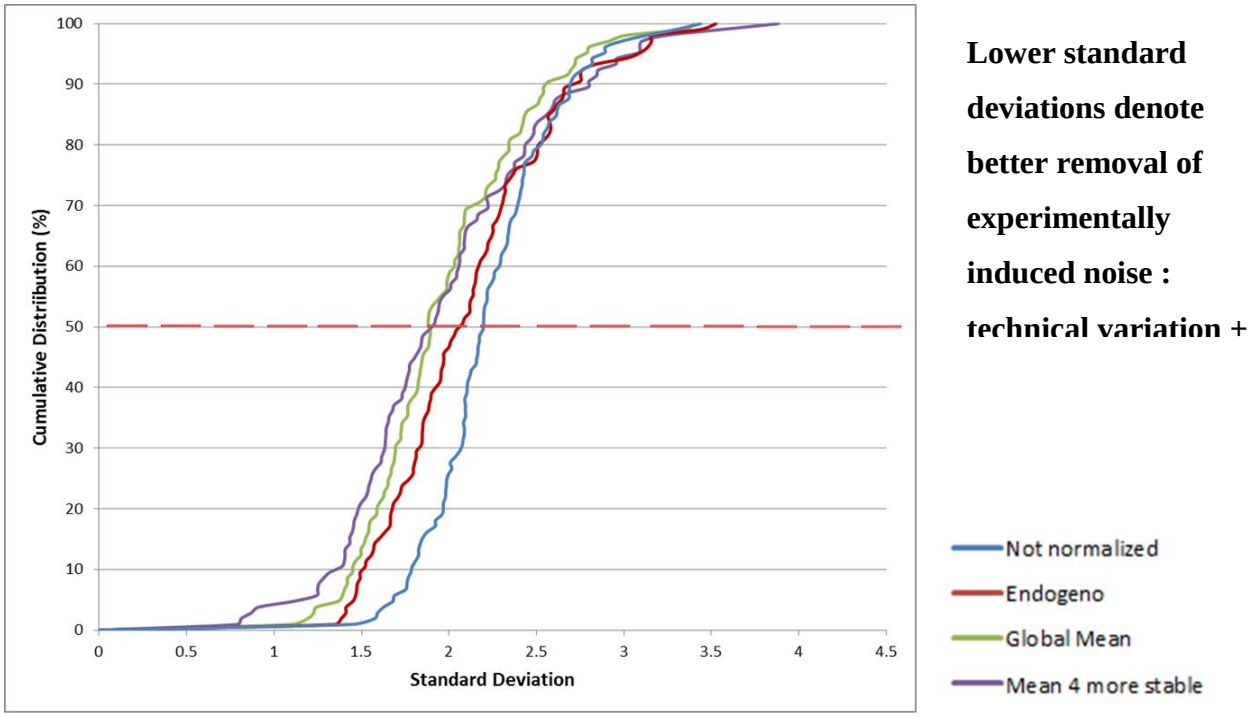


Figure 17: Cumulative distribution of deltaCrt SD values upon applying different normalization procedures applied on miRNAs SET3.

The comparison of the mean standard deviation between the three normalization strategies for the 50% least and 50% more variable miRNAs was performed by means of paired ttest (Figure 18), the results can be summarized as follows:

- ✓ For the 50% least variable miRNAs all three normalization strategies result in a significant decrease of the mean SD value respect to Not normalized data;
- ✓ For the 50% most variable miRNAs only d and c normalization strategy result in a significant decrease of the mean SD value respect to Not normalized data;
- ✓ For both 50% least and most variable miRNAs Global mean result in a significant decrease in of the mean SD value respect to Mean 4 more miRNAs data ($p < 0.001$);
- ✓ As true differentially expressed miRNAs predominantly reside in the most variable half of the dataset, only Global mean normalization is capable of reducing the number of false negatives. Reduction of false positives is possible with both normalization strategies (d and c) but to different extents as Global mean normalization results in a stronger decrease of technical variation for the 50% least variable miRNAs.

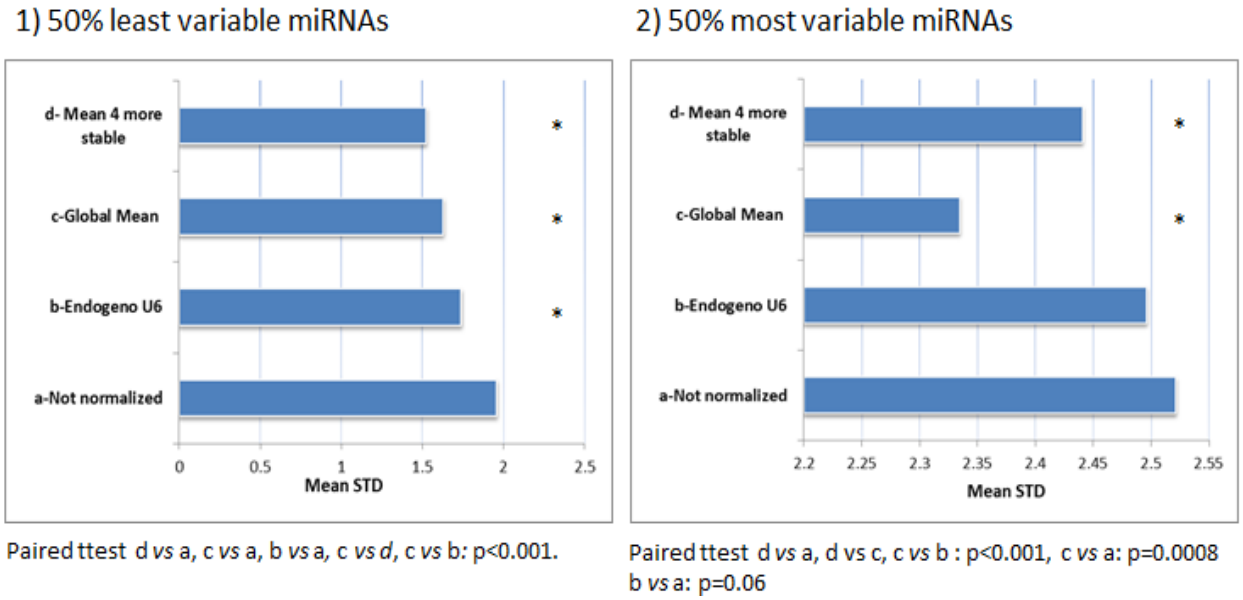


Figure 18: Comparison of Mean SD between the three normalization strategies for the 50% least and 50% more variable miRNAs was performed by means of paired ttest.

The point (2) was assessed by a new experiment aimed to evaluate how different normalization strategies affect biological changes. We consider a single OpenArray plate with three sample and proceeded in the whole-genome profiling of 733 human miRNAs for the three samples, with two replicates performed by the same operator in the same day. Repeated measures analysis by means of proc mixed in SAS was performed in order to:

SAS code

```
PROC MIXED DATA=miRNA ;
class time sample ;
model log2_RQ_1 = sample time / ddfm=kenwardroger;
repeated time ;
random samplename;
lsmestimate Replicates "replicate 2 vs replicate 1" -1 1 /;
lsmestimate Sample "sample 1 vs sample 2" 1 -1 0;
lsmestimate Sample "sample 1 vs sample 3" 1 0 -1;
lsmestimate Sample "sample 2 vs sample 3" 0 1 -1;
run;
```

1) *Evaluate the normalization strategy performance in reducing technical variation on SET3 (136miRNAs):*

Comparison between the two replicates in terms of miRNAs expression obtained for the three samples. The results are summarized in Table 4 and showed that the lowest percentage (8.82%) of miRNAs differentially expressed is obtained with the Global Mean normalization strategy, as well as the lowest Fold Change range (0.2-1.1).

Table 6: Results of evaluation of normalization strategy performance in reducing technical variation on SET3 (136 miRNAs).

Normalization strategy	% of miRNAs differentially expressed replicate 2 vs replicate 1	% of miRNAs differentially expressed replicate 2 vs replicate 1 after FDR adjustment	Fold change range replicate 2 vs replicate 1
Endogeno	13.50%	0%	0.4-1.4
Global Mean	8.82%	0%	0.2-1.1
Mean of 4 more stable	12.20%	0%	0.4-1.4

2) *Evaluate the normalization strategy performance in extracting true biological variation on SET3 (136 miRNAs):*

Comparison between the three samples in terms of miRNAs expression obtained at the two replicates. Results were summarized in Table5. The comparison between the three samples in terms of miRNAs expression obtained at the two replicates showed that the higher percentage (1vs2:47.8%, 1vs3:78.7%, 2vs3:50.0%) of miRNAs differentially expressed is obtained with the Global Mean normalization strategy, as well as the higher Fold Change range (1vs2:0.37-5.7, 1vs3:0.7-9.14, 2vs3:0.6-6.3).

Table 7: Results of evaluation of normalization strategy performance in in extracting true biological variation on SET3 (136 miRNAs).

Normalization strategy	% of miRNAs differentially expressed			% of miRNAs differentially expressed after FDR adjustment			Fold Chage range		
Sample	1vs2	1vs3	2vs3	1vs2	1vs3	2vs3	1vs2	1vs3	2vs3
Endogeno	41.9%	47.8%	61.8%	24.3%	20.6%	50.0%	0.31-5.2	0.16-2.4	0.17-1.8
Global Mean	47.8%	78.7%	50.0%	27.2%	74.2%	35.3%	0.37-5.7	0.7-9.14	0.6-6.3
Mean of 4 more stable	49.2%	31.6%	43.4%	17.6%	-	5.1%	0.24-3.8	0.27-3.6	0.35-3.7

In conclusion we can state that for large scale miRNA expression profiling Global Mean normalization strategy outperforms the other normalization strategy in terms of:

- ✓ better reduction of technical variation:
  - lower % of miRNAs differentially expressed before and after FDR adjustment
  - lower Fold change range;
- ✓ more accurate appreciation of biological changes.
  - higher % of miRNAs differentially expressed before and after FDR adjustment;
  - higher Fold Change range;

5.4 Association between miRNAs expression and PM_{10} exposure

In order to verify the association between miRNAs expression and PM_{10} we first fitted multiple linear regression models. As exposure was considered the daily PM_{10} exposure estimate (µg/m^3) from Eulerian model for the 4x4 km cell containing the address of the Center for Obesity and Weight Control. The exposure lag period chosen for the analysis is of zero days (daily exposure of blood collection day). As outcome was considered the Relative quantification RQ of each miRNAs. log2 transformation was applied in order to satisfy the normality and linearity assumptions of linear regression model. We tested the normality assumption of errors using the normal probability plot and the Shapiro-Wilks statistic. Finally, for each miRNA, plots of residuals versus predicted values and Lack of Fit tests were used to explore potential nonlinearity of PM_{10} and apparent temperature. Moreover, test we tested for nonlinearity of PM_{10} and apparent temperature using penalized splines in generalized linear models for all miRNAs included in the analysis by mean of graphical inspection and by formal test of linearity. We excluded a nonlinear relation between PM_{10} or apparent temperature and miRNAs expression. In particular Figure19 reports the results of non-linearity testing for the first nine top miRNAs selected.

The following adjusting variables were selected age, body mass index, cigarette smoking (never, former, or current), and pack-years. We adjusted for percent of granulocytes (to control for possible shifts in leukocyte differential count), date, Seasonality (using sine and cosine) and apparent temperature. Since in each run of OpenArray were simultaneous analysed up to 4 OpenArray plates, identified by a barcode, for a total of 12 samples (3 per plate) it was possible identify an hierarchical data structure with three levels: sample level (level-1), barcode level (level-2) and run level (level-3). We developed three-levels HLM using the MIXED procedure in SAS.

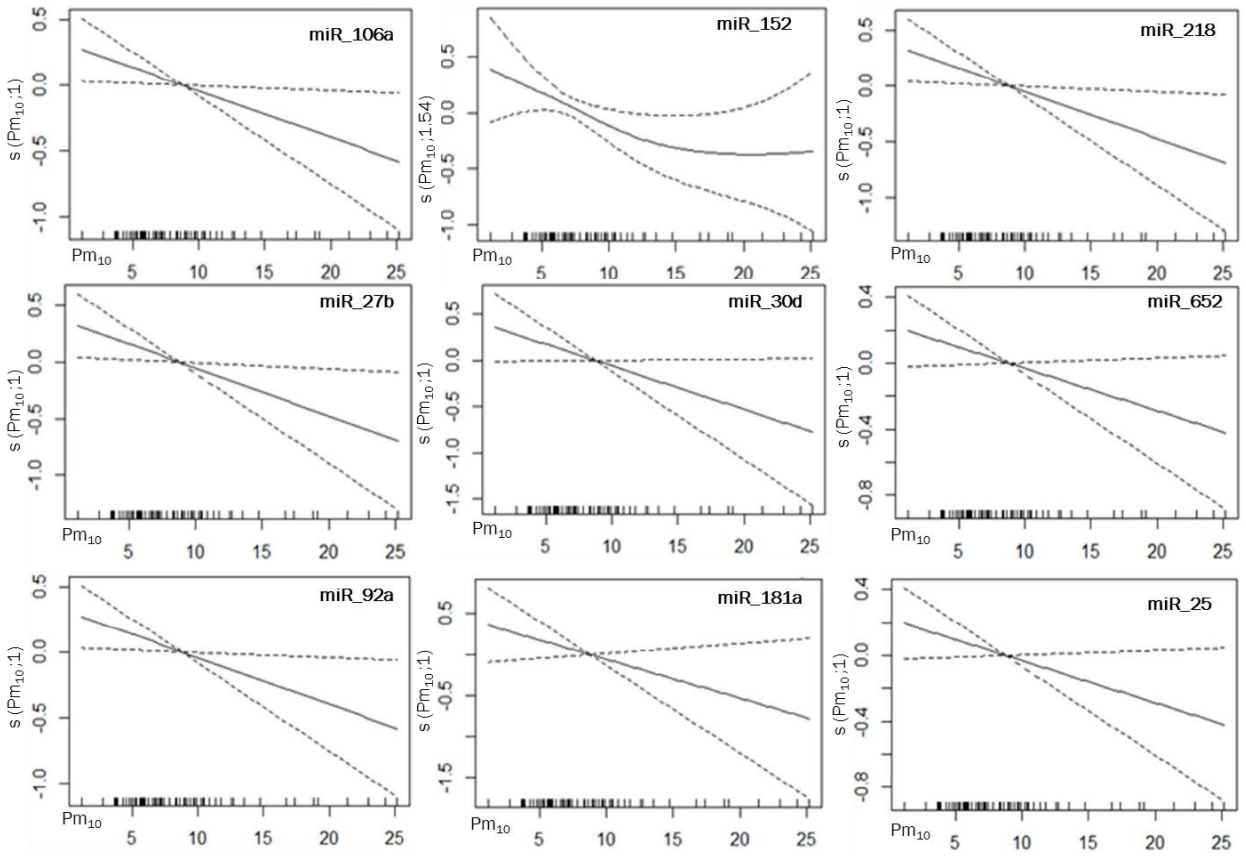


Figure 19: Penalized Splines: testing Non linearity on the association of PM_{10} of and first 9 top miRNAs selected.

The use of three-levels hierarchical linear models allowed to investigate other variability sources linked to the outcome. In particular we inspect the following research questions:

1)how much of the variability in miRNAs expression is attributable to barcodes and runs?

2)does the association between of the level-1 predictor PM_{10} vary among barcode or run?

To answer to this questions we proceeded according the following model selection strategy:

- **Model1- Unconditional model:** We fit the following model to answer to the first question. As miRNA variable we use miR_106a_002169 which is the first top miRNA identified in the multivariable simple regression analysis

  SAS code

proc mixed data=dataset method=ml;

class barcode run;

model mirna=/solution;

random intercept/sub=run type=vc;

random intercept/sub=barcode(run) type=vc;run;

We used the three variance estimates to calculate the intraclass correlation coefficients ICCs for barcode and for run:

$$ICC_{barcode} = \frac{\sigma^2_{barcode}}{\sigma^2_{barcode} + \sigma^2_{run} + \sigma^2_{error}} = \frac{0.1407}{0.1407 + 0.7912 + 0.6875} = 0.0868$$

$$ICC_{run} = \frac{\sigma^2_{run}}{\sigma^2_{barcode} + \sigma^2_{run} + \sigma^2_{error}} = \frac{0.7912}{0.1407 + 0.7912 + 0.6875} = 0.4885$$

The 8.6% of the variation in miRNAs expression exists between barcode and 48.8% exist between run, leaving 42.6% of the variance in miRNAs expression within samples. Thus a practically meaningful proportion of the variance in miRNA expression exists at the barcode and above all at run levels, providing support for the use of a three-level analytical model.

- **Model 2: Model1 + level-1 fixed effect**. We included the sample-level predictor PM_{10}

  SAS code

  proc mixed *data= dataset covtest method=ml;*

  class barcode run;

  model mirna=PM10_policlinico /solution;

  random intercept /sub=run type=vc;

  *random intercept /sub=barcode(run) type=vc;**run**;*

  Results from fixed effects indicate the relationship between level-1 predictor PM_{10} and the outcome miRNAs expression

- **Model 3: Model2 + random slopes for level-1 predictor PM_{10}.** We expanded Model 2 specifying the PM_{10} predictor as random slope at both barcode and run level.

  SAS code

  proc mixed *data=dataset covtest method=ml;*

  class barcode run;

  model mirna=PM10_policlinico /solution;

  random intercept PM10_policlinico /sub=run type=vc;

  *random intercept PM10_policlinico /sub=barcode(run) type=vc;**run**;*

This model allows to answer to the second research question: fixed effects results provide the same information as Model2, random slope results reveal if the relationships between level-1 predictor PM_{10} and the outcome miRNAs expression vary between barcode and run.

Results from the three models can be summarize in Table 8:

In both Model2 and Model3 sample level predictor PM_{10} is significant, suggesting that an increase of 1 (μg/m^^{3}) in PM_{10} is associated with a decrease (respectively β=-0.020 Model2 and β=--0.012) in miR_106a_002169 expression. Examining the random effects section, we see that significant variability in barcode and run intercept, as well as the coefficients associated with $PM_{10,}$ existed even after controlling for the sample level fixed effect.

Table 8. Estimates from three-level hierarchical linear model predicting miRNA expression (miR_106a_002169).

	Model1	**Model2**	**Model3**
Fixed Effect			
Intercept	11.84	12.13*	12.05
	(0.09)	(0.10)	(0.11)
PM_{10}		-0.020*	-0.012*
		(0.002)	(0.003)
Error variance			
Level-1	0.6875*	0.6786*	0.6576*
	(0.04)	(0.04)	(0.04)
Intercept(barcode)	0.1407*	0.1434*	0.1334*
	(0.04)	(0.04)	(0.04)
Intercept (run)	0.7912	0.7529	0.8327
	(0.06)	(0.06)	(0.06)
Slope (PM_{10})B			0.03*
			(0.04)
Slope (PM_{10})R			0.08
			(0.05)
Model Fit			
AIC	2418.7	2410.2	2254.8
BIC	2529.1	2521.9	2389.7

*Note: *Statistically significant p<0.05; Barcode ICC=, Run ICC= B=random effect at barcode level, R=random effect at run level. Value based on SAS PROC MIXED. Entries show parameter estimates with standard error in parentheses. Estimation Method=ML; Satterthwaite degrees of freedom.*

Thus the association between PM_{10} and miRNAs expression varies significantly among barcode and run (as denoting by sub-B and sub R respectively). Thus coefficient associated with this variable may be stronger/weaker from barcode to barcode within a run. Examining the AIC and BIC values at the bottom of Table 6, we can see that each progressive model exhibited better fit to the data. The results obtained from the final model, Model3 adjusted for age, body mass index, cigarette smoking, percent of granulocytes, date, Seasonality (using sine and cosine) and

apparent temperature, were reported in Table7, Table8 and Table9, one for each set of miRNAs used (Set1, Set2 and Set3). Each table reports the significant associations found between miRNAs expression and exposure according to the FDR p-value threshold 0.10 for the first 10 top miRNAs. We can observe that for each set of miRNAs we obtain almost the same first 10 top miRNAs even if with a different order given by the raw and FDR p-value. Looking at the results obtained on the three sets of miRNAs the following list of first 10 top miRNAs were identified: miR_106a_002169, miR_152_000475, miR_181a_2__002317, miR_218_000521, miR_27b_000409, miR_30d_000420, miR_652_002352, miR_92a_000431, miR_25_000403, miR_375_000564. These miRNAs are now being confirmed by Real Time PCR on the next 1000 enrolled patients enrolled.

RESULTS: miRNAs SET 1

Table 9: Association between FARM model estimate of PM_{10} daily exposure and miRNAs expression (SET1: 527 miRNAs)

miRNAs	Estimate	95% CI		VARIATION (%)	RAW pvalue	FDR pvalue
miR_106a_002169	-0.012	-0.017	-0.007	-8.11	<0.001	<0.001
miR_152_000475	-0.013	-0.019	-0.007	-8.69	<0.001	<0.001
miR_181c__002333	0.01	0.006	0.014	6.99	<0.001	<0.001
miR_218_000521	-0.015	-0.022	-0.008	-9.72	<0.001	<0.001
miR_27b_000409	-0.016	-0.024	-0.009	-10.74	<0.001	<0.001
miR_30d_000420	-0.016	-0.022	-0.009	-10.21	<0.001	<0.001
miR_652_002352	-0.023	-0.033	-0.013	-14.88	<0.001	<0.001
miR_674_002021	0.01	0.005	0.014	6.97	<0.001	<0.001
miR_92a_000431	-0.011	-0.017	-0.006	-7.53	<0.001	<0.001
miR_181a_2__002317	-0.015	-0.022	-0.007	-9.79	<0.001	<0.001

*Three-level HLM model adjusted for age, body mass index, cigarette smoking, percent of granulocytes, date, Seasonality (using sine and cosine) and apparent temperature. Only significant FDR-pvalues according to the threshold of 0.10 were shown. Estimate=2^(β*10), Variation(%)=[2^(β*10) -1]x100 expresses the percentage variation in miRNAs expression associated with an increase of 10 (µg/m^3) in PM_{10}.*

RESULTS: miRNAs SET 2

Table 10: Association between FARM model estimate of PM_{10} daily exposure and miRNAs expression (SET2: 152 miRNAs)

miRNAs	Estimate	95% CI		VARIATION (%)	RAW pvalue	FDR pvalue
miR_106a_002169	-0.012	-0.017	-0.007	-8.11	<0.001	<0.001

miR_152_000475	-0.013	-0.019	-0.007	-8.69	<0.001	<0.001
miR_218_000521	-0.015	-0.022	-0.008	-9.72	<0.001	<0.001
miR_27b_000409	-0.016	-0.024	-0.009	-10.74	<0.001	<0.001
miR_30d_000420	-0.016	-0.022	-0.009	-10.21	<0.001	<0.001
miR_652_002352	-0.023	-0.033	-0.013	-14.88	<0.001	<0.001
miR_92a_000431	-0.011	-0.017	-0.006	-7.53	<0.001	<0.001
miR_181a_2__002317	-0.015	-0.022	-0.007	-9.79	0.0001	0.0014
miR_25_000403	-0.012	-0.018	-0.006	-8.12	0.0001	0.0014
miR_375_000564	-0.017	-0.026	-0.009	-11.38	0.0001	0.0014

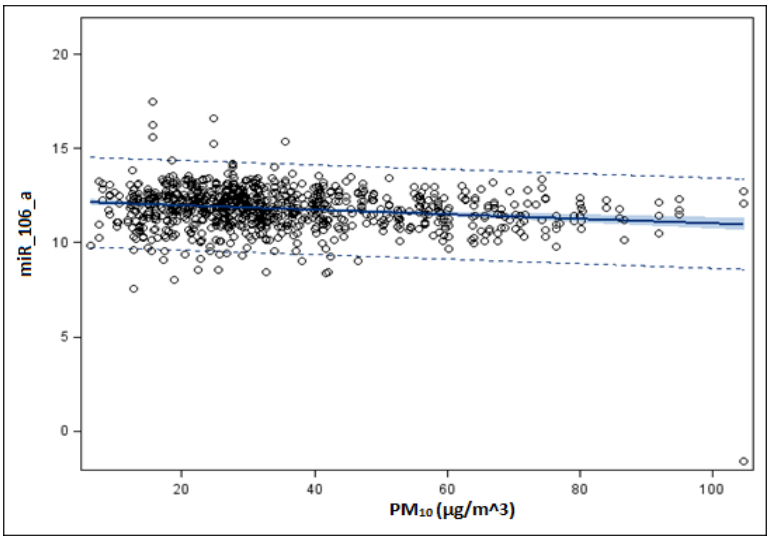
*Three-level HLM model adjusted for age, body mass index, cigarette smoking, percent of granulocytes, date, Seasonality (using sine and cosine) and apparent temperature. Only significant FDR-pvalues according to the threshold of 0.10 were shown. Estimate=2^(β*10), Variation(%)=[2^(β*10) -1]x100 expresses the percentage variation in miRNAs expression associated with an increase of 10 (µg/m^3) in PM_{10}.*

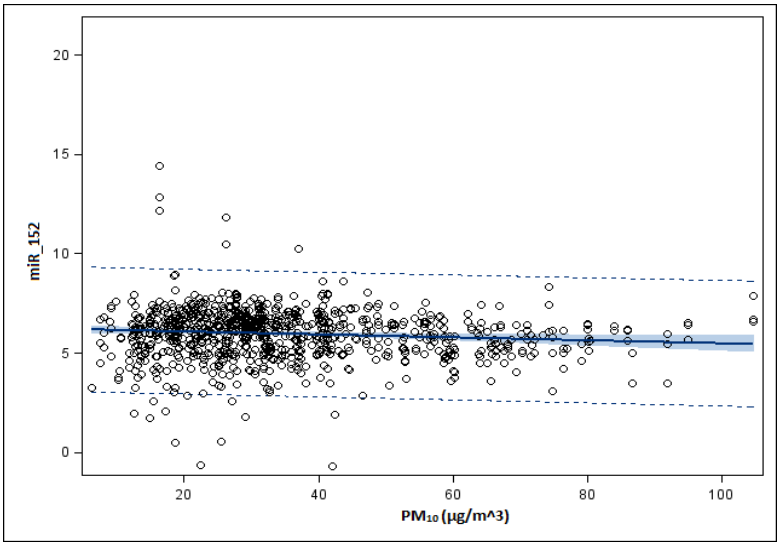
RESULTS: miRNAs SET 3

Figure 19: Association between FARM model estimate of PM_{10} daily exposure and miRNAs expression (SET2: 105 miRNAs)

miRNAs	Estimate	95% CI		VARIATION (%)	RAW pvalue	FDR pvalue
miR_106a_002169	-0.012	-0.017	-0.007	-8.11	<0.001	<0.001
miR_152_000475	-0.013	-0.019	-0.007	-8.69	<0.001	<0.001
miR_218_000521	-0.015	-0.022	-0.008	-9.72	<0.001	<0.001
miR_27b_000409	-0.016	-0.024	-0.009	-10.74	<0.001	<0.001
miR_30d_000420	-0.016	-0.022	-0.009	-10.21	<0.001	<0.001
miR_652_002352	-0.023	-0.033	-0.013	-14.88	<0.001	<0.001
miR_92a_000431	-0.011	-0.017	-0.006	-7.53	<0.001	<0.001
miR_25_000403	-0.012	-0.018	-0.006	-8.12	0.0001	0.0012
miR_720_002895	-0.011	-0.016	-0.006	-7.32	0.0001	0.0012
let_7c_000379	-0.014	-0.022	-0.007	-9.45	0.0002	0.0016

*Three-level HLM model adjusted for age, body mass index, cigarette smoking, percent of granulocytes, date, Seasonality (using sine and cosine) and apparent temperature. Only significant FDR-pvalues according to the threshold of 0.10 were shown. Estimate=2^(β*10), Variation(%)=[2^(β*10) -1]x100 expresses the percentage variation in miRNAs expression associated with an increase of 10 (µg/m^3) in PM_{10}.*


miR_106_a
20
15
10
5
0
20
40
60
80
100
PM10 (µg/m^3)


miR_152
20
15
10
5
0
20
40
60
80
100
PM10 (µg/m^3)

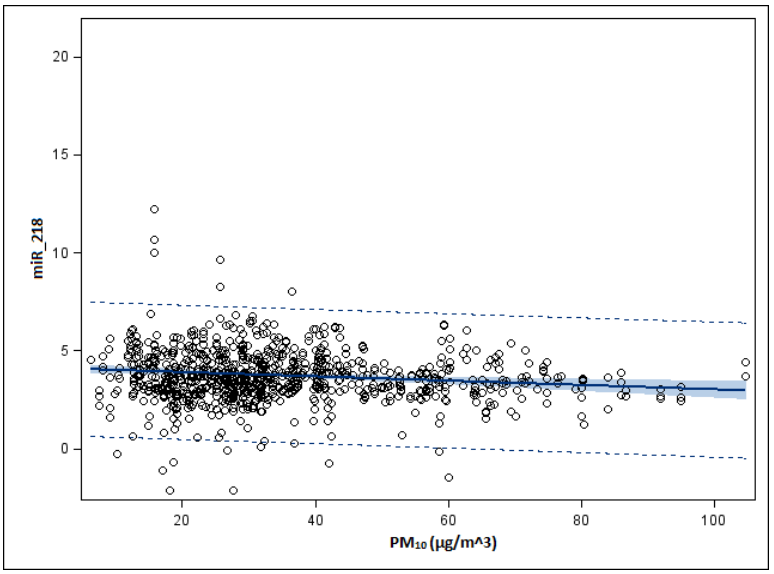

miR_218
20
15
10
5
0
20
40
60
80
100
PM10 (µg/m^3)

Figure 20: Association between eulerian estimate of PM_{10} daily exposure and miR_106a,miR_152, mir_218, expression. for age, body mass index, cigarette smoking, percent of granulocytes, date, Seasonality (using sine and cosine) and apparent temperature.

5.5 Mediation Analysis to investigate the role of miRNAs expression as mediator of the effect of PM_{10} on respiratory, cardiac and inflammatory outcome.

5.5.1 Simple Mediation Models

Simple mediation models were applied in order to investigate the role of miRNAs expression as potential mediator on the effect of PM_{10} on respiratory, cardiac and inflammatory outcomes such as: single breath carbon monoxide diffusing capacity DLco, Forced expiratory volume in the 1st second FEV1, Forced Vital Capacity FVC, Heart Rate, Sistolic Blood Pressure SBP, Diastolic Blood Pressure DBP, C-Reactive Protein CRP, and Fibrinogen. Following tables reports the results of simple mediation model involving miRNAs showed a significant indirect effect of PM_{10} on respiratory, cardiac and inflammatory outcomes. The results obtained for the remaining miRNAs were reported in Appendix1.

DLco

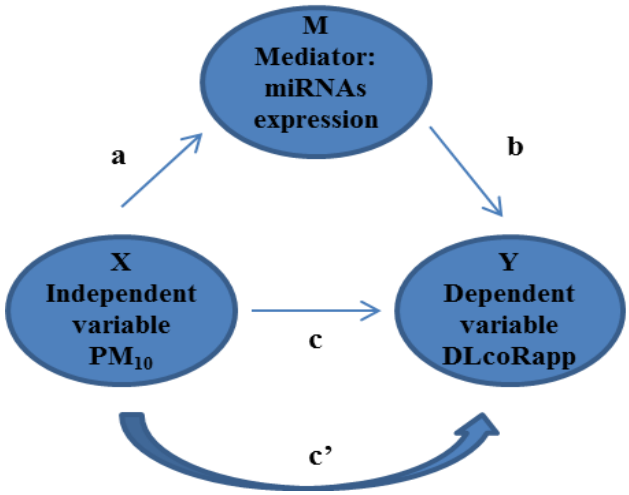


Figure 21: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and DLcoRapp= (Measured DLco / Theoretical DLco)*100 as dependent variable.

mir_106a_002169: Results of Simple Mediation Analysis for mediator mir_106a_002169 were reported in Table11:

- the regression analysis representing path c is significant. The F-statistic was 10.428 and the p-value was <0.001. PM_{10} is positively associated with DLcoRapp and the regression coefficient for PM_{10} (β=0.063 95% CI: -0.0001-0.127) is statistically significantly

different from zero (p=0.051), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.063 in DLcoRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher DLcoRapp level. This results is quite controversial suggesting the intrigiung possibility that short term PM_{10} exposure differentially altercytokine pathways;

								Consequent				
		M(miR_106a_002169)						Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM_{10})		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2=0.078				
								F(7,743)=10.428, p<0.001				
X(PM_{10})	a	-0.011	0.003	-0.016	-0.006	<0.001	c'	0.074	0.033	0.010	0.138	0.023
M(miR_106a_002169)		-	-	-	-	-	b	0.978	0.468	0.059	1.897	0.037
costant	i_1	13.363	0.732	11.926	14.800	<0.001	i_2	37.577	11.245	15.500	59.653	<0.001
		R^2=0.041						R^2=0.083				
		F(6,744)=5.351, p<0.001						F(7,743)=9.602, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM_{10} on DLcoRapp	c	0.063	0.032	0.000	0.127	0.051
Direct Effect of PM_{10} on DLcoRapp	c'	0.074	0.033	0.010	0.138	0.023
Indirect Effect of PM_{10} on DLcoRapp		-0.011	0.006	-	-	0.064*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM_{10} on DLcoRapp		-0.011	0.005	-0.024	-0.003	-

Table 11: Simple Mediation Analysis results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLco / Theoretical DLco)*100
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 5.351 and the p-value was <0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with miR_106a_002169 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that miR_106a_002169 expression decreases by 0.773% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 9.602 and the p-value was <0.001. The regression coefficient for miR_106a_002169 is statistically significantly different from zero (p=0.037) and miR_106a_002169 expression is positively correlated with DLcoRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in miR_106a_002169 expression is associated with an increase of 0.014 in DLcoRapp;

- the regression analysis representing path c' estimated a direct effect c'=0.074 (95% CI:0.010-0.138 pvalue=0.023). This represents the estimated difference in DLcoRapp between two patients with the same miR_106a_002169 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same miR_106a_002169 expression level is estimated to be 0.074 units higher in DLcoRapp. The indirect effect -0.011 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ -0.011 in DLcoRapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_106a_002169 expression level (because a is negative), which in turn translates into higher DLcoRapp level (because b is positive). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely below zero (-0.024; -0.003). In this case the normal theory-based Sobel test (Z= -1.852, p=0.064) does not agree with the inference made using a biased correct bootstrap confidence interval. ***mir_152_000475:*** Results of Simple Mediation Analysis for mediator mir_152_000475 were reported in Table11.

							Consequent					
		M(miR_152_000475)						***Y(DLcoRapp)***				
Antecedent		***Coeff.***	***SE***	***95% CI***		***p***		***Coeff.***	***SE***	***95% CI***		***p***
X(PM $_{10}$)		-	-	-	-	-	*c*	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2=0.078				
								F(7,743)=10.428, p<0.001				
X(PM $_{10}$)	*a*	-0.007	0.003	-0.014	-0.001	0.0319	*c'*	0.070	0.032	0.007	0.133	0.030
M(miR_152_000475)		-	-	-	-	-	*b*	0.949	0.355	0.252	1.646	0.008
costant	i_1	6.589	0.963	4.698	8.479	<0.001	i_2	44.392	9.617	25.513	63.272	<0.001
		R^2=0.021						***R^2=0.086***				
		F(6,744)=2.623, p=0.016						***F(7,743)=10.032, p<0.001***				

		Effect	***SE***	***95% CI***		***p***
Total effect of PM $_{10}$ on DLcoRapp	*c*	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	*c'*	0.070	0.032	0.007	0.133	0.030
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.007	0.004	-	-	0.108*
		Effect	***Boot SE***	***Boot 95% CI***		***p***
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.007	0.004	-0.016	-0.002	-

Table 12: Simple Mediation Analysis results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLco / Theoretical DLco)*100
*Sobel test.

- the regression analysis representing path c is significant. The F-statistic was 10.428 and the p-value was <0.001. PM_{10} is positively associated with DLcoRapp and the regression coefficient for PM_{10} (β=0.063 95% CI: -0.0001-0.127) is statistically and significantly

different from zero (p=0.051), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.063 in DLcoRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher DLcoRapp level;

- the regression analysis representing path a is significant. The F-statistic was 2.623 and the p-value was 0.016. The regression coefficient for PM_{10} is significantly different from zero (p=0.0319) and PM_{10} is negatively correlated with mir_152_000475 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_152_000475 expression decreases by 0.491% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 10.032 and the p-value was p<0.001. The regression coefficient for mir_152_000475 is statistically significantly different from zero (p=0.008) and mir_152_000475 expression is positively correlated with DLcoRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_152_000475 expression is associated with an increase of 0.014 in DLcoRapp;
- the regression analysis representing path c' estimated a direct effect c'=0.070 (95% CI:0.007-0.133 pvalue=0.030). This represents the estimated difference in DLcoRapp between two patients with the same mir_152_000475 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_152_000475 expression level is estimated to be 0.070 units higher in DLcoRapp.

The indirect effect -0.007 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ -0.007 in DLcoRapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_152_000475 expression level (because a is negative), which in turn translates into higher DLcoRapp level (because b is positive). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely below zero (-0.016; -0.002). In this case the normal theory-based Sobel test (Z= -1.608, p=0.108) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_218_000521: Results of Simple Mediation Analysis for mediator mir_218_000521 were reported in Table13:

- the regression analysis representing path c is significant. The F-statistic was 10.428 and the p-value was <0.001. PM_{10} is positively associated with DLcoRapp and the regression coefficient for PM_{10} (β=0.063 95% CI: -0.0001-0.127) is statistically significantly

different from zero (p=0.051), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.063 in DLcoRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher DLcoRapp level;

		Consequent									
		M(miR_218_000521)					Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM 10)		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
							R^2=0.078 F(7,743)=10.428, p<0.001				
X(PM 10)	a	-0.012	0.004	-0.019	-0.005	0.001	c'	0.050	0.032	-0.013	0.113	0.121
M(miR_218_000521)		-	-	-	-	-	b	-1.103	0.320	-1.730	-0.475	<0.001
costant	i_1	2.962	1.067	0.868	5.056	0.006	i_2	53.911	9.346	35.563	72.260	<0.001
		R^2=0.022 F(6,744)=2.773, p=0.011					R^2=0.092 F(7,743)=10.770, p<0.001					

		Effect	SE	95% CI		p
Total effect of PM 10 on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM 10 on DLcoRapp	c'	0.050	0.032	-0.013	0.113	0.121
Indirect Effect of PM 10 on DLcoRapp		0.013	0.006	-	-	0.021*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM 10 on DLcoRapp		0.013	0.006	0.004	0.027	-

Table 13: Simple Mediation Analysis results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.miRNAs expression was log2 transformed. Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLco / Theoretical DLco)*100.
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 2.773 and the p-value was p=0.011. The regression coefficient for PM_{10} is statistically significantly different from zero (p=0.001) and PM_{10} is negatively correlated with mir_218_000521 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that miR_218_000521 expression decreases by 0.821% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 10.770 and the p-value was <0.001. The regression coefficient for miR_218_000521 is statistically significantly different from zero (p=0.037) and miR_218_000521 expression is negatively correlated with DLcoRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in miR_218_000521 expression is associated with a decrease of 0.016 in DLcoRapp.
- the regression analysis representing path c' estimated a direct effect c'=0.050 (95% CI:-0.013-0.113 pvalue=0.121). This represents the estimated difference in DLcoRapp between two patients with the same miR_218_000521 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients

with higher PM_{10} exposure but with the same miR_218_000521 expression level is estimated to be 0.050 units higher in DLcoRapp.

The indirect effect 0.013 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.013 in DLcoRapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_218_000521 expression level (because a is negative), which in turn translates into lower DLcoRapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely below zero (-0.004; -0.027). In this case the normal theory-based Sobel test (Z= 2.313, p=0.027) agrees with the inference made using a biased correct bootstrap confidence interval.

FEV_1Rapp

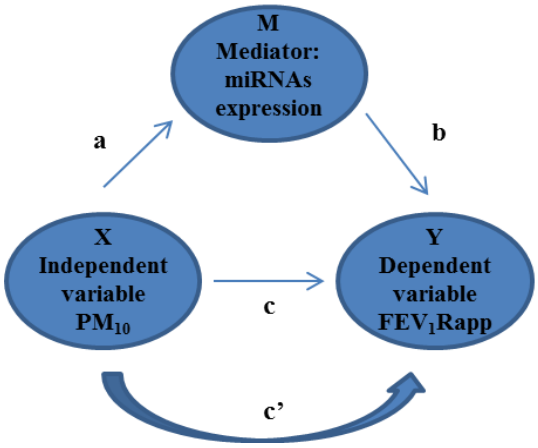


Figure 22: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and FEV_1Rapp= (Measured FEV_1 / Theoretical FEV_1)*100 as dependent variable.

mir_27b_000409: Results of Simple Mediation Analysis for mediator mir_27b_000409 were reported in Table14:

- the regression analysis representing path c is significant. The F-statistic was 7.217 and the p-value was <0.001. PM_{10} is positively associated with FEV1Rapp and the regression coefficient for PM_{10} (β=0.025 95% CI: -0.044-0.094) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this relationship is not statistically significantly different from zero (p=0.473);

		Consequent									
		M(miR_27b_000409)					Y(FEV$_1$ Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm$_{10}$)	a	-0.014	0.004	-0.022	-0.005	0.001	c'	0.016	0.035	-0.053	0.085	0.644
M(miR_27b_000409)		-	-	-	-	-	b	-0.655	0.303	-1.249	-0.060	0.031
costant	i_1	7.967	1.140	5.728	10.206	<0.001	i_2	105.923	9.937	86.416	125.430	<0.001
		R^2 = 0.053						R^2 =0.066				
		F(7,780)=6.203, p<0.001						F(8,779)=6.930, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$ Rapp c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$ Rapp c'	0.016	0.035	-0.053	0.085	0.644
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp	0.009	0.005	-	-	0.080*
	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp	0.009	0.005	0.002	0.020	-

Table 14: Simple Mediation Analysis results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM10.
Back transforming in model with FEV1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV1Rapp = (Measured FEV1 / Theoretical FEV1)*100
*Sobel test

- the regression analysis representing path a is significant. The F-statistic was 6.203 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p=0.001) and PM_{10} is negatively correlated with mir_27b_000409 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_27b_000409 expression decreases by 0.931% for an increase of 1 μg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 6.930 and the p-value was <0.001. The regression coefficient for mir_27b_000409 is statistically significantly different from zero (p=0.031) and mir_27b_000409 expression is negatively correlated with FEV1Rapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_27b_000409 expression is associated with a decrease of 0.009 in FEV1Rapp;
- the regression analysis representing path c' estimated a direct effect c'=0.016 (95% CI:-0.053-0.085 pvalue=0.644). This represents the estimated difference in FEV1Rapp between two patients with the same mir_27b_000409 expression level but who differ by 1 μg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_27b_000409 expression level is estimated to be 0.016 units higher in FEV1Rapp. The indirect effect 0.009 means that two patients who differ by 1 μg/m^3 in their PM_{10} exposure level are estimated to by

differ 0.009 in FEV1Rapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_27b_000409 expression level (because a is negative), which in turn translates into lower FEV1Rapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.002; 0.020). In this case the normal theory-based Sobel test (Z= 1.748, p=0.080) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_30d_000420: Results of Simple Mediation Analysis for mediator mir_30d_000420 were reported in Table15:

							Consequent					
		M(miR_30d_000420)						Y(FEV 1 Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM 10)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm 10)	a	-0.015	0.004	-0.023	-0.008	<0.001	c'	0.015	0.035	-0.054	0.084	0.673
M(miR_30d_000420)		-	-	-	-	-	b	-0.663	0.336	-1.322	-0.005	0.048
costant	i_1	9.233	1.029	7.213	11.253	<0.001	i_2	106.833	10.130	86.947	126.719	<0.001
		R^2 =0.035						R^2 =0.066				
		F(7,780)=4.073, p<0.001						F(8,779)=6.827, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm 10 on FEV 1 Rapp	c	0.025	0.035	-0.0436	0.094	0.473						
Direct Effect of Pm 10 on FEV 1 Rapp	c'	0.015	0.035	-0.054	0.084	0.673						
Indirect Effect of Pm 10 on FEV 1 Rapp		0.010	0.006	-	-	0.082*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm 10 on FEV 1 Rapp		0.010	0.006	0.001	0.023	-						

Table 15: Simple Mediation Analysis results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FEV1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV1Rapp = (Measured FEV1 / Theoretical FEV1)*100
*Sobel test.

- the regression analysis representing path c is significant. The F-statistic was 7.217 and the p-value was <0.001. PM_{10} is positively associated with FEV1Rapp and the regression coefficient for PM_{10} (β=0.025 95% CI: -0.044-0.094) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this relationship is not statistically significantly different from zero (p=0.473);
- the regression analysis representing path a is significant. The F-statistic was 4.073 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly

different from zero (p<0.001) and PM_{10} is negatively correlated with mir_30d_000420 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_30d_000420 expression decreases by 1.062% for an increase of 1 µg/m^3 in PM_{10};

- the regression analysis representing path b is also significant. The F-statistic was 6.827 and the p-value was <0.001. The regression coefficient for mir_30d_000420 is statistically significantly different from zero (p=0.048) and mir_30d_000420 expression is negatively correlated with FEV1Rapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_30d_000420 expression is associated with a decrease of 0.010 in FEV1Rapp.
- the regression analysis representing path c' estimated a direct effect c'=0.015 (95% CI:-0.054-0.084 pvalue=0.673). This represents the estimated difference in FEV1Rapp between two patients with the same mir_30d_000420 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_30d_000420 expression level is estimated to be 0.015 units higher in FEV1Rapp.

The indirect effect 0.010 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.010 in FEV_1Rapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_30d_000420 expression level (because a is negative), which in turn translates into lower FEV_1Rapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.023). In this case the normal theory-based Sobel test (Z= 1.742, p=0.082) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_92a_000431: Results of Simple Mediation Analysis for mediator mir_92a_000431 were reported in Table16:

- the regression analysis representing path c is significant. The F-statistic was 7.217 and the p-value was <0.001. PM_{10} is positively associated with FEV1Rapp and the regression coefficient for PM_{10} (β=0.025 95% CI: -0.044-0.094) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this relationship is not statistically significantly different from zero (p=0.473);

						Consequent						
		M(miR_92a_000431)						Y(FEV $_1$ Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	0.014	0.035	-0.055	0.083	0.694
M(miR_92a_000431)		-	-	-	-	-	b	-0.961	0.409	-1.765	-0.158	0.019
costant	i_1	12.841	0.843	11.187	14.495	<0.001	i_2	113.051	10.976	91.506	134.596	<0.001
		R^2 =0.036						R^2 =0.0674				
		F(7,780)=4.177, p<0.001						F(8,779)=7.041, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm $_{10}$ on FEV $_1$ Rapp	c	0.025	0.035	-0.0436	0.094	0.473						
Direct Effect of Pm $_{10}$ on FEV $_1$ Rapp	c'	0.014	0.035	-0.055	0.083	0.694						
Indirect Effect of Pm $_{10}$ on FEV $_1$ Rapp		0.011	0.006	-	-	0.051*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm $_{10}$ on FEV $_1$ Rapp		0.011	0.006	0.003	0.027	-						

Table 16: Simple Mediation Analysis results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FEV1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV1Rapp = (Measured FEV1 / Theoretical FEV1)*100
*Sobel test

- the regression analysis representing path a is significant. The F-statistic was 4.177 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_92a_000431 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_92a_000431 expression decreases by 0.808% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 7.041 and the p-value was <0.001. The regression coefficient for mir_92a_000431 is statistically significantly different from zero (p=0.019) and mir_92a_000431 expression is negatively correlated with FEV1Rapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_92a_000431 expression is associated with a decrease of 0.014 in FEV1Rapp.
- the regression analysis representing path c' estimated a direct effect c'=0.014 (95% CI:-0.055-0.083 pvalue=0.694). This represents the estimated difference in FEV1Rapp between two patients with the same mir_92a_000431 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_92a_000431 expression level is estimated to be 0.014 units higher in FEV1Rapp.

The indirect effect 0.011 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.011 in FEV_1Rapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_92a_000431 expression level (because a is negative), which in turn translates into lower FEV_1Rapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.003; 0.027). In this case the normal theory-based Sobel test (Z= 1.953, p=0.080) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_181a_2_002317: Results of Simple Mediation Analysis for mediator mir_181a_2_002317 were reported in Table17:

- the regression analysis representing path c is significant. The F-statistic was 7.217 and the p-value was <0.001. PM_{10} is positively associated with FEV1Rapp and the regression coefficient for PM_{10} (β=0.025 95% CI: -0.044-0.094) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this relationship is not statistically significantly different from zero (p=0.473);

		Consequent									
		M(miR_181a_2_002317)					Y(FEV$_1$ Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
							R^2 = 0.061					
							F(7,780)=7.217, p<0.001					
X(Pm$_{10}$)	a	-0.017	0.004	-0.025	-0.008	<0.001	c'	0.011	0.035	-0.058	0.080	0.754
M(miR_181a_2_002317)		-	-	-	-	-	b	-0.854	0.299	-1.441	-0.268	0.004
costant	i_1	1.956	1.153	-0.307	4.219	0.0901	i_2	102.379	9.636	83.463	121.295	<0.001
		R^2 =0.025						R^2 =0.071				
		F(7,780)=2.870, p=0.006						F(8,779)=7.395, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$ Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$ Rapp	c'	0.011	0.035	-0.058	0.080	0.754
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp		0.014	0.006	-	-	0.023*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp		0.014	0.005	0.005	0.026	-

Table 17: Simple Mediation Analysis results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FEV1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV1Rapp = (Measured FEV1 / Theoretical FEV1)*100
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 2.870 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly

different from zero (p<0.001) and PM_{10} is negatively correlated with mir_181a_2_002317 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_181a_2_002317 expression decreases by 1.137% for an increase of 1 µg/m^3 in PM_{10};

- the regression analysis representing path b is also significant. The F-statistic was 7.395 and the p-value was <0.001. The regression coefficient for mir_181a_2_002317 is statistically significantly different from zero (p=0.004) and mir_181a_2_002317 expression is negatively correlated with FEV1Rapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_181a_2_002317 expression is associated with a decrease of 0.012 in FEV1Rapp.
- the regression analysis representing path c' estimated a direct effect c'=0.011 (95% CI:-0.058-0.080 pvalue=0.754). This represents the estimated difference in FEV1Rapp between two patients with the same mir_181a_2_002317 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_181a_2_002317 expression level is estimated to be 0.011 units higher in FEV1Rapp.

The indirect effect 0.014 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.014 in FEV_1Rapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_181a_2_002317 expression level (because a is negative), which in turn translates into lower FEV_1Rapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.005; 0.026). In this case the normal theory-based Sobel test (Z= 2.269, p=0.023) agrees with the inference made using a biased correct bootstrap confidence interval.

mir_218_000521: Results of Simple Mediation Analysis for mediator mir_218_000521 were reported in Table18:

- the regression analysis representing path c is significant. The F-statistic was 7.217 and the p-value was <0.001. PM_{10} is positively associated with FEV1Rapp and the regression coefficient for PM_{10} (β=0.025 95% CI: -0.044-0.094) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this relationship is not statistically significantly different from zero (p=0.473);

		Consequent									
		M(miR_218_000521)					Y(FEV$_1$ Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm$_{10}$)	a	-0.016	0.004	-0.024	-0.009	<0.001	c'	0.015	0.035	-0.055	0.084	0.682
M(miR_218_000521)		-	-	-	-	-	b	-0.658	0.329	-1.303	-0.013	0.046
costant	i_1	3.583	1.051	1.520	5.646	<0.001	i_2	103.066	9.716	83.994	122.139	<0.001
		R^2 =0.033						R^2 =0.066				
		F(7,780)=3.773, p<0.001						F(8,779)=6.841, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$ Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$ Rapp	c'	0.015	0.035	-0.055	0.084	0.682
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp		0.0106	0.0060	-	-	0.077*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$ Rapp		0.011	0.006	0.001	0.023	-

Table 18: Simple Mediation Analysis results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FEV1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV1Rapp = (Measured FEV1 / Theoretical FEV1)*100
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 3.773 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_218_000521 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_218_000521 expression decreases by 1.110% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 6.841 and the p-value was p<0.001. The regression coefficient for mir_218_000521 is statistically significantly different from zero (p=0.046) and mir_218_000521 expression is negatively correlated with FEV1Rapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_218_000521 expression is associated with a decrease of 0.009 in FEV1Rapp.
- the regression analysis representing path c' estimated a direct effect c'=0.015 (95% CI:-0.055-0.084 pvalue=0.682). This represents the estimated difference in FEV1Rapp between two patients with the same mir_218_000521 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_218_000521 expression level is estimated to be 0.015 units higher in FEV1Rapp.

The indirect effect 0.011 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.011 in FEV_1Rapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_218_000521 expression level (because a is negative), which in turn translates into lower FEV_1Rapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.023). In this case the normal theory-based Sobel test (Z= 1.771, p=0.077) does not agree with the inference made using a biased correct bootstrap confidence interval.

FVCRapp

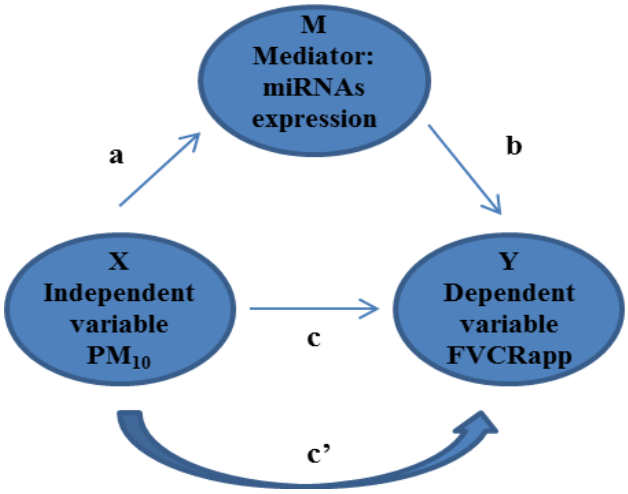


Figure 23: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and FVCRapp=(Measured FVC / Theoretical FVC)*100 as dependent variable.

mir_27b_000409: Results of Simple Mediation Analysis for mediator mir_27b_000409 were reported in Table19:

- the regression analysis representing path c is significant. The F-statistic was 15.999 and the p-value was <0.001. PM_{10} is positively associated with FVCRapp and the regression coefficient for PM_{10} (β=0.051 95% CI: -0.016-0.117) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.051 in FVCRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FVCRapp level. However this relationship is not statistically significantly different from zero (p=0.134);

							Consequent					
		M(miR_27b_000409)					Y(FVCRapp)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	a	-0.014	0.004	-0.022	-0.005	0.001	c'	0.042	0.034	-0.025	0.108	0.220
M(miR_27b_000409)		-	-	-	-	-	b	-0.669	0.291	-1.240	-0.098	0.0216
costant	i_1	7.967	1.140	5.728	10.206	<0.001	i_2	102.841	9.549	84.096	121.585	<0.001
		R^2 =0.053						R^2 =0.132				
		F(7,780)=6.203, p<0.001						F(8,779)=14.739, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134						
Direct Effect of Pm10 on FVCRapp	c'	0.042	0.034	-0.025	0.108	0.220						
Indirect Effect of Pm10 on FVCRapp		0.009	0.005	-	-	0.068*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm10 on FVCRapp		0.009	0.005	0.001	0.021	-						

Table 19: Simple Mediation Analysis results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 6.203 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p=0.001) and PM_{10} is negatively correlated with mir_27b_000409 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_27b_000409 expression decreases by 0.931% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 14.739 and the p-value was p<0.001. The regression coefficient for mir_27b_000409 is statistically significantly different from zero (p=0.022) and mir_27b_000409 expression is negatively correlated with FVCRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_27b_000409 expression is associated with a decrease of 0.010 in FVCRapp.
- the regression analysis representing path c' estimated a direct effect c'=0.042 (95% CI:-0.025-0.108 pvalue=0.222). This represents the estimated difference in FVCRapp between two patients with the same mir_27b_000409 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_27b_000409 expression level is estimated to be 0.042 units higher in FVCRapp.

The indirect effect 0.009 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.009 in FVCRapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_27b_000409 expression level (because a is negative), which in turn translates into lower FVCRapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.021). In this case the normal theory-based Sobel test (Z= 1.826, p=0.068) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_92a_000431*:* Results of Simple Mediation Analysis for mediator mir_92a_000431 were reported in Table20:

- the regression analysis representing path c is significant. The F-statistic was 15.999 and the p-value was <0.001. PM_{10} is positively associated with FVCRapp and the regression coefficient for PM_{10} (β=0.051 95% CI: -0.016-0.117) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.051 in FVCRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher FVCRapp level. However this relationship is not statistically significantly different from zero (p=0.134);

							Consequent					
		M(miR_92a_000431)					Y(FVCRapp)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM_{10})		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm_{10})	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	0.042	0.034	-0.025	0.108	0.221
M(miR_92a_000431)		-	-	-	-	-	b	-0.773	0.394	-1.546	0.001	0.050
costant	i_1	12.841	0.843	11.187	14.495	<0.001	i_2	107.428	10.562	86.694	128.162	<0.001
		R^2 =0.036						R^2 =0.130				
		F(7,780)=4.177, p<0.001						F(8,779)=14.531, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134						
Direct Effect of Pm10 on FVCRapp	c'	0.042	0.034	-0.025	0.108	0.221						
Indirect Effect of Pm10 on FVCRapp		0.009	0.005	-	-	0.089*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm10 on FVCRapp		0.009	0.005	0.001	0.023	-						

Table 20: Simple Mediation Analysis results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 4.177 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_92a_000431 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_92a_000431 expression decreases by 0.808% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 14.531 and the p-value was p<0.001. The regression coefficient for mir_92a_000431 is statistically significantly different from zero (p=0.050) and mir_92a_000431 expression is negatively correlated with FVCRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_92a_000431 expression is associated with a decrease of 0.011 in FVCRapp.
- the regression analysis representing path c' estimated a direct effect c'=0.042 (95% CI:-0.025-0.108 pvalue=0.222). This represents the estimated difference in FVCRapp between two patients with the same mir_92a_000431 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_92a_000431 expression level is estimated to be 0.042 units higher in FVCRapp.

The indirect effect 0.009 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.009 in FVCRapp level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_92a_000431 expression level (because a is negative), which in turn translates into lower FVCRapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.023). In this case the normal theory-based Sobel test (Z= 1.699, p=0.089) does not agree with the inference made using a biased correct bootstrap confidence interval.

mir_181a_2_002317: Results of Simple Mediation Analysis for mediator mir_181a_2_002317 were reported in Table 21:

- the regression analysis representing path c is significant. The F-statistic was 15.999 and the p-value was <0.001. PM_{10} is positively associated with FVCRapp and the regression coefficient for PM_{10} (β=0.051 95% CI: -0.016-0.117) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.051 in FVCRapp level, the positive sign suggests that patients with higher PM_{10} exposure show higher

FVCRapp level. However this relationship is not statistically significantly different from zero (p=0.134);

- the regression analysis representing path a is significant. The F-statistic was 2.870 and the p-value was p=0.006. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is positively correlated with mir_181a_2_002317 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_181a_2_002317 expression increases by 0.292% for an increase of 1 µg/m^3 in PM_{10};

		Consequent									
		M(miR_181a_2_002317)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM 10)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm 10)	a	0.004	-0.017	-0.025	-0.008	<0.001	c'	0.038	0.034	-0.029	0.104	0.266
M(miR_181a_2_002317)		-	-	-	-	-	b	-0.778	0.287	-1.342	-0.214	0.007
costant	i_1	1.956	1.153	-0.307	4.219	0.0901	i_2	99.029	9.269	80.835	117.224	<0.001
		R^2 =0.025						R^2 =0.134				
		F(7,780)=2.870, p=0.006						F(8,779)=15.029, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134						
Direct Effect of Pm10 on FVCRapp	c'	0.038	0.034	-0.029	0.104	0.266						
Indirect Effect of Pm10 on FVCRapp		0.013	0.006	-	-	0.029*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm10 on FVCRapp		0.013	0.006	0.003	0.026	-						

Table 21: Simple Mediation Analysis results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test.

- the regression analysis representing path b is also significant. The F-statistic was 15.029 and the p-value was p<0.001. The regression coefficient for mir_181a_2_002317 is statistically significantly different from zero (p=0.007) and mir_181a_2_002317 expression is negatively correlated with FVCRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_181a_2_002317 expression is associated with a decrease of 0.011 in FVCRapp.
- the regression analysis representing path c' estimated a direct effect c'=0.038 (95% CI:-0.029-0.104 pvalue=0.266). This represents the estimated difference in FVCRapp between two patients with the same mir_181a_2_002317 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that

patients with higher PM_{10} exposure but with the same mir_181a_2_002317 expression level is estimated to be 0.038 units higher in FVCRapp.

The indirect effect 0.013 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.013 in FVCRapp level as a result of the tendency of those with higher PM_{10} exposure level to have higher mir_181a_2_002317 expression level (because a is positive), which in turn translates into lower FVCRapp level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.003; 0.026). In this case the normal theory-based Sobel test (Z=2.186, p=0.029) agrees with the inference made using a biased correct bootstrap confidence interval.

Heart Rate

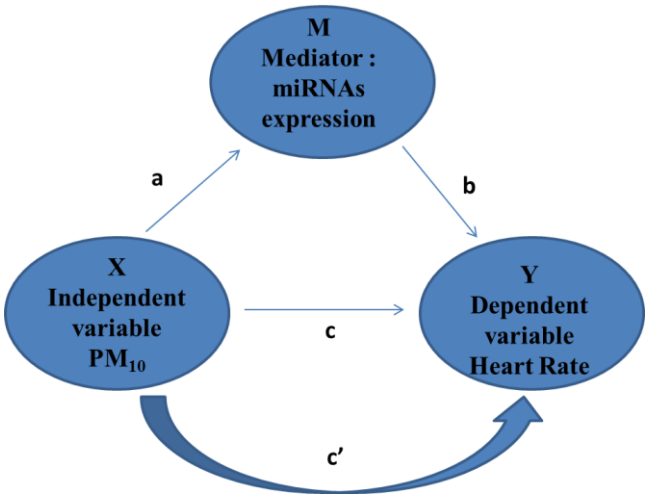


Figure 24: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and Heart Rate as dependent variable.

mir_218_000521: Results of Simple Mediation Analysis for mediator mir_218_000521 were reported in Table 22:

- the regression analysis representing path c is significant. The F-statistic was 10.428 and the p-value was <0.001. The regression coefficient for PM_{10} is significantly different from zero (p=0.026) and PM_{10} is positively associated with Heart Rate and the regression coefficient for PM_{10} (β=0.044 95% CI: -0.005-0.082) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.044 bpm in Heart Rate, the positive sign suggests that patients with higher PM_{10} exposure show higher Heart Rate ;
- the regression analysis representing path a is significant. The F-statistic was 3.411 and the p-value was p=0.020. The regression coefficient for PM_{10} is statistically significantly

different from zero (p<0.001) and PM_{10} is negatively correlated with mir_218_000521 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_218_000521 expression decreases by 1.151% for an increase of 1 µg/m^3 in PM_{10};

							Consequent					
		M(miR_218_000521)					Y(Heart Rate)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM 10)		-	-	-	-	-	c	0.044	0.020	0.0051	0.082	0.026
costant		-	-	-	-	-	i_3	50.191	3.161	43.987	56.394	<0.001
							R^2=0.060					
							F(5,850)=10.428, p<0.001					
X(PM 10)	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	0.039	0.020	0.001	0.078	0.048
M(miR_218_000521)		-	-	-	-	-	b	-0.371	0.204	-0.771	0.028	0.068
costant	i_1	4.110	0.532	3.066	5.154	<0.001	i_2	51.717	3.265	45.308	58.126	<0.001
		R^2=0.020						R^2=0.064				
		F(5,850)=3.411, p=0.005						F(6,849)=9.686, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM 10 on Heart Rate	c	0.044	0.020	0.005	0.082	0.026
Direct Effect of PM 10 on Heart Rate	c'	0.039	0.020	0.001	0.078	0.048
Indirect Effect of PM 10 on Heart Rate		0.004	0.003	-	-	0.114*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM 10 on Heart Rate		0.004	0.002	0.001	0.010	-

Table 22: Simple Mediation Analysis results for mediator mir_218_000521on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

- the regression analysis representing path b is also significant. The F-statistic was 9.686 and the p-value was p<0.001. The regression coefficient for mir_218_000521 is not statistically significantly different from zero (p=0.068) and mir_218_000521 expression is negatively correlated with FVCRapp, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_218_000521 expression is associated with a decrease of 0.006 bpm in Heart Rate.
- the regression analysis representing path c' estimated a direct effect c'=0.039 (95% CI:-0.001-0.078, pvalue=0.048). This represents the estimated difference in Heart Rate between two patients with the same mir_218_000521 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_218_000521 expression level is estimated to be 0.039 bpm higher in Heart Rate.

The indirect effect 0.004 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.004 bpm in Heart Rate as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_218_000521 expression level (because a is

negative), which in turn translates into lower Heart Rate (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.010). In this case the normal theory-based Sobel test (Z=1.762, p=0.114) does not agree with the inference made using a biased correct bootstrap confidence interval.

SBP: Sistolic Blood Pressure

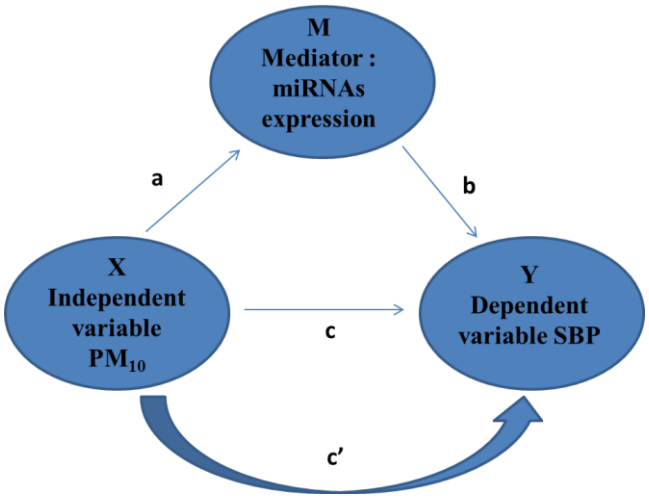


Figure 25: : Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression Systolic Blood Pressure as dependent variable.

mir_92a_000431: Results of Simple Mediation Analysis for mediator mir_92a_000431 were reported in Table 23:

- the regression analysis representing path c is significant. The F-statistic was 27.761 and the p-value was <0.001. PM_{10} is negatively associated with SBP and the regression coefficient for PM_{10} (β=-0.012 95% CI: -0.072;-0.047) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.012 mmHg in SBP level, the negative sign suggests that patients with higher PM_{10} exposure show lower SBP level. However this relationship is not statistically significantly different from zero (p=0.682);
- the regression analysis representing path a is significant. The F-statistic was 5.454 and the p-value was p=0.037. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negativelyy correlated with mir_92a_000431 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_92a_000431 expression decreases by 0.821% for an increase of 1 µg/m^3 in PM_{10};

		Consequent									
		M(miR_92a_000431)						Y(SBP)			
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI	p
$X(PM_{10})$		-	-	-	-	-	c	-0.012	0.030	-0.0716 0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093 108.621	<0.001
								R^2=0.164			
								F(6,851)=27.761, p<0.001			
$X(Pm_{10})$	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	-0.019	0.030	-0.079 0.041	0.533
M(miR_92a_000431)		-	-	-	-	-	b	-0.555	0.360	-1.261 0.152	0.124
costant	i_1	13.433	0.449	12.552	14.315	<0.001	i_2	106.806	6.754	93.549 120.063	<0.001
		R^2=0.037						R^2=0.166			
		F(6,851)=5.454, p<0.001						F(7,850)=24.172, p<0.001			

		Effect	SE	95% CI		p
Total effect of Pm_{10} on SBP	c	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm_{10} on SBP	c'	-0.019	0.030	-0.079	0.041	0.5333
Indirect Effect of Pm_{10} on SBP		0.007	0.004	-	-	0.159*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on SBP	0.007	0.004	0.001	0.017	-

Table 23: Simple Mediation Analysis results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

- the regression analysis representing path b is also significant. The F-statistic was 24.172 and the p-value was p<0.001. The regression coefficient for mir_92a_000431 is not statistically significantly different from zero (p=0.124) and mir_92a_000431 expression is negatively correlated with SBP, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in mir_92a_000431 expression is associated with a decrease of 0.011 mmHg in SBP level.
- the regression analysis representing path c' estimated a direct effect c'=-0.019 (95% CI:-0.079-0.041 pvalue=0.533). This represents the estimated difference in SBP between two patients with the same mir_92a_000431 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is negative, meaning that patients with higher PM_{10} exposure but with the same mir_92a_000431 expression level is estimated to be 0.019 mmHg lower in SBP level.

The indirect effect 0.007 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.007mmHg in SBP level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_92a_000431 expression level (because a is negative), which in turn translates into lower SBP level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.001; 0.017). In this case the normal theory-

based Sobel test (Z=1.409, p=0.159) does not agree with the inference made using a biased correct bootstrap confidence interval.

DBP:Diastolic Blood Pressure

No miRNAs showed a significant indirect effect of PM_{10} on Diastolic Blood Pressure

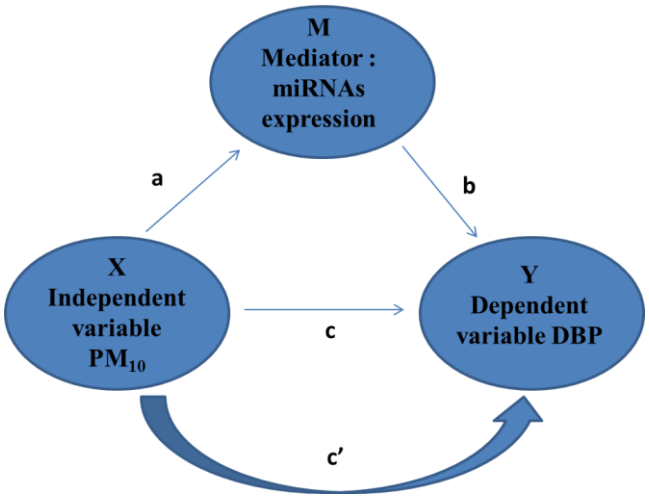


Figure 26: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and Diastolic Blood Pressure as dependent variable.

CRP: C-Reactive Protein

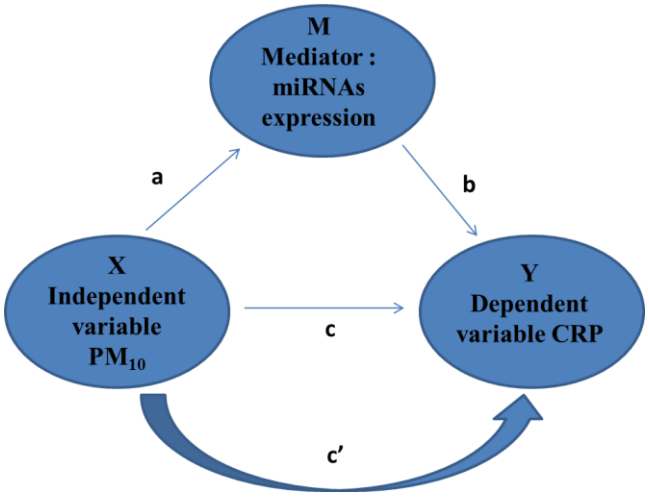


Figure 27: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and C-reactive protein as dependent variable.

mir_106a_002169: Results of Simple Mediation Analysis for mediator mir_106a_002169 were reported in Table 24:

- the regression analysis representing path c is significant. The F-statistic was 25.951 and the p-value was <0.001. and PM_{10} is positively associated with Heart Rate and the regression coefficient for PM_{10} (β=0.002 95% CI: -0.002-0.006) this means that two

patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.002 mg/l in CRP, the positive sign suggests that patients with higher PM_{10} exposure show higher CRP. However the regression coefficient for PM_{10} is not significantly different from zero (p=0.351);

							Consequent					
		M(miR_106a_002169)					Y(CRP)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM 10)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								$R^2 = 0.156$				
								F(6,840)=25.951, p<0.001				
X(Pm 10)	a	-0.012	0.003	-0.017	-0.007	<0.001	c'	0.001	0.002	-0.003	0.005	0.581
M(miR_106a_002169)		-	-	-	-	-	b	-0.062	0.028	-0.116	-0.007	0.027
costant	i_1	13.102	0.394	12.330	13.875	<0.001	i_2	-3.539	0.482	-4.485	-2.593	<0.001
		R^2=0.045						R^2=0.163				
		F(6,840)=6.532, p<0.001						F(7,839)=23.049, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm 10 on CRP	c	0.002	0.002	-0.002	0.006	0.351						
Direct Effect of Pm 10 on CRP	c'	0.001	0.002	-0.003	0.005	0.581						
Indirect Effect of Pm 10 on CRP		0.0008	0.0004	-	-	0.047*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm 10 on CRP		0.0008	0.0004	0.0002	0.0017	-						

Table 24: Simple Mediation Analysis results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test.

- the regression analysis representing path a is significant. The F-statistic was 6.532 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_106a_002169 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_106a_002169 expression decreases by 0.849% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 23.049 and the p-value was p<0.001. The regression coefficient for mir_106a_002169 is statistically significantly different from zero (p=0.027) and mir_106a_002169 expression is negatively correlated with CRP, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression and CRP level appears that 1% change in mir_106a_002169 expression is associated with a decrease of 0.03 mg/l in CRP level.
- the regression analysis representing path c' estimated a direct effect c'=0.001 (95% CI:-0.003-0.005 pvalue=0.581). This represents the estimated difference in CRP between two

patients with the same mir_92a_000431 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_106a_002169 expression level is estimated to be 0.001 mg/l lower in CRP level.

The indirect effect 0.0008 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.0008 mg/l in CRP level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_106a_002169 expression level (because a is negative), which in turn translates into lower CRP level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.0002; 0.0017). In this case the normal theory-based Sobel test (Z=1.985, p=0.047) agrees with the inference made using a biased correct bootstrap confidence interval.

mir_652_002352: Results of Simple Mediation Analysis for mediator mir_652_002352 were reported in Table 24:

- the regression analysis representing path c is significant. The F-statistic was 25.951 and the p-value was <0.001. and PM_{10} is positively associated with Heart Rate and the regression coefficient for PM_{10} (β=0.002 95% CI: -0.002-0.006) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.002 mg/l in CRP, the positive sign suggests that patients with higher PM_{10} exposure show higher CRP. However the regression coefficient for PM_{10} is not significantly different from zero (p=0.351);
- the regression analysis representing path a is significant. The F-statistic was 5.596 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_652_002352 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_652_002352 expression decreases by 1.513% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 22.949 and the p-value was p<0.001. The regression coefficient for mir_652_002352 is statistically significantly different from zero (p=0.038) and mir_652_002352 expression is negatively correlated with CRP, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression appears that 1% change in mir_652_002352 expression is associated with a decrease of 0.004 mg/l in CRP level.

		Consequent										
		M(miR_652_002352)						Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	a	-0.022	0.005	-0.032	-0.012	<0.001	c'	0.001	0.002	-0.003	0.005	0.534
M(miR_652_002352)		-	-	-	-	-	b	-0.028	0.014	-0.055	-0.002	0.038
costant	i_1	8.086	0.807	6.502	9.670	<0.001	i_2	-4.117	0.335	-4.774	-3.460	<0.001
		R^2 =0.038						R^2 =0.161				
		F(6,840)=5.596, p<0.001						F(7,839)=22.949, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on CRP	c	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	c'	0.001	0.002	-0.003	0.005	0.534
Indirect Effect of Pm $_{10}$ on CRP		0.0006	0.0003	-	-	0.068*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on CRP		0.0006	0.0003	0.0001	0.0014	-

Table 25: Simple Mediation Analysis results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test.

- the regression analysis representing path c' estimated a direct effect c'=0.001 (95% CI:-0.003-0.005 pvalue=0.534). This represents the estimated difference in CRP between two patients with the same mir_652_002352 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_652_002352 expression level is estimated to be 0.001 mg/l higher in CRP level.

The indirect effect 0.0006 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.0006 mg/l in CRP level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_652_002352 expression level (because a is negative), which in turn translates into lower SBP level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.0001; 0.0014). In this case the normal theory-based Sobel test (Z=1.828, p=0.068) does not agree with the inference made using a biased correct bootstrap confidence interval.

Fibrinogen

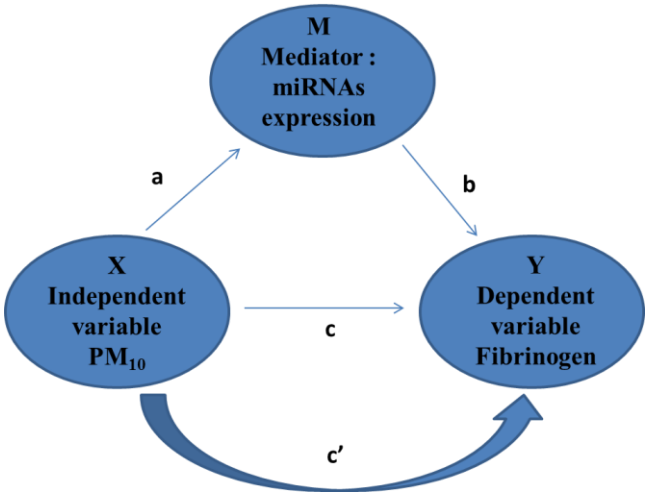


Figure 28: Statistical Diagram for the Simple mediation model with PM_{10} as independent variable, M as miRNAs expression and Fibrinogen as dependent variable.

mir_375_000564: Results of Simple Mediation Analysis for mediator mir_375_000564 were reported in Table 26:

- the regression analysis representing path c is significant. The F-statistic was 18.709 and the p-value was <0.001. PM_{10} is positively associated with Fibrinogen and the regression coefficient for PM_{10} (β=0.217 95% CI: -0.016-0.450) this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.217 g/l in Fibrinogen, the positive sign suggests that patients with higher PM_{10} exposure show higher Fibrinogen level. Hower the regression coefficient for PM_{10} is not significantly different from zero (p=0.068)
- the regression analysis representing path a is significant. The F-statistic was 5.116 and the p-value was p<0.001. The regression coefficient for PM_{10} is statistically significantly different from zero (p<0.001) and PM_{10} is negatively correlated with mir_375_000564 expression, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that mir_375_000564 expression decreases by 1.185% for an increase of 1 µg/m^3 in PM_{10};
- the regression analysis representing path b is also significant. The F-statistic was 16.865 and the p-value was p<0.001. The regression coefficient for mir_375_000564 is statistically significantly different from zero (p=0.023) and mir_375_000564 expression is negatively correlated with Fibrinogen, in particular, back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in

mir_375_000564 expression is associated with a decrease of 0.030 g/l in Fibrinogen level.

		Consequent									
		M(miR_375_000564)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	a	-0.017	0.005	-0.026	-0.008	<0.001	c'	0.181	0.119	-0.054	0.415	0.130
M(miR_375_000564)		-	-	-	-	-	b	-2.108	0.923	-3.920	-0.297	0.023
costant	i_1	3.912	0.709	2.521	5.303	<0.001	i_2	157.897	19.049	120.506	195.287	<0.001
		R^2 =0.036						R^2 =0.126				
		F(6,818)=5.116, p<0.001						F(7,817)=16.865, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.181	0.119	-0.054	0.415	0.130
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.036	0.024	-	-	0.056*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.036	0.024	0.002	0.099	-

Table 26: Simple Mediation Analysis results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

- the regression analysis representing path c' estimated a direct effect c'=0.181 (95% CI:-0.054-0.415 pvalue=0.130). This represents the estimated difference in Fibrinogen between two patients with the same mir_375_000564 expression level but who differ by 1 µg/m^3 in their PM_{10} exposure level. The coefficient is positive, meaning that patients with higher PM_{10} exposure but with the same mir_375_000564 expression level is estimated to be 0.181 g/l higher in Fibrinogen level.

The indirect effect 0.036 means that two patients who differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to by differ 0.036 g/l in Fibrinogen level as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_375_000564 expression level (because a is negative), which in turn translates into lower Fibrinogen level (because b is negative). Finally, the indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence interval that is interely above zero (0.002; 0.099). In this case the normal theory-based Sobel test (Z=1.914, p=0.056) agrees with the
inference made using a biased correct bootstrap confidence interval.

5.5.2 Parallel Multiple Mediation Models

DLco

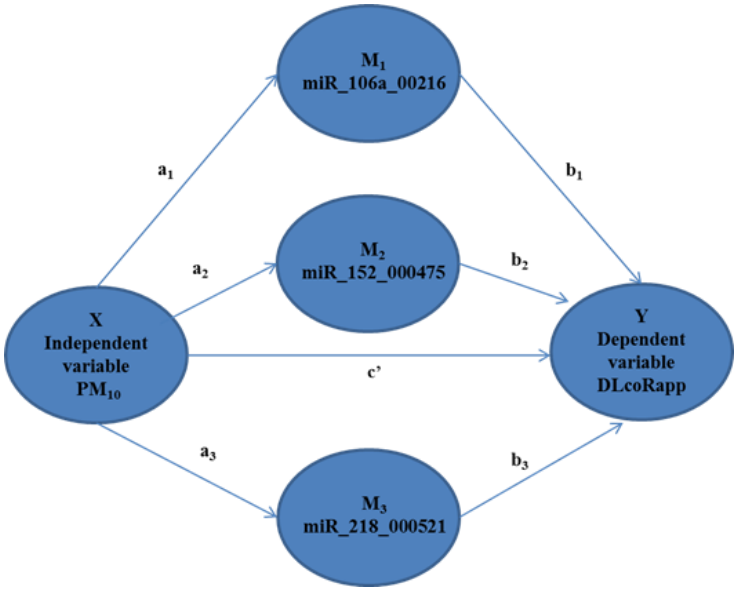


Figure 29: Statistical Diagram for the Parallel Multiple Mediation model with PM_{10} as independent variable, M_1 (miR_106a_002169), M_2 (miR_152_000475) and M_3 (miR_218_000521) as miRNAs expression and DLcoRapp as dependent variable.

Table 27 shows the results of the the four best fitting OLS regression models that define the parallel multiple mediator model reresented in Figure 29. Regression analysis representing paths a_1,a_2 and a_3 are significant as show the p-values for the F statistics. Moreover, back-trasforming results due to the log2 trasformation of miRNAs expression data, appears that:

- mir_106a_00216 expression decreases by 0.773% for an increase of 1 µg/m^3 in PM_{10};
- mir_152_000475 expression decreases by 0.491% for an increase of 1 µg/m^3 in PM_{10};
- mir_218_000521 expression decreases by 0.821% for an increase of 1 µg/m^3 in PM_{10};

Regression analysis representing paths b_1,b_2 and b_3 is also significant (F statistic 12.973, $p<0.001$) and back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in:

- mir_375_000564 expression is associated with a decrease of 0.016 in DLcoRapp;
- mir_152_000475 expression is associated with an increase of 0.048 in DLcoRapp;
- mir_218_000521 expression is associated with a of 0.037 in DLcoRapp;

The total effect from estimating DLcoRapp from PM_{10} alone is statistically significantly different from zero β=0.064 (95%CI: 0.0004;0.127; p-value=0.049), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.064 in DLcoRapp, the positive sign suggests that patients with higher PM_{10} exposure show higher DLcoRapp level. Very little of the variance in mir_106a_00216 or mir_152_000475 or mir_218_000521

expression is explained by PM_{10} (respectively $R^2 = 0.041$, $R^2 = 0.021$, $R^2 = 0.022$), and about a sixth of the variance in DLcoRapp is accounted for by proposed mediators and PM_{10}, $R^2 = 0.136$.

The most relevant information pertinent to the process being modeled is the direct and indirect effects of PM_{10} on DLcoRapp. Starting first with the indirect effect through mir_106a_00216 expression, this indirect effect is estimated as 0.012, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.012 units in their DLcoRapp through mir_106a_00216 expression, having higher DLcoRapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have higher mir_106a_00216 expression level (because a_1 is positive), which in turn translates into higher DLcoRapp level (because b_1 is positive).

The second indirect effect of PM_{10} exposure on DLcoRapp modeled through mir_152_000475 expression, is estimated as -0.024, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by -0.024 in their DLcoRapp through mir_152_000475 expression, having lower DLcoRapp (because the indirect effect is negative). This indirect effect is negative as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_152_000475 expression level (because a_2 is negative), which in turn translates into higher DLcoRapp level (because b_2 is positive).

The third indirect effect of PM_{10} exposure on DLcoRapp modeled through mir_2018_000521 expression, is estimated as 0.031, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.031 in their DLcoRapp through mir_218_000521 expression, having higher DLcoRapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_218_000521 expression level (because a_3 is negative), which in turn translates into lower DLcoRapp level (because b_3 is negative).

The total indirect effect of PM_{10} exposure on DLcoRapp obtained summed the indirect effects across all mediators is 0.020, this is positive, meaning that that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.020 in their DLcoRapp as a result of the effect of PM_{10} exposure on the mediators, which in turn influence DLcoRapp. The total indirect effect often is not of much interest in a multiple mediator model, and sometimes it will be small even when the specific indirect effects are relatively large, which seems paradoxical. The direct effect, $c' = 0.044$, quantifies the effect of PM_{10} exposure on DLcoRapp independent of the effect of the proposed mediators on DLcoRapp. Irrespective of the effects of PM_{10} exposure on mediators (mir_106a_00216, mir_152_000475 and mir_218_000521

expression level) and how those mediators relate to DLcoRapp, an increase of 1 µg/m^3 in PM_{10} exposure is associated with an increase of 0.044 in DLcoRapp (because c′ is positive). The total effect of PM_{10} exposure on DLcoRapp is not determined at all by the mediators proposed as intervening between X and Y. As it was in the simple mediation model, c = 0.064. As promised, this total effect partitions cleanly into the direct effect plus the sum of the specific indirect effects:

$$c = c' + a_1b_1 + a_2b_2 + a_3b_3 = 0.044 + (-0.011*-1.105)+(-0.007*-3.332) + (-0.012*-2.599) = 0.064$$

meaning that the total indirect effect of PM_{10} exposure (i.e., the sum of the specific indirect effects) is difference between the total and direct effects of PM_{10} exposure:

$$c - c' = a_1b_1 + a_2b_2 + a_3b_3 = 0.064 - 0.044 = 0.012 - 0.024 + 0.031 = 0.020$$

The total indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals (0.001;0.039), this supports the claim that mir_106a_00216, mir_152_000475 and mir_218_000521 expression mediate the effect of PM_{10} exposure on DLcoRapp. Also the indirect effects for the mediators mir_152_000475 and mir_2018_000521 expression are statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals. Respectively for mir_152_000475 expression it is interely below zero (-0.005; -0.004) and for mir_2018_000521 expression it is interely above zero (0.016;0.051). These results agree with the inferences made using the normal theory-based Sobel test. The estimate for the specific indirect effects (C1) through miR_106A_002169 minus the specific indirect effect through miR_152_000475 (a_1b_1 - a_2b_2) is 0.012-(-0.024)=0.036 and the 95% BC bootstrap confidence interval is entirely above zero (0.009;0.082) meaning that these specific indirect effect are statistically different from each other.

The estimate for the specific indirect effects (C2) through miR_106A_002169 minus the specific indirect effect through miR_218_000521 (a_1b_1 - a_3b_3) is 0.012-(-0.031)=0.0.19 and the 95% BC bootstrap confidence interval straddles zero (-0.040;0.000) meaning that these specific indirect effect are not statistically different from each other. Finally, the estimate for the specific indirect effects (C3) through mir_152_000475 minus the specific indirect effect through miR_218_000521 (a_2b_2 - a_3b_3) is (-0.024 - 0.031)=-0.055 and the 95% BC bootstrap confidence interval is entirely below zero (-0.099;-0.024) meaning that these specific indirect effect are statistically different from each other. It is tempting to treat this as a test of the difference in strength of the mechanisms at work linking X to Y, or that one indirect effect is larger than another in an absolute sense.

However, such an interpretation is justified only if the point estimates for the two specific indirect effects being compared are of the same sign. Consider, for instance, the case where aibi = −0.30 and ajbj = 0.30. A test of the difference between these specific indirect effects may lead

to the claim that their difference is not zero, but this does not imply the mechanisms are of different strength or that one indirect effect is bigger. The point estimates suggest one mechanism results in a positive difference in Y, whereas the other yields a negative difference of equal magnitude. In an absolute sense, they are equal in size by the point estimates, yet statistically different by an inferential test which considers their sign. But one indirect effect is not stronger than the other. Nor can we say that X exerts a larger effect on Y through one of the mediators relative to the other.

		Consequent																				
		M_1 (miR_106a_002169)						M_2 (miR_152_000475)						M_3 (miR_218_000521)						Y(DLCORapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})	a_1	-0.011	0.003	-0.016	-0.006	<0.001	a_2	-0.007	0.003	-0.014	-0.001	0.033	a_3	-0.012	0.004	-0.019	-0.005	0.001	c'	0.044	0.032	-0.019	0.107	0.169
M_1 (miR_106a_002169)		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_1	-1.105	0.781	-2.638	0.429	0.158
M_2 (miR_152_000475)		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_2	3.332	0.682	1.994	4.670	<0.001
M_3 (miR_218_000521)		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_3	-2.599	0.396	-3.376	-1.822	<0.001
costant	i_M	13.390	0.733	11.951	14.828	<0.001	i_M	6.603	0.964	4.710	8.496	<0.001	i_M	2.959	1.068	0.862	5.057	0.006	i_Y	51.435	11.832	28.207	74.662	<0.001
		R^2=0.041 F(6,743)=5.307, p<0.001						R^2=0.021 F(6,743)=2.599, p=0.017						R^2=0.022 F(6,743)=2.766, p=0.0115						R^2=0.136 *F(9,740)=12.973, p<0.001*				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on DLcoRapp	0.064	0.032	0.0004	0.127	0.049
Direct Effect of Pm_{10} on DLcoRapp	0.044	0.032	-0.019	0.107	0.169
Indirect Effect of Pm_{10} on DLcoRapp					
M_1 (miR_106a_002169)	0.012	0.009	-	-	0.188*
M_2 (miR_152_000475)	-0.024	0.012	-	-	0.054*
M_3 (miR_218_000521)	0.031	0.011	-	-	0.004*
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm_{10} on DLcoRapp					
Total	0.020	0.010	0.001	0.039	-
M_1 (miR_106a_002169)	0.012	0.008	-0.001	0.029	-
M_2 (miR_152_000475)	-0.024	0.013	-0.055	-0.004	-
M_3 (miR_218_000521)	0.031	0.009	0.016	0.051	-
(C_1)	0.036	0.018	0.009	0.082	-
(C_2)	-0.019	0.010	-0.040	0.000	-
(C_3)	-0.055	0.019	-0.099	-0.024	-

Table 27: Parallel Multiple Mediation Analysis results for mediators M1 (miR_106a_002169), M2 (miR_152_000475) and M3 (miR_218_000521) on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp: β log2(101/100) associated with 1%change in miRNAs expression.
Contrasts for pairwaise comparisons between specific indirect effects: (C1) miR_106A_002169 minus miR_152_000475, (C2) miR_106A_002169 minus miR_218_000521, (C3) miR_152_000475 minus miR_218_000521.
*Sobel test.

FEV$_1$

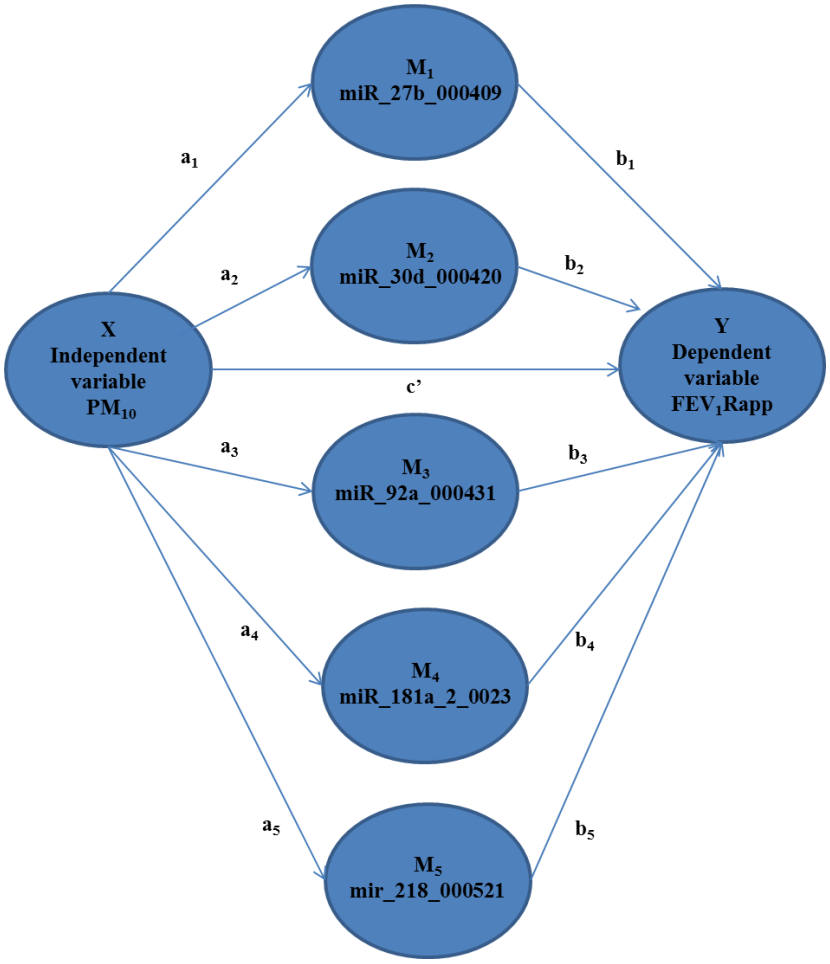


Figure 30: Statistical Diagram for the Parallel Multiple Mediation model with PM_{10} as independent variable, M_1 (miR_27b_000409), M_2 (miR_30d_000420), M_3 (miR_92a_000431), M_4 (miR_181a_2_002317), M_5 (miR_218_000521) as miRNAs expression and FEV_1 as dependent variable.

Table 28 shows the results of the the four best fitting OLS regression models that define the parallel multiple mediator model reresented in Figure 30. Regression analysis representing paths a1, a2, a3, a4, a5 are significant as show the p-values for the F statistics. Moreover, back-trasforming results due to the log2 trasformation of miRNAs expression data, appears that:

- miR_27b_000409 expression decreases by 0.931 for an increase of 1 µg/m^3 in PM_{10};
- miR_30d_000420 expression decreases by 1.062 for an increase of 1 µg/m^3 in PM_{10};
- miR_92a_000431 expression decreases by 0.808 for an increase of 1 µg/m^3 in PM_{10};
- miR_181a_2_002317 expression decreases by 1.137 for an increase of 1 µg/m^3 in PM_{10};
- miR_218_000521 expression decreases by 1.110 for an increase of 1 µg/m^3 in PM_{10};

Regression analysis representing paths b1, b2, b3, b4 and b5 is also significant (F statistic 5.078, $p<0.001$) and back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in:

- miR_27b_000409 expression is associated with a decrease of 0.017 in FEV1Rapp;
- miR_30d_000420 expression is associated with an increase of 0.603 in FEV1Rapp;
- miR_92a_000431 expression is associated with a decrease of 0.730 in FEV1Rapp;
- miR_181a_2_002317 expression is associated with a decrease of 0.877 in FEV1Rapp;
- miR_218_000521 expression is associated with a decrease of 0.157 of in FEV1Rapp;

The total effect from estimating FEV1Rapp from PM_{10} alone is 0.025 (95%CI: -0.044;0.094; p-value=0.473), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.025 in FEV1Rapp, the positive sign suggests that patients with higher PM_{10} exposure show higher FEV1Rapp level. However this total effect is not statistically significant.

The most relevant information pertinent to the process being modeled is the direct and indirect effects of PM10 on FEV1Rapp. Starting first with the indirect effect through miR_27b_000409 expression, this indirect effect is estimated as 0.0002, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.0002 in their FEV1Rapp level through miR_27b_000409 expression, having higher FEV1Rapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_27b_000409 expression level (because a1 is negative), which in turn translates into lower FEV1Rapp level (because b1 is negative).

The second indirect effect of PM_{10} exposure on FEV1Rapp modeled through miR_30d_000420 expression, is estimated as -0.009, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by -0.009 in their FEV1Rapp through miR_30d_000420 expression, having lower FEV1Rapp (because the indirect effect is negative). This indirect effect is negative as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_30d_000420 expression level (because a2 is negative), which in turn translates into higher FEV1Rapp level (because b2 is positive). The third indirect effect of PM_{10} exposure on FEV1Rapp modeled through miR_92a_000431 expression, is estimated as 0.009, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.009 in their FEV1Rapp through miR_92a_000431 expression, having higher FEV1Rapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_92a_000431

expression level (because a3 is negative), which in turn translates into lower FEV1Rapp level (because b3 is negative).

The fourth indirect effect of PM_{10} exposure on FEV1Rapp modeled through miR_181a_2_002317 expression, is estimated as 0.015, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.015 in their FEV1Rapp through miR_181a_2_002317 expression, having higher FEV1Rapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_181a_2_002317 expression level (because a4 is negative), which in turn translates into lower FEV1Rapp level (because b4 is negative).

The fifth indirect effect of PM_{10} exposure on FEV1Rapp modeled through miR_218_000521 expression, is estimated as 0.003, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.003 in their FEV1Rapp through miR_218_000521 expression, having higher FEV1Rapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower miR_218_000521 expression level (because a5 is negative), which in turn translates into lower FEV1Rapp level (because b5 is negative).

The total indirect effect of PM_{10} exposure on FEV1Rapp obtained summed the indirect effects across all mediators is 0.017, this is positive, meaning that that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.017 in their FEV1Rapp as a result of the effect of PM_{10} exposure on the mediators, which in turn influence FEV1Rapp. The direct effect, $c' = 0.009$, quantifies the effect of PM_{10} exposure on FEV1Rapp independent of the effect of the proposed mediators on FEV1Rapp. Irrespective of the effects of PM_{10} exposure on mediators (miR_27b_000409, miR_30d_000420, miR_92a_000431, miR_181a_2_002317 and miR_218_000521 expression level) and how those mediators relate to FEV1Rapp, an increase of 1 µg/m^3 in PM_{10} exposure is associated with an increase of 0.009 in FEV1Rapp (because c' is positive). The total effect of PM_{10} exposure on DLcoRapp is not determined at all by the mediators proposed as intervening between X and Y. As it was in the simple mediation model, c = 0.025. As promised, this total effect partitions cleanly into the direct effect plus the sum of the specific indirect effects:

$$c = c' + a_1b_1 + a_2b_2 + a_3b_3 = 0.044 + (-0.011*-1.105)+(-0.007*-3.332) + (-0.012*-2.599) = 0.064$$

meaning that the total indirect effect of PM_{10} exposure (i.e., the sum of the specific indirect effects) is difference between the total and direct effects of PM_{10} exposure:

$$c - c' = a_1b_1 + a_2b_2 + a_3b_3 = 0.064 - 0.044 = 0.012 - 0.024+0.031 = 0.020$$

The total indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals (0.001;0.039), this supports the claim that mir_106a_00216,

mir_152_000475 and mir_218_000521 expression mediate the effect of PM_{10} exposure on DLcoRapp. Also the indirect effects for the mediators mir_152_000475 and mir_2018_000521 expression are statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals. Respectively for mir_152_000475 expression it is interely below zero (-0.005; -0.004) and for mir_2018_000521 expression it is interely above zero (0.016;0.051). These results agree with the inferences made using the normal theory-based Sobel test. The estimate for the specific indirect effects (C1) through miR_106A_002169 minus the specific indirect effect through miR_152_000475 (a_1b_1 - a_2b_2) is 0.012-(-0.024)=0.036 and the 95% BC bootstrap confidence interval is entirely above zero (0.009;0.082) meaning that these specific indirect effect are statistically different from each other.

The estimate for the specific indirect effects (C2) through miR_106A_002169 minus the specific indirect effect through miR_218_000521 (a_1b_1 - a_3b_3) is 0.012-(-0.031)=0.0.19 and the 95% BC bootstrap confidence interval straddles zero (-0.040;0.000) meaning that these specific indirect effect are not statistically different from each other. Finally, the estimate for the specific indirect effects (C3) through mir_152_000475 minus the specific indirect effect through miR_218_000521 (a_2b_2 - a_3b_3) is (-0.024 - 0.031)=-0.055 and the 95% BC bootstrap confidence interval is entirely below zero (-0.099;-0.024) meaning that these specific indirect effect are statistically different from each other. It is tempting to treat this as a test of the difference in strength of the mechanisms at work linking X to Y, or that one indirect effect is larger than another in an absolute sense.

	Consequent																																	
	M_1 (miR_27b_000409)						M_2 (miR_30d_000420)						M_3 (miR_92a_000431)						M_4 (miR_181a_2_002317)						M_5 (miR_218_000521)						Y(FEV_1 Rapp)					
Antecedent	Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})	a_1 -0.014	0.004	-0.022	-0.005	0.001	a_2	-0.015	0.004	-0.023	-0.008	<0.001	a_3	-0.012	0.003	-0.018	-0.006	<0.001	a_4	-0.017	0.004	-0.025	-0.008	<0.001	a_5	-0.016	0.004	-0.024	-0.009	<0.001	c'	0.009	0.035	-0.061	0.078	0.808
M_1 (miR_27b_000409)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_1	-0.017	0.482	-0.963	0.928	0.971
M_2 (miR_30d_000420)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_2	0.603	0.626	-0.626	1.832	0.336
M_3 (miR_92a_000431)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_3	-0.730	0.596	-1.900	0.440	0.221
M_4 (miR_181a_2_002317)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_4	-0.877	0.535	-1.926	0.173	0.101
M_5 (miR_218_000521)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-	b_5	-0.157	0.430	-1.001	0.687	0.715
costant	i_M 7.967	1.140	5.728	10.206	<0.001	i_M	9.233	1.029	7.213	11.253	<0.001	i_M	12.841	0.843	11.187	14.495	<0.001	i_M	1.956	1.153	-0.307	4.219	0.090	i_M	3.583	1.051	1.520	5.646	<0.001	i_Y	106.934	11.420	84.516	129.351	<0.001
	R^2=0.053						R^2=0.035						R^2=0.036						R^2=0.025						R^2=0.033						R^2=0.073				
	F(7,780)=6.203, p<0.001						F(7,780)=4.073, p<0.001						F(7,780)=4.177, p<0.001						F(7,780)=2.870, p=0.006						F(7,780)=3.773, p<0.001						F(12,755)=5.078, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on FEV_1 Rapp	0.025	0.035	-0.044	0.094	0.473
Direct Effect of Pm_{10} on FEV_1 Rapp	0.009	0.035	-0.061	0.078	0.808
Indirect Effect of Pm_{10} on FEV1Rapp					
M_1 (miR_27b_000409)	0.0002	0.007	-	-	0.973*
M_2 (miR_30d_000420)	-0.009	0.010	-	-	0.362*
M_3 (miR_92a_000431)	0.009	0.008	-	-	0.258*
M_4 (miR_181a_2_002317)	0.015	0.010	-	-	0.140*
M_5 (miR_218_000521)	0.003	0.007	-	-	0.723*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on FEV1Rapp					
Total	0.017	0.007	0.003	0.032	-
M_1 (miR_27b_000409)	0.0002	0.007	-0.014	0.013	-
M_2 (miR_30d_000420)	-0.009	0.011	-0.033	0.009	-
M_3 (miR_92a_000431)	0.009	0.007	-0.002	0.024	-
M_4 (miR_181a_2_002317)	0.015	0.010	-0.003	0.037	-
M_5 (miR_218_000521)	0.003	0.008	-0.015	0.018	-
(C_1)	0.010	0.014	-0.017	0.040	-
(C_2)	-0.008	0.010	-0.031	0.010	-
(C_3)	-0.014	0.014	-0.045	0.010	-
(C_4)	-0.002	0.010	-0.021	0.018	-
(C_5)	-0.018	0.014	-0.048	0.008	-
(C_6)	-0.024	0.017	-0.062	0.006	-
(C_7)	-0.012	0.014	-0.041	0.016	-
(C_8)	-0.006	0.011	-0.029	0.016	-
(C_9)	0.006	0.010	-0.012	0.029	-
(C_{10})	0.012	0.016	-0.016	0.046	-

Table 28: Parallel Multiple Mediation Analysis results for mediators M1 (miR_106a_002169), M2 (miR_152_000475), M3 (miR_218_000521), M4 (miR_181a_2_002317), M5 (miR_218_000521) on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.

miRNAs expression was log2 transformed.

Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.

Back transforming in model with FEV1Rapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.

Contrasts for pairwaise comparisons between specific indirect effects: (C1) miR_27B_000409 minus miR_30D_000420, (C2) miR_27B_000409 minus miR_92A_000431, (C3) miR_27B_000409 minus miR_181A_2__002317, (C4) miR_27B_000409 minus miR_218_000521, (C5) miR_30D_000420 minus miR_92A_000431, (C6) miR_30D_000420 minus miR_181A_2__002317, (C7) miR_30D_000420 minus miR_218_000521, (C8) miR_92A_000431 minus miR_181A_2__002317, (C9) miR_92A_000431 minus miR_218_000521, (C10) miR_181A_2__002317 minus miR_218_000521.

*Sobel test.

FVCRapp

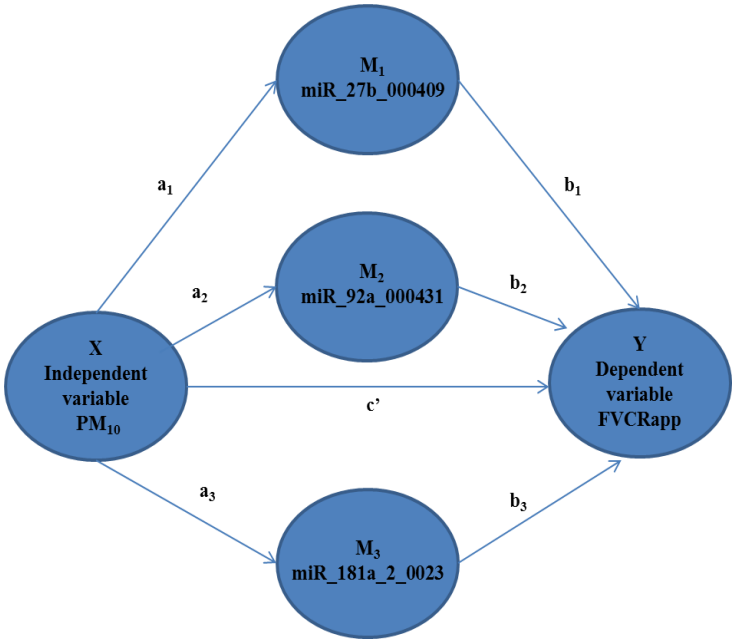


Figure 31: Statistical Diagram for the Parallel Multiple Mediation model with PM_{10} as independent variable, M_1 (miR_27b_000409), M_2 (miR_92a_000431), M_3 (miR_181a_2_002317) as miRNAs expression and FVCRapp as dependent variable.

Table 29 shows the results of the the four best fitting OLS regression models that define the parallel multiple mediator model reresented in Figure 31. Regression analysis representing paths a_1,a_2 and a_3 are significant as show the p-values for the F statistics. Moreover, back-trasforming results due to the log2 trasformation of miRNAs expression data, appears that:

- mir_27b_000409 expression decreases by 0.931% for an increase of 1 µg/m^3 in PM_{10};
- mir_92a_000431 expression decreases by 0.808% for an increase of 1 µg/m^3 in PM_{10};
- mir_181a_2_002317 expression decreases by 1.137% for an increase of 1 µg/m^3 in PM_{10};

Regression analysis representing paths b_1,b_2 and b_3 is also significant (F statistic 12.050, $p<0.001$) and back-trasforming results due to the log2 trasformation of miRNAs expression data appears that 1% change in:

- mir_27b_000409 expression is associated with a decrease of 0.003 g/l in FVCRapp;
- mir_92a_000431 expression is associated with a decrease of 0.003 g/l in FVCRapp;
- mir_181a_2_002317 expression is associated with an increase of 0.006 g/l in FVCRapp;

The estimate for the total effect obtained estimating FVCRapp from PM_{10} alone is $\beta=0.051$ (95%CI: -0.016;0.117; p-value=0.134), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.051 in FVCRapp, the positive sign suggests that patients with higher PM_{10} exposure show higher FVCRapp level. However the total effect is not

statistically significantly different from zero. Very little of the variance in mir_106a_00216 or mir_152_000475 or mir_2018_000521 expression is explained by PM_{10} (respectively R^2 = 0.053, R^2 = 0.036, R^2 = 0.025), and about a sixth of the variance in FVCRapp is accounted for by proposed mediators and PM_{10}, R^2 = 0.134.

The most relevant information pertinent to the process being modeled is the direct and indirect effects of PM_{10} on FVCRapp. Starting first with the indirect effect through mir_27b_000409 expression, this indirect effect is estimated as 0.002, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.002 in their FVCRapp through mir_27b_000409 expression, having higher FVCRapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_27b_000409 expression level (because a_1 is negative), which in turn translates into lower FVCRapp level (because b_1 is negative).

The second indirect effect of PM_{10} exposure on FVCRapp modeled through mir_92a_000431 expression, is estimated as 0.002, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.002 in their FVCRapp through mir_92a_000431 expression, having lower FVCRapp (because the indirect effect is negative). This indirect effect is negative as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_92a_000431 expression level (because a_2 is negative), which in turn translates into lower FVCRapp level (because b_2 is negative).

The third indirect effect of PM_{10} exposure on FVCRapp modeled through mir_181a_2_002317 expression, is estimated as 0.010, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.010 in their FVCRapp through mir_181a_2_002317 expression, having higher FVCRapp (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_181a_2_002317 expression level (because a_3 is negative), which in turn translates into lower FVCRapp level (because b_3 is negative).

The total indirect effect of PM_{10} exposure on FVCRapp obtained summed the indirect effects across all mediators is 0.014, this is positive, meaning that that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.014 in their FVCRapp as a result of the effect of PM_{10} exposure on the mediators, which in turn influence FVCRapp. The direct effect, $c' = 0.036$, quantifies the effect of PM_{10} exposure on FVCRapp independent of the effect of the proposed mediators on FVCRapp. Irrespective of the effects of PM_{10} exposure on mediators (mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression level) and how those mediators relate to FVCRapp, an increase of 1 µg/m^3 in PM_{10} exposure is associated

with an increase of 0.036 in FVCRapp (because c′ is positive). The total effect of PM_{10} exposure on FVCRapp is not determined at all by the mediators proposed as intervening between X and Y. As it was in the simple mediation model, c = 0.051. As promised, this total effect partitions cleanly into the direct effect plus the sum of the specific indirect effects:

$$c = c' + a_1b_1 + a_2b_2 + a_3b_3 = 0.036 + (-0.014*-0.175)+(-0.012*-0.182) + (-0.017*-0.588) = 0.051$$

meaning that the total indirect effect of PM_{10} exposure (i.e., the sum of the specific indirect effects) is difference between the total and direct effects of PM_{10} exposure:

$$c - c' = a_1b_1 + a_2b_2 + a_3b_3 = 0.051 - 0.036 = 0.002 +0.002+0.010 = 0.051$$

The total indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals (0.002;0.029), this supports the claim that mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression mediate the effect of PM_{10} exposure on FVCRapp. However, the indirect effects for all the mediators are not statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals which straddle zero. These results agree with the inferences made using the normal theory-based Sobel test.

The estimate for the specific indirect effects (C1) through miR_27b_000409 minus the specific indirect effect through miR_92a_000431 (a_1b_1 - a_2b_2) is 0.0024-0.0021=0.0003.

The estimate for the specific indirect effects (C2) through miR_27b_000409 minus the specific indirect effect through miR_181a_2__002317 (a_1b_1 - a_3b_3) is 0.0024-0.0097=-0.007.

The estimate for the specific indirect effects (C3) through miR_92a_000431 minus the specific indirect effect through miR_181a_2__002317 (a_2b_2 - a_3b_3) is 0.0021-0.0097=-0.008.

These specific indirect effect are not statistically different from zero as shown by the 95% BC bootstrap confidence intervals.

		Consequent																			
		M_1 (miR_27b_000409)					M_2 (miR_92a_000431)					M_3 (miR_181a_2_002317)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p	Coeff.	SE	95% CI		p	Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})	a_1	-0.014	0.004	-0.022	-0.005	0.001	a_2 -0.012	0.003	-0.02	-0.006	<0.001	a_3 -0.017	0.004	-0.025	-0.008	<0.001	c'	0.036	0.034	-0.030	0.103	0.285
M_1 (miR_27b_000409)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	b_1	-0.175	0.451	-1.060	0.711	0.699
M_2 (miR_92a_000431)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	b_2	-0.182	0.530	-1.222	0.858	0.732
M_3 (miR_181a_2_002317)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	b_3	-0.588	0.400	-1.373	0.198	0.142
costant	i_M	7.967	1.140	5.728	10.206	<0.001	i_M 12.841	0.843	11.187	14.495	<0.001	i_M 1.956	1.153	-0.307	4.219	0.090	i_Y	102.383	10.861	81.062	123.704	<0.001
		R^2=0.053 F(7,780)=6.203, p<0.001					R^2=0.036 F(7,780)=4.177, p<0.001					R^2=0.025 F(7,780)=2.870, p=0.006						R^2=0.134 F(10,777)=12.050, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on FVCRapp	0.051	0.034	-0.016	0.117	0.134
Direct Effect of Pm_{10} on FVCRapp	0.036	0.034	-0.030	0.103	0.285
Indirect Effect of Pm_{10} on FVCRapp					
M_1 (miR_27b_000409)	0.0024	0.006	-	-	0.713*
M_2 (miR_92a_000431)	0.0021	0.006	-	-	0.741*
M_3 (miR_181a_2_002317)	0.001	0.007	-	-	0.180*
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm_{10} on FVCRapp					
Total	0.0142	0.007	0.002	0.029	-
M_1 (miR_27b_000409)	0.0024	0.006	-0.01	0.015	-
M_2 (miR_92a_000431)	0.0021	0.005	-0.010	0.012	-
M_3 (miR_181a_2_002317)	0.010	0.007	-0.003	0.025	-
(C_1)	0.0003	0.009	-0.016	0.020	-
(C_2)	-0.007	0.011	-0.029	0.013	-
(C_3)	-0.008	0.010	-0.027	0.010	-

Table 29: Parallel Multiple Mediation Analysis results for mediators M1 (miR_27b_000409), M2 (miR_92a_000431) and M3 (miR_181a_2_002317) on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
Contrasts for pairwaise comparisons between specific indirect effects: (C1) miR_27b_000409 minus miR_92A_000431, (C2) miR_27b_000409 minus miR_181a_2__002317, (C3) miR_92a_000431 minus miR_181a_2__002317.
*Sobel test.

CRP

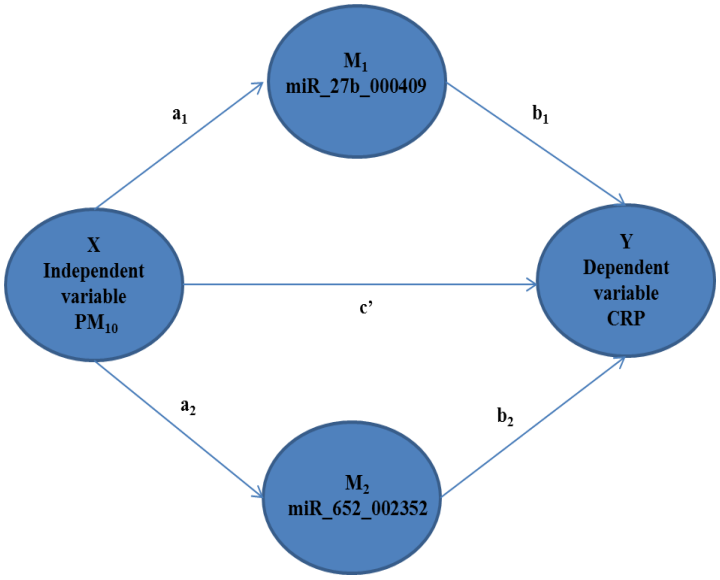


Figure 32: Statistical Diagram for the Parallel Multiple Mediation model with PM_{10} as independent variable, M_1 (miR_27b_000409), M_2 (miR_652_002352) as miRNAs expression and CRP as dependent variable.

Table 30 shows the results of the the three best fitting OLS regression models that define the parallel multiple mediator model reresented in Figure 32. Regression analysis representing paths a_1, a_2 are significant as show the p-values for the F statistics. Moreover, back-trasforming results due to the log2 trasformation of miRNAs expression data, appears that:

- mir_27b_000409 expression decreases by -0.979% for an increase of 1 µg/m^3 in PM_{10};
- mir_652_002352 expression decreases by -1.459% for an increase of 1 µg/m^3 in PM_{10};

Regression analysis representing paths b_1, and b_2 is also significant (F statistic 17.781, $p<0.001$) and back-trasforming results due to the log2 trasformation of miRNAs expression data and CRP levels appears that 1% change in:

- mir_27b_000409 expression is associated with a decrease of 0.011 g/l in CRP;
- mir_652_002352 expression is associated with a decrease of 0.024 g/l in CRP;

The estimate for the total effect obtained estimating CRP from PM_{10} alone is β=0.002 (95%CI: -0.002; 0.006; p-value=0.3946), this means that two patients who differ by 1 µg/m^3 in PM_{10} exposure level are estimated to differ by 0.002 mg/l in CRP level, the positive sign suggests that patients with higher PM_{10} exposure show higher CRP level. However the total effect is not statistically significantly different from zero. Very little of the variance in mir_27b_000409 or mir_92a_000431 expression is explained by PM_{10} (respectively R^2 = 0.053, R^2 = 0.037), and about a sixth of the variance in FVCRapp is accounted for by proposed mediators and PM_{10}, R^2 = 0.162.

The most relevant information pertinent to the process being modeled is the direct and indirect effects of PM_{10} on CRP. Starting first with the indirect effect through mir_27b_000409 expression, this indirect effect is estimated as 0.0002, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.0002 mg/l in their CRP through mir_27b_000409 expression, having higher CRP (because the indirect effect is positive). This indirect effect is positive as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_27b_000409 expression level (because a_1 is negative), which in turn translates into lower CRP level (because b_1 is negative).

The second indirect effect of PM_{10} exposure on CRP modeled through mir_652_002352 expression, is estimated as 0.0005, meaning that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.0005 in their CRP through mir_652_002352 expression, having higher CRP (because the indirect effect is positive). This indirect effect is negative as a result of the tendency of those with higher PM_{10} exposure level to have lower mir_652_002352 expression level (because a_2 is negative), which in turn translates into lower CRP level (because b_2 is negative).

The total indirect effect of PM_{10} exposure on CRP obtained summed the indirect effects across all mediators is 0.0007, this is positive, meaning that that two patients that differ by 1 µg/m^3 in their PM_{10} exposure level are estimated to differ by 0.0007 in their CRP as a result of the effect of PM_{10} exposure on the mediators, which in turn influence CRP. The direct effect, $c' = 0.001$, quantifies the effect of PM_{10} exposure on CRP independent of the effect of the proposed mediators on CRP. Irrespective of the effects of PM_{10} exposure on mediators (mir_27b_000409, mir_652_002352 expression level) and how those mediators relate to CRP, an increase of 1 µg/m^3 in PM_{10} exposure is associated with an increase of 0.001 in CRP (because c' is positive). The total effect of PM_{10} exposure on CRP is not determined at all by the mediators proposed as intervening between X and Y. As it was in the simple mediation model, $c = 0.002$. As promised, this total effect partitions cleanly into the direct effect plus the sum of the specific indirect effects:

$$c = c' + a_1b_1 + a_2b_2 = 0.001 + (-0.014*-0.011)+(-0.021*-0.024) = 0.002$$

meaning that the total indirect effect of PM_{10} exposure (i.e., the sum of the specific indirect effects) is difference between the total and direct effects of PM_{10} exposure:

$$c - c' = a_1b_1 + a_2b_2 = 0.002 - 0.001 = 0.0002 + 0.0005 = 0.0007$$

The total indirect effect is statistically different from zero, as revealed by the 95% BC bootstrap confidence intervals (0.0001;0.0014), this supports the claim that mir_27b_000409, mir_652_002352 expression mediate the effect of PM_{10} exposure on CRP. However, the indirect effects for all the mediators are not statistically different from zero, as revealed by the

95% BC bootstrap confidence intervals which straddle zero. These results agree with the inferences made using the normal theory-based Sobel test.

The estimate for the specific indirect effects (C1) through miR_27b_000409 minus the specific indirect effect through miR_652_002352 (a_1b_1 - a_2b_2) is 0.0002-0.0005=-0.0004, and the 95% BC bootstrap confidence interval straddles zero meaning that it is not statistically different from zero.

		Consequent														
		M_1 (miR_27b_000409)					M_2 (miR_652_002352)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p	Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})	a_1	-0.014	0.004	-0.022	-0.006	<0.001	a_2 -0.021	0.005	-0.032	-0.011	<0.001	c'	0.001	0.002	-0.003	0.005	0.603
M_1 (miR_27b_000409)		-	-	-	-	-	-	-	-	-	-	b_1	-0.011	0.023	-0.055	0.034	0.638
M_2 (miR_652_002352)		-	-	-	-	-	-	-	-	-	-	b_2	-0.024	0.017	-0.059	0.010	0.162
costant	i_M	8.183	1.085	6.053	10.314	<0.001	i_M 8.388	1.414	5.612	11.164	<0.001	i_Y	-3.485	0.575	-4.615	-2.356	<0.001
		R^2=0.053					R^2=0.037						R^2 =0.162				
		F(6,831)=6.572, p<0.001					F(7,831)=4.611, p<0.001						F(9,829)= 17.781, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on CRP	0.002	0.002	-0.002	0.006	0.3946
Direct Effect of Pm_{10} on CRP	0.001	0.002	-0.003	0.005	0.603
Indirect Effect of Pm_{10} on CRP					
M_1 (miR_27b_000409)	0.0002	0.0003	-	-	0.6529*
M_2 (miR_652_002352)	0.0005	0.0004	-	-	0.197*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on CRP					
Total	0.0007	0.0003	0.0001	0.0014	-
M_1 (miR_27b_000409)	0.0002	0.0003	-0.0004	0.0008	-
M_2 (miR_652_002352)	0.0005	0.0004	0.0000	0.0014	-
(C_1)	-0.0004	0.0006	-0.0016	0.0006	-

Table 30: Parallel Multiple Mediation Analysis results for mediators M1 (miR_27b_000409) and M2 (miR_652_002352) on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
Contrasts for pairwaise comparisons between specific indirect effects: (C1) miR_27b_000409 minus miR_652_002352
*Sobel test.

6. DISCUSSION AND FUTURE DIRECTIONS

The proposed research will help to shed light on the chain of events that from air pollution exposure leads to CVD trying to explore a new mechanism which involves alteration of extracellular vesicles production and content. Our findings, if confirmed, could lead to the identification of potentially-reversible alterations that might be also considered as potential target for new diagnostic and therapeutic interventions. Results on the first 1000 enrolled subjects have highlighted a statistical significant association at level 0.10 after FDR adjustment of miR_106a_002169, miR_152_000475, miR_181a_2__002317, miR_218_000521, miR_27b_000409, miR_30d_000420, miR_652_002352, miR_92a_000431,miR_25_000403, miR_375_000564 expression with PM_{10} exposure estimated by Eulerian model of the cell containing the address of the Center for Obesity. The use of the daily FARM PM_{10} exposure estimate of the 4*4 km cell of subject residence for the same day of blood collection produced similar results. The candidate miRNAs were subjected to pathway exploration using the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA) . Using this software, top-ranked pathways were also determined. Moreover are provided the biological functions associated with these miRNAs, such as the diseases in which they are involved (Figure 33).

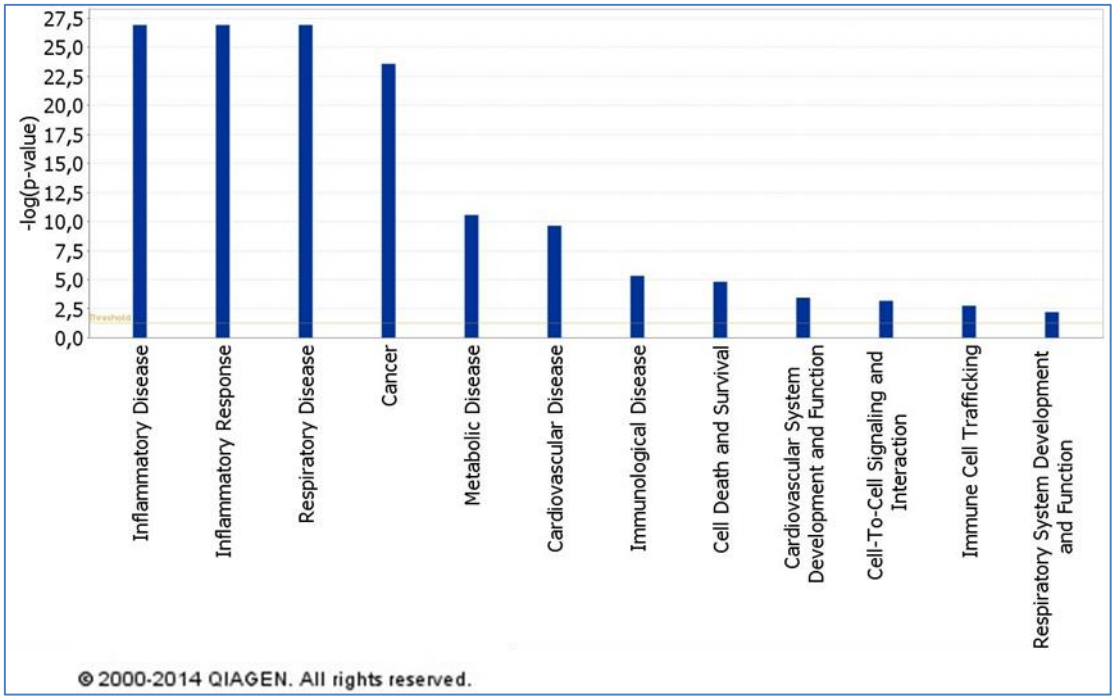


Figure 33: Biological functions and diseases associated with candidate miRNAs.

Inflammatory diseases, inflammatory response and respiratory disease resulted the first three top-ranked pathways.

A new experiment was set up in order to evaluate the performance of different normalization strategies in reducing technical variation and extracting true biological variation. Results showed that for large scale miRNA expression profiling Global Mean normalization strategy outperforms the other normalization strategy in terms of :

- ✓ better reduction of technical variation:
  - lower % of miRNAs differentially expressed before and after FDR adjustment
  - lower Fold change range;
- ✓ more accurate appreciation of biological changes.
  - higher % of miRNAs differentially expressed before and after FDR adjustment;
  - higher Fold Change range;

Individual air pollution exposure assessment is determined using two sources of information: actual monitor measurements for each and every day starting from January 1st, 1990 and The FARM model, a regional well validated modelling systems applied starting from 2007. The winter months (October to February) are those where there is a more obvious difference between the two methods of exposure assessment; for the rest of the year the distributions are similar. The examination of model performance of FARM model suggests the fulfillment of the objective of performance, although with a tendency to underestimate the observed concentrations. However the differences between the two methods are consistent with what reported by ARPA Lombardy in the Annual Assessment Of Air Quality Modeling for years 2009-2011, and the huge amount of available data offer the opportunity to implement new modelling techniques as long as they might come out.

Potential non linearity, was investigated by means of generalized additive models using penalized splines. We tested for nonlinearity of PM_{10} and apparent temperature using penalized splines in generalized linear models. We excluded a nonlinear relation between PM_{10} or apparent temperature and miRNAs expression. Mixed effect models was developed in order to take into account of other variability sources linked to the outcome due to the hierarchical data structure with three levels: sample level (level-1), barcode level (level-2) and run level (level-3). Results showed that the association between PM_{10} and miRNAs expression varies significantly among barcode and run, providing inputs for the biologists in order to improve the repeatability of the experiment in future analysis.

Simple Mediation Models and Parallel Multiple Mediation Models have proven useful and appropriate tools to investigate the role of miRNAs expression as mediator of the effect of PM_{10}

on respiratory, cardiac and inflammatory outcomes. We estimated the Indirect Effect of PM_{10} on outcome through the mediator as the product a*b, and applied Sobel test for testing the null hypothesis that the ‘‘true’’ indirect effect is zero, with the p-value derived from the standard normal distribution (Normal Theory Approach). However the sampling distribution of the indirect effect a*b tends to be asymmetric, with nonzero skewness and kurtosis. We applied bootstrapping as alternative more powerful than the Normal Theory Approach. In particular we calculated *percentiles bootstrap confidence intervals*, because they are based entirely on values of *ab** that demarcate the upper and lower (100 − ci)/2% of the distribution of k bootstrap estimates of the indirect effect, and *bias-corrected bootstrap confidence intervals (BC bootstrap confidence intervals).* They are like percentile confidence intervals but the endpoints are adjusted as a function of the proportion of k values of *ab** that are less than ab, the point estimate of the indirect effect calculated in the original data. The endpoints will be adjusted upward or downward to varying degrees depending on that proportion. Although bootstrapping is recommended, it does have a few weaknesses, among them that it requires the original data (not usually a real problem typically), the endpoints of the confidence interval will vary from run to run (but not if you seed the random number generator yourself), and it isn’t implemented in all software one might choose to use. An alternative to get around these problems: Monte Carlo confidence intervals. A significant indirect effect of PM_{10} on:

- DLcoRapp, was found through the following mediators: mir_106a_002169, mir_152_000475, mir_218_000521 expression;
- FEV1Rapp was found through the following mediators: mir_27b_000409 mir_30d_000420 mir_92a_000431 mir_181a_2_002317 mir_218_000521 expression;
- FVCRapp was found through the following mediators: mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression;
- Heart Rate was found through the following mediator: mir_218_000521 expression;
- Sistolic Blood Pressure was found through the following mediator: mir_92a_000431 expression;
- *CRP* was found through the following mediator: mir_106a_002169 and mir_652_002352 expression.
- Fibrinogeno was found through the following mediator: mir_375_000564 expression.

Finally, a the total indirect effect of PM_{10} exposure:

- on DLcoRapp obtained summed the indirect effects across all mediators: mir_106a_002169, mir_152_000475, and mir_218_000521 expression was statistically different from zero;

- on FEV1Rapp obtained summed the indirect effects across all mediators: mir_27b_000409 mir_30d_000420 mir_92a_000431 mir_181a_2_002317 mir_218_000521 expression was statistically different from zero;
- on FVCRapp obtained summed the indirect effects across all mediators mir_27b_000409, mir_92a_000431 and mir_181a_2_002317 expression was statistically different from zero;
- on CRP obtained summed the indirect effects across all mediators mir_106a_002169 and mir_652_002352 expression was statistically different from zero;

From a a literature review *[115,118]* , has emerged an important role of these miRNAs, for example mir_106a_002169, mir_92a_000431 and mir_652_002352 effectively regulate macrophage inflammatory responses through modulating leukocyte SIRPa synthesis. As an important signaling molecule, SIRPa modulates many aspects of leukocyte inflammatory responses, including activation, chemotaxis, and phagocytosis. These miRNAs are involved in macrophage infiltration through the direct suppression of expression of signal-regulatory protein α (SIRP α). A downregulation of mir_106a_002169 and mir_92a_000431 seems to act in favor of the differentiation of monocytes into macrophages. Macrophage infiltration occurs in many tissue types, such as adipose tissue, the vascular system, liver and muscle and it is involved in the processes of formation of atherosclerotic plaques. The resident macrophages in tissues and organs play critical roles in controlling physiological functions and systemic homeostasis in tissues. miRNAs are a set of potent regulators of macrophage differentiation, infiltration, activation and cell-cell interactions.

Future directions in research activities will be:

- Apply mediation analysis to the three-levels HLM models developed.
- Deepen and apply a the modern approach to mediation analysis based on counterfactual framework;
- Replicate the analysis on data coming from standard real time-PCR validation on the set of 10 top miRNAs identified in stage 1 on 1000 subjects enrolled in stage2;
- Using exposure measured by personal passive samplers on a subgroup of 200 selected subjects;
- Using exposure to metals as measured in urine and hair to estimate short/medium term exposure;

7. APPENDIX 1: RESULTS

DLcoRapp: Single breath carbon monoxide diffusing capacity

mir_25_000403

								Consequent				
		M(miR_25_000403)						Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2 =0.076				
								F(7,743)=10.428, p<0.001				
X(PM $_{10}$)	a	-0.007	0.003	-0.014	-0.001	0.0244	c'	0.068	0.032	0.004	0.131	0.037
M(miR_25_000403)		-	-	-	-	-	b	0.585	0.366	-0.133	1.303	0.110
costant	i_1	8.959	0.938	7.118	10.799	<0.001	i_2	45.405	9.914	25.943	64.868	<0.001
		R^2 =0.122						R^2 =0.083				
		F(6,744)=1.533, p=0.040						F(7,743)=9.322, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM $_{10}$ on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	c'	0.068	0.032	0.004	0.131	0.037
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.004	0.004	-	-	0.220*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.004	0.003	-0.0126	0.0003	-

Table 1: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin.
miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test

mir_27b_000409

								Consequent				
		M(miR_27b_000409)						Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2 =0.078				
								F(7,743)=10.428, p<0.001				
X(PM $_{10}$)	a	-0.005	0.004	-0.013	0.003	0.2064	c'	0.069	0.032	0.006	0.132	0.033
M(miR_27b_000409)		-	-	-	-	-	b	1.053	0.297	0.470	1.636	<0.001
costant	i_1	6.436	1.148	4.182	8.690	<0.001	i_2	43.869	9.488	25.241	62.496	<0.001
		R^2 =0.017						R^2 =0.083				
		F(6,744)=2.113, p=0.049						F(7,743)=9.602, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM $_{10}$ on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	c'	0.069	0.032	0.006	0.132	0.033
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.005	0.005	-	-	0.250*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.005	0.004	-0.015	0.001	-

Table 2: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin.
miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test

mir_30d_000420

		Consequent									
		M(miR_30d_000420)					Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2=0.078				
								F(7,743)=8.936, p<0.001				
X(PM $_{10}$)	a	0.004	-0.009	-0.016	-0.002	0.013	c'	0.063	0.032	-0.001	0.126	0.054
M(miR_30d_000420)		-	-	-	-	-	b	-0.085	0.330	-0.733	0.563	0.797
costant	i_1	8.459	1.041	6.415	10.502	<0.001	i_2	51.363	9.779	32.165	70.561	<0.001
		R^2=0.012						R^2=0.083				
		F(6,744)=1.545, p=0.021						F(7,743)=9.602, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM $_{10}$ on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	c'	0.063	0.032	-0.001	0.126	0.054
Indirect Effect of PM $_{10}$ on DLcoRapp		0.001	0.003	-	-	0.812*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM $_{10}$ on DLcoRapp		0.001	0.003	-0.006	0.008	-

Table 3: Simple Mediation Analysis Results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated hemoglobin.
miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test.

mir_92a_000431

		Consequent									
		M(miR_92a_000431)					Y(DLcoRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
								R^2=0.078				
								F(7,743)=8.936, p<0.001				
X(PM $_{10}$)	a	-0.007	0.003	-0.012	-0.001	0.0259	c'	0.068	0.032	0.004	0.131	0.036
M(miR_92a_000431)		-	-	-	-	-	b	0.715	0.402	-0.075	1.504	0.076
costant	i_1	12.122	0.853	10.448	13.796	<0.001	i_2	41.983	10.547	21.277	62.689	<0.001
		R^2=0.012						R^2=0.082				
		F(6,744)=1.484, p<0.001						F(7,743)=9.415, p<0.001				

		Effect	SE	95% CI		p
Total effect of PM $_{10}$ on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	c'	0.068	0.032	0.004	0.131	0.036
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.005	0.004	-	-	0.190*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM $_{10}$ on DLcoRapp		-0.005	0.004	-0.016	0.000	-

Table 4: Simple Mediation Analysis Results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated hemoglobin.
miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test.

mir_181a_2_0002317

								Consequent				
			M(miR_181a_2_002317)						Y(DLcoRapp)			
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(PM_{10})$		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
									R^2 =0.078			
									F(7,743)=10.428, p<0.001			
$X(PM_{10})$	a	-0.014	0.004	-0.022	-0.006	0.0005	c'	0.062	0.033	-0.002	0.126	0.059
M(miR_181a_2_002317)		-	-	-	-	-	b	-0.116	0.297	-0.699	0.467	0.697
costant	i_1	1.628	1.157	-0.644	3.900	0.160	i_2	50.834	9.384	32.411	69.256	<0.001
			R^2 =0.022						R^2 =0.078			
			F(6,744)=2.823, p=0.010						F(7,743)=9.949, p<0.001			

		Effect	SE	95% CI		p
Total effect of PM_{10} on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM_{10} on DLcoRapp	c'	0.062	0.033	-0.002	0.126	0.059
Indirect Effect of PM_{10} on DLcoRapp		0.002	0.004	-	-	0.709*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM_{10} on DLcoRapp		0.002	0.005	-0.007	0.012	-

Table 5: Simple Mediation Analysis Results for mediator mir_181a_2_0002317on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated hemoglobin. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test.

mir_375_000564

								Consequent				
			M(miR_375_000564)						Y(DLcoRapp)			
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(PM_{10})$		-	-	-	-	-	c	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
									R^2 =0.078			
									F(7,743)=10.428, p<0.001			
$X(PM_{10})$	a	-0.017	0.004	-0.026	-0.009	<0.001	c'	0.062	0.033	-0.002	0.126	0.058
M(miR_375_000564)		-	-	-	-	-	b	-0.075	0.270	-0.604	0.455	0.783
costant	i_1	1.43	1.2730	1.3151	6.3135	0.003	i_2	50.929	9.429	32.420	69.439	<0.001
			R^2 =0.040						R^2 =0.078			
			F(6,744)=4.736, p<0.001						F(7,743)=8.939, p<0.001			

		Effect	SE	95% CI		p
Total effect of PM_{10} on DLcoRapp	c	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM_{10} on DLcoRapp	c'	0.062	0.033	-0.002	0.126	0.058
Indirect Effect of PM_{10} on DLcoRapp		0.001	0.005	-	-	0.790*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of PM_{10} on DLcoRapp		0.001	0.005	-0.009	0.012	-

Table 6: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated hemoglobin. miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100
*Sobel test

mir_652_002352

							Consequent					
			M(miR_652_002352)						**Y(DLcoRapp)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.063	0.032	-0.0001	0.127	0.051
costant		-	-	-	-	-	i_3	50.645	9.366	32.257	69.033	<0.001
									R^2=0.078			
									F(7,743)=10.428, p<0.001			
X(PM $_{10}$)	*a*	-0.009	0.004	-0.016	-0.002	0.0131	*c'*	0.063	0.032	-0.001	0.126	0.054
M(miR_652_002352)		-	-	-	-	-	*b*	-0.085	0.330	-0.733	0.563	0.797
costant	i_1	8.459	1.041	6.415	10.502	<0.001	i_2	51.363	9.779	32.165	70.561	<0.001
			R^2=0.012						**R^2=0.078**			
			F(6,744)=1.145, p=0.040						**F(7,743)=8.936, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of PM $_{10}$ on DLcoRapp	*c*	0.063	0.032	-0.0001	0.127	0.051
Direct Effect of PM $_{10}$ on DLcoRapp	*c'*	0.063	0.032	-0.001	0.126	0.054
Indirect Effect of PM $_{10}$ on DLcoRapp		0.001	0.003	-	-	0.812*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of PM $_{10}$ on DLcoRapp		0.001	0.003	-0.006	0.008	-

Table 7: Simple Mediation Analysis Results for mediator mir_652_002352on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated hemoglobin.
miRNAs expression was $\log_2$ transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DLcoRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in DLcoRapp : β log2(101/100) associated with 1%change in miRNAs expression.
DLcoRapp= (Measured DLcoRapp / Theoretical DLcoRapp)*100.
*Sobel test.

FEV1 Rapp:

mir_25_000403

							Consequent					
			M(miR_25_000403)						**Y(FEV $_1$ Rapp)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
									R^2 = 0.061			
									F(7,780)=7.217, p<0.001			
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.019	-0.006	<0.001	*c'*	0.022	0.035	-0.048	0.091	0.537
M(miR_25_000403)		-	-	-	-	-	*b*	-0.269	0.374	-1.003	0.466	0.473
costant	i_1	9.740	0.926	7.924	11.557	<0.001	i_2	103.323	10.329	83.047	123.600	<0.001
			R^2=0.033						**R^2=0.158**			
			F(7,780)=7.217, p<0.001						**F(8+K37,839)=22.459, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on FEV $_1$ Rapp	*c*	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm $_{10}$ on FEV $_1$ Rapp	*c'*	0.022	0.035	-0.048	0.091	0.537
Indirect Effect of Pm $_{10}$ on FEV $_1$ Rapp		0.003	0.005	-	-	0.496*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on FEV $_1$ Rapp		0.003	0.005	-0.006	0.014	-

Table 8: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with FEV_1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV_1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV_1Rapp = (Measured FEV_1 / Theoretical FEV_1)*100
*Sobel test

mir_106a_002169

		Consequent									
		M(miR_106a_002169)					Y(FEV$_1$Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm$_{10}$)	a	-0.012	0.003	-0.017	-0.007	<0.001	c'	0.019	0.036	-0.051	0.088	0.597
M(miR_106a_002169)		-	-	-	-	-	b	-0.519	0.474	-1.448	0.411	0.274
costant	i_1	13.463	0.730	12.029	14.896	<0.001	i_2	107.688	11.576	84.965	130.412	<0.001
		R^2=0.044						R^2=0.062				
		F(7,780)=5.136, p<0.001						F(8,779)=6.467, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$Rapp	c'	0.019	0.036	-0.051	0.088	0.597
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.006	0.006	-	-	0.297*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.006	0.006	-0.003	0.021	-

Table 9: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FEV_1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV_1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV_1Rapp = (Measured FEV_1 / Theoretical FEV_1)*100
*Sobel test

mir_152_000475

		Consequent									
		M(miR_152_000475)					Y(FEV$_1$Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
								R^2 = 0.061				
								F(7,780)=7.217, p<0.001				
X(Pm$_{10}$)	a	-0.012	0.004	-0.019	-0.005	<0.001	c'	0.020	0.035	-0.050	0.089	0.580
M(miR_152_000475)		-	-	-	-	-	b	-0.464	0.362	-1.176	0.247	0.201
costant	i_1	7.308	0.954	5.435	9.182	<0.001	i_2	104.099	10.015	84.439	123.760	<0.001
		R^2=0.038						R^2=0.063				
		F(7,780)=4.437, p<0.001						F(8,779)=6.525, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$Rapp	c'	0.020	0.035	-0.050	0.089	0.580
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.006	0.005	-	-	0.246*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.006	0.005	-0.002	0.016	-

Table 10: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FEV_1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV_1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV_1Rapp = (Measured FEV_1 / Theoretical FEV_1)*100
*Sobel test

mir_375_000564

		Consequent									
		M(miR_375_000564)					Y(FEV$_1$Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
							R^2 = 0.061					
							F(7,780)=7.217, p<0.001					
X(Pm$_{10}$)	a	-0.018	0.005	-0.027	-0.009	<0.001	c'	0.022	0.035	-0.047	0.092	0.531
M(miR_375_000564)		-	-	-	-	-	b	-0.162	0.270	-0.692	0.368	0.548
costant	i_1	4.004	1.282	1.487	6.521	0.002	i_2	101.357	9.727	82.263	120.451	<0.001
		R^2 =0.038						R^2 =0.061				
		F(7,780)=4.378, p<0.001						F(8,779)=6.355, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$Rapp	c'	0.022	0.035	-0.047	0.092	0.531
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.003	0.005	-	-	0.565*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp	0.003	0.005	-0.006	0.014	-

Table 11: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FEV_1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV_1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV_1Rapp = (Measured FEV_1 / Theoretical FEV_1)*100
*Sobel test

mir_652_002352

		Consequent									
		M(miR_652_002352)					Y(FEV$_1$Rapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM$_{10}$)		-	-	-	-	-	c	0.025	0.035	-0.0436	0.094	0.473
costant		-	-	-	-	-	i_3	100.708	9.663	81.740	119.676	<0.001
							R^2 = 0.061					
							F(7,780)=7.217, p<0.001					
X(Pm$_{10}$)	a	-0.021	0.005	-0.032	-0.011	<0.001	c'	0.023	0.035	-0.047	0.092	0.517
M(miR_652_002352)		-	-	-	-	-	b	-0.103	0.234	-0.562	0.356	0.659
costant	i_1	8.632	1.480	5.726	11.538	<0.001	i_2	101.600	9.876	82.213	120.987	<0.001
		R^2 =0.037						R^2 =0.061				
		F(7,780)=4.290, p<0.001						F(8,779)=6.333, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm$_{10}$ on FEV$_1$Rapp	c	0.025	0.035	-0.0436	0.094	0.473
Direct Effect of Pm$_{10}$ on FEV$_1$Rapp	c'	0.023	0.035	-0.047	0.092	0.517
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp		0.002	0.005	-	-	0.670*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm$_{10}$ on FEV$_1$Rapp	0.002	0.005	-0.007	0.013	-

Table 12: Simple Mediation Analysis Results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FEV_1 as independent variable and miRNAs expression as dependent variable it was obtained the change in FEV_1Rapp: β log2(101/100) associated with 1%change in miRNAs expression.
FEV_1Rapp = (Measured FEV_1 / Theoretical FEV_1)*100
*Sobel test

FVCRapp:

mir_27b_000409

		Consequent									
		M(miR_25_000403)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	*c*	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.019	-0.006	<0.001	*c'*	0.048	0.034	-0.019	0.115	0.158
M(miR_25_000403)		-	-	-	-	-	*b*	-0.202	0.360	-0.908	0.504	0.575
costant	i_1	9.740	0.926	7.924	11.557	<0.001	i_2	99.475	9.931	79.980	118.969	<0.001
		R^2 =0.033						R^2 =0.126				
		F(7,780)=3.759, p<0.001						F(8,779)=14.027, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	*c*	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	*c'*	0.048	0.034	-0.019	0.115	0.158
Indirect Effect of Pm10 on FVCRapp		0.003	0.005	-	-	0.592*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.003	0.005	-0.008	0.013	-

Table 13: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test

mir_27b_000409

		Consequent									
		M(miR_30d_000420)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	*c*	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	*a*	-0.015	0.004	-0.023	-0.008	<0.001	*c'*	0.042	0.034	-0.025	0.109	0.219
M(miR_30d_000420)		-	-	-	-	-	*b*	-0.565	0.323	-1.199	0.068	0.080
costant	i_1	9.233	1.029	7.213	11.253	<0.001	i_2	102.726	9.744	83.600	121.853	<0.001
		R^2 =0.035						R^2 =0.129				
		F(7,780)=4.073, p<0.001						F(8,779)=14.420, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	*c*	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	*c'*	0.042	0.034	-0.025	0.109	0.219
Indirect Effect of Pm10 on FVCRapp		).0087	0.0055	-	-	0.116*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.009	0.006	-0.001	0.022	-

Table 14: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test

mir_106a_002169

		Consequent									
		M(miR_106a_002169)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.017	-0.007	<0.001	c'	0.046	0.034	-0.021	0.113	0.178
M(miR_106a_002169)		-	-	-	-	-	b	-0.370	0.455	-1.264	0.525	0.418
costant	i_1	13.463	0.730	12.029	14.896	<0.001	i_2	102.482	11.132	80.630	124.334	<0.001
		R^2 =0.044						R^2 =0.126				
		F(7,780)=5.136, p<0.001						F(8,779)=14.076, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	c'	0.046	0.034	-0.021	0.113	0.178
Indirect Effect of Pm10 on FVCRapp		0.005	0.006	-	-	0.435*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.005	0.006	-0.005	0.018	-

Table 15: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM10 as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM10.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test.

mir_152_000475

		Consequent									
		M(miR_152_000475)					Y(FVCRapp)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.004	-0.019	-0.005	<0.001	c'	0.046	0.034	-0.021	0.112	0.180
M(miR_152_000475)		-	-	-	-	-	b	-0.418	0.348	-1.101	0.267	0.231
costant	i_1	7.308	0.954	5.435	9.182	<0.001	i_2	100.559	9.629	81.657	119.461	<0.001
		R^2 =0.038						R^2 =0.127				
		F(7,780)=4.437, p<0.001						F(8,779)=14.187, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	c'	0.046	0.034	-0.021	0.112	0.180
Indirect Effect of Pm10 on FVCRapp		0.005	0.005	-	-	0.274*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.005	0.005	-0.004	0.016	-

Table 16: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test

mir_218_000521

							Consequent					
		M(miR_218_000521)					Y(FVCRapp)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	a	-0.016	0.004	-0.024	-0.009	<0.001	c'	0.042	0.034	-0.025	0.109	0.218
M(miR_218_000521)		-	-	-	-	-	b	-0.533	0.316	-1.153	0.088	0.092
costant	i_1	3.583	1.051	1.520	5.646	<0.001	i_2	99.416	9.347	81.069	117.764	<0.001
		R^2 =0.033						R^2 =0.129				
		F(7,780)=3.773, p<0.001						F(8,779)=14.386, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	c'	0.042	0.034	-0.025	0.109	0.218
Indirect Effect of Pm10 on FVCRapp		0.009	0.006	-	-	0.126*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.009	0.006	-0.003	0.021	-

Table 17: Simple Mediation Analysis Results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test.

mir_375_000564

							Consequent					
		M(miR_375_000564)					Y(FVCRapp)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
								R^2 = 0.126				
								F(7,780)=15.999, p<0.001				
X(Pm $_{10}$)	a	-0.018	0.005	-0.027	-0.009	<0.001	c'	0.049	0.034	-0.018	0.115	0.153
M(miR_375_000564)		-	-	-	-	-	b	-0.102	0.260	-0.611	0.407	0.694
costant	i_1	4.004	1.282	1.487	6.521	0.002	i_2	97.916	9.352	79.558	116.274	<0.001
		R^2 =0.038						R^2 =0.126				
		F(7,780)=4.378, p<0.001						F(8,779)=14.004, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm10 on FVCRapp	c	0.051	0.034	-0.016	0.117	0.134
Direct Effect of Pm10 on FVCRapp	c'	0.049	0.034	-0.018	0.115	0.153
Indirect Effect of Pm10 on FVCRapp		0.002	0.005	-	-	0.705*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm10 on FVCRapp		0.002	0.005	-0.009	0.013	-

Table 18: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test

mir_652_002352

							Consequent					
		M(miR_652_002352)					**Y(FVCRapp)**					
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.051	0.034	-0.0155	0.117	0.134
costant		-	-	-	-	-	i_3	97.508	9.289	79.274	115.742	<0.001
										R^2 = 0.126		
										F(7,780)=15.999, p<0.001		
X(Pm $_{10}$)	*a*	-0.021	0.005	-0.032	-0.011	<0.001	*c'*	0.049	0.034	-0.018	0.115	0.152
M(miR_652_002352)		-	-	-	-	-	*b*	-0.084	0.225	-0.525	0.357	0.709
costant	i_1	8.632	1.480	5.726	11.538	<0.001	i_2	98.232	9.494	79.594	116.869	<0.001
				R^2 =0.037						**R^2 =0.126**		
				F(7,780)=4.290, p<0.001						**F(8,779)=14.001, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm10 on FVCRapp	*c*	0.051	0.034	-0.0155	0.117	0.134
Direct Effect of Pm10 on FVCRapp	*c'*	0.049	0.034	-0.018	0.115	0.152
Indirect Effect of Pm10 on FVCRapp		0.002	0.005	-	-	0.719*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm10 on FVCRapp		0.002	0.005	-0.008	0.012	-

Table 19: Simple Mediation Analysis Results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with FVCRapp as independent variable and miRNAs expression as dependent variable it was obtained the change in FVCRapp: β log2(101/100) associated with 1%change in miRNAs expression.
FVCRapp = (Measured FVC / Theoretical FVC)*100
*Sobel test

Heart Rate:

mir_25_000403

							Consequent					
		M(miR_25_000403)					**Y(Heart Rate)**					
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
										R^2 =0.061		
										F(6,848)=9.242, p<0.001		
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.019	-0.006	<0.001	*c'*	0.035	0.022	-0.007	0.077	0.105
M(miR_25_000403)		-	-	-	-	-	*b*	-0.052	0.231	-0.506	0.402	0.823
costant	i_1	9.809	0.496	8.836	10.783	<0.001	i_2	51.705	4.038	43.780	59.630	<0.001
				R^2 =0.034						**R^2 =0.061**		
				F(6,848)=4.938, p<0.001						**F(7,847)=7.920, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on Heart Rate	*c*	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm $_{10}$ on Heart Rate	*c'*	0.035	0.022	-0.007	0.077	0.105
Indirect Effect of Pm $_{10}$ on Heart Rate		0.001	0.003	-	-	0.829*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on Heart Rate		0.001	0.003	-0.004	0.006	-

Table 20: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_27_000409

							Consequent					
			M(miR_27b_000409)						Y(Heart Rate)			
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm $_{10}$)		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2 =0.061			
									F(6,848)=9.242, p<0.001			
X(Pm $_{10}$)	a	-0.016	0.004	-0.024	-0.008	<0.001	c'	0.035	0.022	-0.008	0.077	0.110
M(miR_27b_000409)		-	-	-	-	-	b	-0.070	0.186	-0.436	0.295	0.7065
costant	i_1	8.550	0.616	7.340	9.759	<0.001	i_2	51.797	3.700	44.535	59.059	<0.001
			R^2 =0.054						R^2 =0.062			
			F(6,848)=7.989, p<0.001						F(7,847)=7.934, p<0.001			

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Heart Rate	c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm $_{10}$ on Heart Rate	c'	0.035	0.022	-0.008	0.077	0.110
Indirect Effect of Pm $_{10}$ on Heart Rate		0.001	0.003	-	-	0.716*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Heart Rate		0.001	0.003	-0.005	0.007	-

Table 21: Simple Mediation Analysis Results for mediator mir_27_000409 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_30d_000420

							Consequent					
			M(miR_30d_000420)						Y(Heart Rate)			
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm $_{10}$)		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2 =0.061			
									F(6,848)=9.242, p<0.001			
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.010	<0.001	c'	0.034	0.022	-0.008	0.076	0.116
M(miR_30d_000420)		-	-	-	-	-	b	-0.094	0.204	-0.495	0.307	0.646
costant	i_1	9.084	0.562	7.981	10.187	<0.001	i_2	52.051	3.820	44.553	59.549	<0.001
			R^2 =0.039						R^2 =0.062			
			F(6,848)=5.664, p<0.001						F(7,847)=7.944, p<0.001			

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Heart Rate	c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm $_{10}$ on Heart Rate	c'	0.034	0.022	-0.008	0.076	0.116
Indirect Effect of Pm $_{10}$ on Heart Rate		0.002	0.004	-	-	0.654*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Heart Rate		0.002	0.004	-0.005	0.009	-

Table 22: Simple Mediation Analysis Results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_92a_000431

		Consequent									
		M(miR_92a_000431)					Y(Heart Rate)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(Pm_{10})$		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
								R^2=0.061				
								F(6,848)=9.242, p<0.001				
$X(Pm_{10})$	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	0.035	0.022	-0.007	0.077	0.106
M(miR_92a_000431)		-	-	-	-	-	b	-0.060	0.254	-0.559	0.439	0.813
costant	i_1	13.438	0.451	12.552	14.323	<0.001	i_2	52.010	4.778	42.629	61.385	<0.001
		R^2=0.038						R^2=0.061				
		F(6,848)=5.608, p=0.0003						F(7,847)=7.921, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm_{10} on Heart Rate	c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm_{10} on Heart Rate	c'	0.035	0.022	-0.007	0.077	0.106
Indirect Effect of Pm_{10} on Heart Rate		0.001	0.003	-	-	0.818*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on Heart Rate		0.001	0.003	-0.005	0.007	-

Table 23: Simple Mediation Analysis Results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_106a_002169

		Consequent									
		M(miR_106a_002169)					Y(Heart Rate)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(Pm_{10})$		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
								R^2=0.061				
								F(6,848)=9.242, p<0.001				
$X(Pm_{10})$	a	-0.013	0.003	-0.018	-0.008	<0.001	c'	0.037	0.022	-0.006	0.080	0.088
M(miR_106a_002169)		-	-	-	-	-	b	0.107	0.294	-0.470	0.684	0.716
costant	i_1	13.115	0.390	12.349	13.880	<0.001	i_2	49.791	5.103	39.775	59.807	<0.001
		R^2=0.047						R^2=0.062				
		F(6,848)=7.039, p<0.001						F(7,847)=7.932, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm_{10} on Heart Rate	c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm_{10} on Heart Rate	c'	0.037	0.022	-0.006	0.080	0.088
Indirect Effect of Pm_{10} on Heart Rate		-0.001	0.004	-	-	0.721*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on Heart Rate		-0.001	0.004	-0.009	0.005	-

Table 24: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_152_000475

		Consequent									
		M(miR_152_000475)					Y(Heart Rate)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2 =0.061			
									F(6,848)=9.242, p<0.001			
X(Pm_{10})	a	-0.013	0.003	-0.019	-0.006	<0.001	c'	0.033	0.022	-0.009	0.075	0.124
M(miR_152_000475)		-	-	-	-	-	b	-0.195	0.224	-0.635	0.245	0.386
costant	i_1	7.031	0.512	6.027	8.035	<0.001	i_2	52.565	3.692	45.318	59.812	<0.001
		R^2 =0.036						R^2 =0.062				
		F(6,848)=5.239, p<0.001						F(7,847)=8.027, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on Heart Rate c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm_{10} on Heart Rate c'	0.033	0.0215	-0.009	0.0753	0.124
Indirect Effect of Pm_{10} on Heart Rate	0.003	0.003	-	-	0.412*
	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on Heart Rate	0.003	0.003	-0.002	0.008	-

Table 25: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_181a_2_002317

		Consequent									
		M(miR_181a_2_002317)					Y(Heart Rate)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm_{10})		-	-	-	-	-	c	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2 =0.061			
									F(6,848)=9.242, p<0.001			
X(Pm_{10})	a	-0.017	0.004	1.206	3.606	<0.001	c'	0.034	0.022	-0.009	0.076	0.117
M(miR_181a_2_002317)		-	-	-	-	-	b	-0.101	0.188	-0.469	0.268	0.591
costant	i_1	2.406	0.611	1.206	3.606	<0.001	i_2	51.440	3.370	44.825	58.055	<0.001
		R^2 =0.028						R^2 =0.062				
		F(6,848)=3.586, p<0.001						F(6,848)=7.956 p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on Heart Rate c	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm_{10} on Heart Rate c'	0.034	0.022	-0.009	0.076	0.117
Indirect Effect of Pm_{10} on Heart Rate	0.002	0.003	-	-	0.603*
	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm_{10} on Heart Rate	0.002	0.003	-0.004	0.008	-

Table 26: Simple Mediation Analysis Results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_375_000564

							Consequent					
			M(miR_375_000564)						**Y(Heart Rate)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2=0.061			
									F(6,848)=9.242, p<0.001			
X(Pm $_{10}$)	*a*	-0.018	0.004	-0.027	-0.009	<0.001	*c'*	*0.033*	*0.022*	*-0.009*	*0.075*	0.126
M(miR_375_000564)		-	-	-	-	-	*b*	*-0.144*	*0.167*	*-0.472*	*0.184*	*0.389*
costant	i_1	3.7337	0.686	2.387	5.080	<0.001	i_2	*51.735*	*3.397*	*45.068*	*58.402*	<0.001
			R^2=0.0356						**R^2=0.0622**			
			F(6,848)=4.5185, p<0.001						**F(7,847)=8.025, p<0.001**			

	Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on Heart Rate *c*	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm $_{10}$ on Heart Rate *c'*	0.033	0.022	-0.009	0.075	0.126
Indirect Effect of Pm $_{10}$ on Heart Rate	0.003	0.003	-	-	0.412
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on Heart Rate	0.003	0.003	-0.002	0.009	-

Table 27: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_652_002352

							Consequent					
			M(miR_652_002352)						**Y(Heart Rate)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	0.036	0.021	-0.0062	0.077	0.095
costant		-	-	-	-	-	i_3	51.197	3.339	44.645	57.750	<0.001
									R^2=0.061			
									F(6,848)=9.242, p<0.001			
X(Pm $_{10}$)	*a*	-0.023	0.005	-0.033	-0.013	<0.001	*c'*	0.032	0.022	-0.011	0.074	0.142
M(miR_652_002352)		-	-	-	-	-	*b*	-0.173	0.144	-0.455	0.109	0.228
costant	i_1	8.094	0.799	6.527	9.661	<0.001	i_2	52.600	3.534	45.663	59.536	<0.001
			R^2=0.040						**R^2=0.0630**			
			F(6,848)=5.856, p<0.001						**F(7,847)=8.1345, p<0.001**			

	Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on Heart Rate *c*	0.036	0.021	-0.006	0.077	0.095
Direct Effect of Pm $_{10}$ on Heart Rate *c'*	0.032	0.022	-0.011	0.074	0.142
Indirect Effect of Pm $_{10}$ on Heart Rate	0.004	0.003	-	-	0.255
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on Heart Rate	0.004	0.003	-0.002	0.074	-

Table 28: Simple Mediation Analysis Results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% CI were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Heart Rate as independent variable and miRNAs expression as dependent variable it was obtained the change in Heart Rate : β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

DBP: Diastolic Blood Pressure

mir_25_000403

							Consequent					
			M(miR_25_000403)					**Y(DBP)**				
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
									R^2 =0.059			
									F(6,851)=8.960 p<0.001			
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.018	-0.006	<0.001	*c'*	-0.032	0.020	-0.071	0.007	0.106
M(miR_25_000403)		-	-	-	-	-	*b*	0.034	0.214	-0.386	0.453	0.875
costant	i_1	9.796	0.494	8.826	10.765	<0.001	i_2	72.370	3.724	65.061	79.680	<0.001
			R^2 =0.033						**R^2 =0.056**			
			F(6,851)=4.775, p<0.001						**F(7,850)=7.675, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.032	0.020	-0.071	0.007	0.106
Indirect Effect of Pm $_{10}$ on DBP		-0.0004	0.0027	-	-	0.879*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		-0.0004	0.003	-0.005	0.005	-

Table 29: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_27b_000409

							Consequent					
			M(miR_27b_000409)					**Y(DBP)**				
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
									R^2 =0.059			
									F(6,851)=8.960 p<0.001			
X(Pm $_{10}$)	*a*	-0.016	0.004	-0.023	-0.008	<0.001	*c'*	-0.036	0.020	-0.075	0.003	0.074
M(miR_27b_000409)		-	-	-	-	-	*b*	-0.192	0.172	-0.530	0.146	0.2647
costant	i_1	8.546	0.613	7.343	9.748	<0.001	i_2	74.342	3.411	67.647	81.038	<0.001
			R^2 =0.053						**R^2 =0.061**			
			F(6,851)=3.632, p<0.001						**F(7,850)=7.860, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.036	0.020	-0.075	0.003	0.074
Indirect Effect of Pm $_{10}$ on DBP		0.003	0.003	-	-	0.296*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		0.003	0.003	-0.002	0.010	-

Table 30: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_30d_000420

		Consequent									
		M(miR_30d_000420)					Y(DBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm $_{10}$)		-	-	-	-	-	c	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
								R^2 =0.059				
								F(6,851)=8.960 p<0.001				
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.010	<0.001	c'	-0.031	0.020	-0.070	0.009	0.125
M(miR_30d_000420)		-	-	-	-	-	b	0.113	0.189	-0.257	0.483	0.549
costant	i_1	9.072	0.560	7.974	10.170	<0.001	i_2	71.674	3.553	64.760	78.589	<0.001
		R^2 =0.039						R^2 =0.060				
		F(6,851)=5.708, p<0.001						F(7,850)=7.725, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on DBP	c	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm $_{10}$ on DBP	c'	-0.031	0.020	-0.070	0.009	0.125
Indirect Effect of Pm $_{10}$ on DBP		-0.002	0.003	-	-	0.561*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on DBP	-0.002	0.003	-0.008	0.004	-

Table 31: Simple Mediation Analysis Results for mediator mir_30d_000420on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_92a_000431

		Consequent									
		M(miR_92a_000431)					Y(DBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(Pm $_{10}$)		-	-	-	-	-	c	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
								R^2 =0.059				
								F(6,851)=8.960 p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.018	-0.006	<0.001	c'	-0.032	0.020	-0.071	0.008	0.114
M(miR_92a_000431)		-	-	-	-	-	b	0.090	0.235	-0.371	0.552	0.701
costant	i_1	13.433	0.449	12.552	14.315	<0.001	i_2	71.486	4.410	62.829	80.143	<0.001
		R^2 =0.037						R^2 =0.060				
		F(6,851)=5.454, p<0.001						F(7,850)=7.693, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on DBP	c	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm $_{10}$ on DBP	c'	-0.032	0.020	-0.071	0.008	0.114
Indirect Effect of Pm $_{10}$ on DBP		-0.001	0.003	-	-	0.710*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on DBP	-0.001	0.003	-0.007	0.005	-

Table 32: Simple Mediation Analysis Results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_106a_002169

							Consequent					
			M(miR_106a_002169)						**Y(DBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
										R^2 =0.059		
										F(6,851)=8.960 p<0.001		
X(Pm $_{10}$)	*a*	-0.018	0.004	-0.026	-0.009	<0.001	*c'*	-0.013	0.031	-0.073	0.047	0.666
M(miR_106a_002169)		-	-	-	-	-	*b*	-0.045	0.236	-0.509	0.419	0.850
costant	i_1	3.789	0.685	2.445	5.134	<0.001	i_2	99.527	4.807	90.092	108.961	<0.001
			R^2 =0.036							**R^2 =0.164**		
			F(6,851)=4.541, p<0.001							**F(7,850)=23.773, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.012	0.030	-0.072	0.047	0.682
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.013	0.031	-0.073	0.047	0.666
Indirect Effect of Pm $_{10}$ on DBP		0.001	0.003	-	-	0.881*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		0.001	0.003	-0.006	0.008	-

Table 33: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_152_000475

							Consequent					
			M(miR_152_000475)						**Y(DBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
										R^2 =0.059		
										F(6,851)=8.960 p<0.001		
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.019	-0.006	<0.001	*c'*	-0.033	0.020	-0.072	0.006	0.097
M(miR_152_000475)		-	-	-	-	-	*b*	-0.037	0.208	-0.445	0.370	0.857
costant	i_1	7.046	0.509	6.047	8.044	<0.001	i_2	72.963	3.409	66.271	79.654	<0.001
			R^2 =0.036							**R^2 =0.060**		
			F(6,851)=5.240, p<0.001							**F(7,850)=7.676, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.033	0.020	-0.072	0.006	0.097
Indirect Effect of Pm $_{10}$ on DBP		0.001	0.003	-	-	0.862*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		0.001	0.003	-0.004	0.007	-

Table 34: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_181a_2_002317

							Consequent					
			M(miR_181a_2_002317)						**Y(DBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
									R^2 =0.059			
									F(6,851)=8.960 p<0.001			
X(Pm $_{10}$)	*a*	-0.017	0.004	-0.024	-0.009	<0.001	*c'*	-0.035	0.020	-0.074	0.004	0.078
M(miR_181a_2_002317)		-	-	-	-	-	*b*	-0.150	0.173	-0.490	0.190	0.387
costant	i_1	2.360	0.609	1.166	3.555	<0.001	i_2	73.053	3.106	66.958	79.149	<0.001
			R^2 =0.027						**R^2 =0.060**			
			F(6,851)=3.858, p<0.001						**F(7,850)=7.785, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.033	0.0197	-0.071	0.0061	0.098
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.035	0.0199	-0.074	0.004	0.078
Indirect Effect of Pm $_{10}$ on DBP		0.003	0.003	-	-	0.408*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		0.003	0.003	-0.003	0.010	-

Table 35: Simple Mediation Analysis Results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_218_000521

							Consequent					
			M(miR_218_000521)						**Y(DBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(Pm $_{10}$)		-	-	-	-	-	*c*	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
									R^2 =0.059			
									F(6,851)=8.960 p<0.001			
X(Pm $_{10}$)	*a*	-0.016	0.004	-0.023	-0.009	<0.001	*c'*	-0.029	0.020	-0.068	0.010	0.142
M(miR_218_000521)		-	-	-	-	-	*b*	0.201	0.190	-0.172	0.573	0.290
costant	i_1	4.643	0.556	3.551	5.734	<0.001	i_2	71.768	3.201	65.484	78.051	<0.001
			R^2 =0.032						**R^2 =0.061**			
			F(6,851)=4.718, p<0.001						**F(7,850)=7.841, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on DBP	*c*	-0.033	0.0197	-0.071	0.0061	0.0984
Direct Effect of Pm $_{10}$ on DBP	*c'*	-0.029	0.0199	-0.068	0.01	0.142
Indirect Effect of Pm $_{10}$ on DBP		-0.003	0.0034	-	-	0.313*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on DBP		-0.003	0.003	-0.010	0.004	-

Table 36: Simple Mediation Analysis Results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_375_000564

		Consequent									
		M(miR_375_000564)					Y(DBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(Pm_{10})$		-	-	-	-	-	c	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
								$R^2=0.059$				
								$F(6,851)=8.960$ p<0.001				
$X(Pm_{10})$	a	-0.018	0.004	-0.026	-0.009	<0.001	c'	*-0.033*	*0.020*	*-0.072*	*0.006*	0.102
M(miR_375_000564)		-	-	-	-	-	b	*-0.001*	*0.154*	*-0.303*	*0.302*	*0.998*
costant	i_1	3.789	0.685	2.445	5.134	<0.001	i_2	*72.701*	*3.135*	*66.548*	*78.854*	<0.001
		$R^2=0.036$						$R^2=0.059$				
		$F(6,851)=5.322$, p<0.001						$F(7,850)=7.678$, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on DBP c	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm_{10} on DBP c'	-0.033	0.020	-0.072	0.006	0.102
Indirect Effect of Pm_{10} on DBP	0.001	0.003	-	-	0.998*
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm_{10} on DBP	0.001	0.003	-0.005	0.006	-

Table 37: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : $(2^{\beta}-1)*100$ for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: $\beta \log2(101/100)$ associated with 1%change in miRNAs expression.
*Sobel test

mir_652_002352

		Consequent									
		M(miR_652_002352)					Y(DBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
$X(Pm_{10})$		-	-	-	-	-	c	-0.033	0.020	-0.0711	0.006	0.098
costant		-	-	-	-	-	i_3	72.699	3.078	66.658	78.741	<0.001
								$R^2=0.059$				
								$F(6,851)=8.960$ p<0.001				
$X(Pm_{10})$	a	-0.022	0.005	-0.032	-0.012	<0.001	c'	-0.035	0.020	-0.074	0.004	0.078
M(miR_652_002352)		-	-	-	-	-	b	-0.117	0.133	-0.378	0.143	0.377
costant	i_1	8.074	0.794	6.515	9.633	<0.001	i_2	73.647	3.260	67.249	80.045	<0.001
		$R^2=0.039$						$R^2=0.060$				
		$F(6,851)=5.824$, p<0.001						$F(7,850)=7.789$, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm_{10} on DBP c	-0.033	0.020	-0.071	0.006	0.098
Direct Effect of Pm_{10} on DBP c'	-0.035	0.020	-0.074	0.004	0.078
Indirect Effect of Pm_{10} on DBP	0.003	0.003	-	-	0.398*
	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm_{10} on DBP	0.003	0.003	-0.003	0.010	-

Table 38: Simple Mediation Analysis Results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : $(2^{\beta}-1)*100$ for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with DBP as independent variable and miRNAs expression as dependent variable it was obtained the change in DBP: $\beta \log2(101/100)$ associated with 1%change in miRNAs expression.
*Sobel test

SBP: Systolic Blood Pressure

mir_25_000403

							Consequent					
			M(miR_25_000403)						**Y(SBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
										R^2 **=0.164**		
										F(6,851)=27.761, p<0.001		
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.018	-0.006	<0.001	*c'*	-0.015	0.030	-0.075	0.045	0.621
M(miR_25_000403)		-	-	-	-	-	*b*	-0.222	0.328	-0.865	0.421	0.498
costant	i_1	9.796	0.494	8.826	10.765	<0.001	i_2	101.531	5.709	90.326	112.737	<0.001
			R^2 **=0.033**						R^2 **=0.164**			
			F(6,851)=4.775, p<0.001						**F(7,850)=23.84, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on SBP	*c*	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm $_{10}$ on SBP	*c'*	-0.015	0.030	-0.075	0.045	0.6213
Indirect Effect of Pm $_{10}$ on SBP		0.003	0.003	-	-	0.519*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on SBP		0.003	0.003	-0.003	0.009	_

Table 39: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_27b_000409

							Consequent					
			M(miR_27b_000409)						**Y(SBP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
										R^2 **=0.164**		
										F(6,851)=27.761, p<0.001		
X(Pm $_{10}$)	*a*	-0.016	0.004	-0.023	-0.008	<0.001	*c'*	-0.015	0.031	-0.074	0.045	0.635
M(miR_27b_000409)		-	-	-	-	-	*b*	-0.134	0.264	-0.652	0.385	0.6128
costant	i_1	8.546	0.613	7.343	9.748	<0.001	i_2	100.500	5.234	90.227	110.773	<0.001
			R^2 **=0.053**						R^2 **=0.164**			
			F(6,851)=7.969, p<0.001						**F(7,850)=23.811, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on SBP	*c*	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm $_{10}$ on SBP	*c'*	-0.015	0.031	-0.074	0.045	0.6349
Indirect Effect of Pm $_{10}$ on SBP		0.002	0.004	-	-	0.626*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on SBP		0.002	0.004	-0.005	0.010	-

Table 40: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_30d_000420

							Consequent					
		M(miR_30d_000420)					Y(SBP)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
									R^2=0.164			
									F(6,851)=27.761, p<0.001			
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.010	<0.001	c'	-0.017	0.031	-0.077	0.043	0.585
M(miR_30d_000420)		-	-	-	-	-	b	-0.255	0.289	-0.823	0.313	0.378
costant	i_1	9.072	0.560	7.974	10.170	<0.001	i_2	101.671	5.401	91.071	112.271	<0.001
		R^2=0.039						R^2=0.165				
		F(6,851)=5.783, p<0.001						F(7,850)=23.899, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm $_{10}$ on SBP	c'	-0.017	0.031	-0.077	0.043	0.5852
Indirect Effect of Pm $_{10}$ on SBP		0.004	0.004	-	-	0.396*
	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP	0.004	0.004	-0.003	0.014	-

Table 41: Simple Mediation Analysis Results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_106a_002169

							Consequent					
		M(miR_106a_002169)					Y(SBP)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
									R^2=0.164			
									F(6,851)=27.761, p<0.001			
X(Pm $_{10}$)	a	-0.013	0.003	-0.018	-0.008	<0.001	c'	-0.019	0.031	-0.079	0.041	0.527
M(miR_106a_002169)		-	-	-	-	-	b	-0.547	0.417	-1.365	0.271	0.190
costant	i_1	13.100	0.388	12.338	13.861	<0.001	i_2	106.521	7.216	92.357	120.685	<0.001
		R^2=0.047						R^2=0.165				
		F(6,851)=6.951,p<0.001						F(7,850)=24.061, p<0.001				

	Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm $_{10}$ on SBP	c'	-0.019	0.031	-0.079	0.041	0.5274
Indirect Effect of Pm $_{10}$ on SBP		0.007	0.005	-	-	0.212*
	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP	0.007	0.005	-0.001	0.018	-

Table 42: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_152_000475

		Consequent									
		M(miR_152_000475)					Y(SBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
								R^2=0.164				
								F(6,851)=27.761, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.019	-0.006	<0.001	c'	-0.013	0.030	-0.072	0.047	0.681
M(miR_152_000475)		-	-	-	-	-	b	-0.011	0.318	-0.636	0.613	0.972
costant	i_1	7.046	0.509	6.047	8.044	<0.001	i_2	99.437	5.228	89.175	109.698	<0.001
		R^2=0.036						R^2=0.164				
		F(6,851)=5.240, p<0.001						F(7,850)=23.767, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.030	-0.072	0.047	0.6817
Direct Effect of Pm $_{10}$ on SBP	c'	-0.013	0.030	-0.072	0.047	0.681
Indirect Effect of Pm $_{10}$ on SBP		0.001	0.003	-	-	0.973*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP		0.001	0.003	-0.007	0.007	-

Table 43: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_181a_2_002317

		Consequent									
		M(miR_181a_2_002317)					Y(SBP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
								R^2=0.164				
								F(6,851)=27.761, p<0.001				
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.009	<0.001	c'	-0.019	0.031	-0.079	0.041	0.533
M(miR_181a_2_002317)		_	_	_	_	_	b	-0.394	0.266	-0.916	0.127	0.138
costant	i_1	2.360	0.609	1.166	3.555	<0.001	i_2	100.287	4.758	90.948	109.626	<0.001
		R^2=0.027						R^2=0.166				
		F(6,851)=3.858, p<0.001						F(7,850)= 24.143, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.030	-0.072	0.047	0.682
Direct Effect of Pm $_{10}$ on SBP	c'	-0.019	0.031	-0.079	0.041	0.533
Indirect Effect of Pm $_{10}$ on SBP		0.007	0.004	-	-	0.170*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP		0.007	0.004	-0.001	0.015	-

Table 44: Simple Mediation Analysis Results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_218_000521

		Consequent										
		M(miR_218_000521)					Y(SBP)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
								R^2=0.164				
								F(6,851)=27.761, p<0.001				
X(Pm $_{10}$)	a	-0.016	0.004	-0.023	-0.009	<0.001	c'	-0.008	0.031	-0.068	0.052	0.797
M(miR_218_000521)		-	-	-	-	-	b	0.277	0.291	-0.294	0.848	0.341
costant	i_1	4.643	0.556	3.551	5.734	<0.001	i_2	98.071	4.909	88.435	107.707	<0.001
		R^2=0.032						R^2=0.165				
		F(6,851)=4.718, p<0.001						F(7,850)=23.922, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.0302	-0.072	0.0468	0.6817
Direct Effect of Pm $_{10}$ on SBP	c'	-0.008	0.0305	-0.068	0.0521	0.797
Indirect Effect of Pm $_{10}$ on SBP		-0.005	0.0042	-	-	0.362*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP		-0.005	0.004	-0.014	0.003	-

Table 45: Simple Mediation Analysis Results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_375_000564

		Consequent										
		M(miR_375_000564)					Y(SBP)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
								R^2=0.164				
								F(6,851)=27.761, p<0.001				
X(Pm $_{10}$)	a	-0.018	0.004	-0.026	-0.009	<0.001	c'	*-0.013*	*0.031*	*-0.073*	*0.047*	0.666
M(miR_375_000564)		-	-	-	-	-	b	*-0.045*	*0.236*	*-0.509*	*0.419*	*0.850*
costant	i_1	3.789	0.685	2.445	5.134	<0.001	i_2	*99.527*	*4.807*	*90.092*	*108.961*	<0.001
		R^2=0.036						R^2=0.164				
		F(6,851)=5.322, p<0.001						F(7,850)=23.773, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on SBP	c	-0.012	0.030	-0.072	0.047	0.682
Direct Effect of Pm $_{10}$ on SBP	c'	-0.013	0.031	-0.073	0.047	0.666
Indirect Effect of Pm $_{10}$ on SBP		0.001	0.004	-	-	0.854*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on SBP		0.001	0.004	-0.006	0.008	-

Table 46: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_652_002352

							Consequent					
			M(miR_652_002352)						***Y(SBP)***			
Antecedent		***Coeff.***	***SE***	***95% CI***		***p***		***Coeff.***	***SE***	***95% CI***		***p***
X(PM $_{10}$)		-	-	-	-	-	*c*	-0.012	0.030	-0.0716	0.047	0.682
costant		-	-	-	-	-	i_3	99.357	4.720	90.093	108.621	<0.001
									R^2=0.164			
									F(6,851)=27.761, p<0.001			
X(Pm $_{10}$)	*a*	-0.022	0.005	-0.032	-0.012	<0.001	*c'*	-0.011	0.031	-0.071	0.049	0.711
M(miR_652_002352)		-	-	-	-	-	*b*	0.047	0.204	-0.353	0.447	0.817
costant	i_1	8.074	0.794	6.515	9.633	<0.001	i_2	98.975	5.001	89.160	108.791	<0.001
			R^2=0.039						***R^2=0.164***			
			F(6,851)=5.824, p<0.001						***F(7,850)=23.776, p<0.001***			

		Effect	***SE***	***95% CI***		***p***
Total effect of Pm $_{10}$ on SBP	*c*	-0.012	0.030	-0.072	0.047	0.682
Direct Effect of Pm $_{10}$ on SBP	*c'*	-0.011	0.031	-0.071	0.049	0.711
Indirect Effect of Pm $_{10}$ on SBP		-0.001	0.005	-	-	0.821
		Effect	***Boot SE***	***Boot 95% CI***		***p***
Indirect Effect of Pm $_{10}$ on SBP		-0.001	0.005	-0.011	0.007	-

Table 47: : Simple Mediation Analysis Results for mediator mir_652_002352on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with SBP as independent variable and miRNAs expression as dependent variable it was obtained the change in SBP: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

CRP:

mir_25_000403

							Consequent					
			M(miR_25_000403)						***Y(CRP)***			
Antecedent		***Coeff.***	***SE***	***95% CI***		***p***		***Coeff.***	***SE***	***95% CI***		***p***
X(PM $_{10}$)		-	-	-	-	-	*c*	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
									R^2 = 0.156			
									F(6,840)=25.951, p<0.001			
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.018	-0.005	<0.001	*c'*	0.002	0.002	-0.002	0.006	0.436
M(miR_25_000403)		-	-	-	-	-	*b*	-0.026	0.022	-0.069	0.017	0.231
costant	i_1	9.850	0.501	8.867	10.833	<0.001	i_2	-4.087	0.383	-4.839	-3.335	<0.001
			R^2=0.032						***R^2=0.158***			
			F(6,840)=4.619, p<0.001						***F(7,839)=22.459, p<0.001***			

		Effect	***SE***	***95% CI***		***p***
Total effect of Pm $_{10}$ on CRP	*c*	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	*c'*	0.002	0.002	-0.002	0.006	0.436
Indirect Effect of Pm $_{10}$ on CRP		0.0003	0.0002	-	-	0.272*
		Effect	***Boot SE***	***Boot 95% CI***		***p***
Indirect Effect of Pm $_{10}$ on CRP		0.0003	0.0002	-0.0001	0.0008	-

Table 48: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test.

mir_27b_000409

		Consequent									
		M(miR_27b_000409)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	a	-0.014	0.004	-0.022	-0.006	<0.001	c'	0.002	0.002	-0.003	0.006	0.476
M(miR_27b_000409)		-	-	-	-	-	b	-0.031	0.018	-0.066	0.004	0.0813
costant	i_1	8.696	0.617	7.486	9.906	<0.001	i_2	-4.076	0.352	-4.767	-3.384	<0.001
		R^2 =0.052						R^2 =0.159				
		F(6,840)=7.727, p<0.001						F(7,839)=22.733, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on CRP	c	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	c'	0.002	0.002	-0.003	0.006	0.476
Indirect Effect of Pm $_{10}$ on CRP		0.0004	0.0003	-	-	0.128*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on CRP	0.0004	0.0003	0.0000	0.0010	-

Table 49: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_30d_000420

		Consequent									
		M(miR_30d_000420)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	a	-0.015	0.004	-0.022	-0.008	<0.001	c'	0.001	0.002	-0.003	0.006	0.481
M(miR_30d_000420)		_	_	_	_	_	b	-0.030	0.020	-0.068	0.008	0.126
costant	i_1	9.256	0.559	8.159	10.353	<0.001	i_2	-4.067	0.365	-4.783	-3.351	<0.001
		R^2 =0.036						R^2 =0.159				
		F(6,840)=5.205, p<0.001						F(7,839)=22.615, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on CRP	c	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	c'	0.001	0.002	-0.003	0.006	0.481
Indirect Effect of Pm $_{10}$ on CRP		0.0004	0.0003	-	-	0.160*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on CRP	0.0004	0.0003	0.0000	0.0011	-

Table 50: Simple Mediation Analysis Results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_92a_000431

		Consequent									
		M(miR_92a_000431)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI	p
X(PM $_{10}$)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.017	-0.006	<0.001	c'	0.001	0.002	-0.003	0.006	0.482
M(miR_92a_000431)		-	-	-	-	-	b	-0.040	0.024	-0.087	0.008	0.099
costant	i_1	13.424	0.455	12.530	14.317	<0.001	i_2	-3.812	0.452	-4.699	-2.925	<0.001
		R^2 =0.036						R^2 =0.159				
		F(6,840)=5.194, p<0.001						F(7,839)=22.679, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm $_{10}$ on CRP	c	0.002	0.002	-0.002	0.006	0.3512						
Direct Effect of Pm $_{10}$ on CRP	c'	0.001	0.002	-0.003	0.006	0.4824						
Indirect Effect of Pm $_{10}$ on CRP		0.001	0.0003	-	-	0.138*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm $_{10}$ on CRP		0.001	0.0003	0.000	0.001	-						

Table 51: Simple Mediation Analysis Results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_152_000475

		Consequent									
		M(miR_152_000475)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI	p
X(PM $_{10}$)		-	-	-	-	-	c	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.019	-0.006	<0.001	c'	0.002	0.002	-0.002	0.006	0.439
M(miR_152_000475)		_	_	_	_	_	b	-0.026	0.021	-0.067	0.016	0.224
costant	i_1	7.053	0.516	6.040	8.066	<0.001	i_2	-4.163	0.351	-4.851	-3.475	<0.001
		R^2 =0.035						R^2 =0.158				
		F(6,840)=5.001, p<0.001						F(7,839)=22.467, p<0.001				
		Effect	SE	95% CI		p						
Total effect of Pm $_{10}$ on CRP	c	0.002	0.002	-0.002	0.006	0.351						
Direct Effect of Pm $_{10}$ on CRP	c'	0.002	0.002	-0.002	0.006	0.439						
Indirect Effect of Pm $_{10}$ on CRP		0.0003	0.0002	-	-	0.264*						
		Effect	Boot SE	Boot 95% CI		p						
Indirect Effect of Pm $_{10}$ on CRP		0.0003	0.0002	-0.0001	0.0008	-						

Table 52: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_181a_2_002317

							Consequent					
			M(miR_181a_2_002317)						**Y(CRP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
										R^2 = 0.156		
										F(6,840)=25.951, p<0.001		
X(Pm $_{10}$)	*a*	-0.016	0.004	-0.024	-0.008	<0.001	*c'*	0.002	0.002	-0.002	0.006	0.429
M(miR_181a_2_002317)		-	-	-	-	-	*b*	-0.017	0.018	-0.052	0.018	0.340
costant	i_1	2.453	0.617	1.241	3.665	<0.001	i_2	-4.303	0.320	-4.931	-3.675	<0.001
				R^2 =0.025						**R^2 =0.157**		
				F(6,840)=5.001, p<0.001						**F(7,839)=22.371, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on CRP	*c*	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	*c'*	0.002	0.002	-0.002	0.006	0.429
Indirect Effect of Pm $_{10}$ on CRP		0.0003	0.0002	-	-	0.366*

	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on CRP	0.0003	0.0002	-0.0001	0.0007	-

Table 53: Simple Mediation Analysis Results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_218_000521

							Consequent					
			M(miR_218_000521)						**Y(CRP)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
										R^2 = 0.156		
										F(6,840)=25.951, p<0.001		
X(Pm $_{10}$)	*a*	-0.016	0.004	-0.023	-0.009	<0.001	*c'*	0.002	0.002	-0.002	0.006	0.279
M(miR_218_000521)		-	-	-	-	-	*b*	0.020	0.019	-0.018	0.058	0.293
costant	i_1	4.682	0.564	3.574	5.790	<0.001	i_2	-4.440	0.330	-5.087	-3.793	<0.001
				R^2 =0.031						**R^2 =0.158**		
				F(6,840)=4.463, p<0.001						**F(7,839)=22.404, p<0.001**		

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on CRP	*c*	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	*c'*	0.002	0.002	-0.002	0.006	0.279
Indirect Effect of Pm $_{10}$ on CRP		-0.0003	0.0003	-	-	0.317*

	Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on CRP	-0.0003	0.0003	-0.0009	0.0001	-

Table 54: Simple Mediation Analysis Results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

mir_375_000564

		Consequent									
		M(miR_375_000564)					Y(CRP)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	*c*	0.002	0.002	-0.0021	0.006	0.351
costant		-	-	-	-	-	i_3	-4.345	0.317	-4.967	-3.722	<0.001
								R^2 = 0.156				
								F(6,840)=25.951, p<0.001				
X(Pm $_{10}$)	*a*	-0.017	0.004	-0.026	-0.008	<0.001	*c'*	0.002	0.002	-0.002	0.006	0.406
M(miR_375_000564)		-	-	-	-	-	*b*	-0.011	0.016	-0.042	0.020	0.475
costant	i_1	3.829	0.692	2.470	5.187	<0.001	i_2	-4.301	0.323	-4.935	-3.667	<0.001
		R^2 =0.040						R^2 =0.157				
		F(6,840)=5.065, p<0.001						F(7,839)=22.304, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on CRP	*c*	0.002	0.002	-0.002	0.006	0.351
Direct Effect of Pm $_{10}$ on CRP	*c'*	0.002	0.002	-0.002	0.006	0.406
Indirect Effect of Pm $_{10}$ on CRP		0.0002	0.0002	-	-	0.496*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on CRP	0.0002	0.0002	-0.0002	0.0007	-

Table 55: Simple Mediation Analysis Results for mediator mir_375_000564 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression and CRP was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with CRP as independent variable and miRNAs expression as dependent variable it was obtained the percentage change in CRP: 100(1.01^ β -1) associated with 1%change in miRNAs expression.
*Sobel test

Fibrinogen:

mir_25_000403

		Consequent									
		M(miR_25_000403)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	*c*	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	*a*	-0.011	0.003	-0.018	-0.005	<0.001	*c'*	0.204	0.120	-0.031	0.438	0.088
M(miR_25_000403)		-	-	-	-	-	*b*	-1.160	1.281	-3.674	1.355	0.366
costant	i_1	9.871	0.512	8.866	10.876	<0.001	i_2	161.094	22.619	116.696	205.493	<0.001
		R^2 =0.033						R^2 =0.122				
		F(6,818)=4.575, p<0.001						F(6,817)=16.150, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	*c*	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	*c'*	0.204	0.120	-0.031	0.438	0.088
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.013	0.021	-	-	0.399*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen	0.013	0.021	-0.018	0.068	-

Table 56: Simple Mediation Analysis Results for mediator mir_25_000403 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_27b_000409

		Consequent									
		M(miR_27b_000409)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	a	-0.016	0.004	-0.023	-0.008	<0.001		0.204	0.120	-0.031	0.439	0.088
M(miR_27b_000409)		_	_	_	_	_		-0.806	1.038	-2.842	1.231	0.438
costant	i_1	8.703	0.632	7.463	9.944	<0.001		156.663	20.817	115.802	197.523	<0.001
		R^2 =0.058						R^2 = 0.121				
		F(6,818)=8.342, p<0.001						F(7,817)=16.115, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.204	0.120	-0.031	0.439	0.088
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.013	0.023	-	-	0.460*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.013	0.023	-0.027	0.068	-

Table 57: Simple Mediation Analysis Results for mediator mir_27b_000409 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_30d_000420

		Consequent									
		M(miR_30d_000420)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.010	<0.001	c'	0.202	0.120	-0.034	0.438	0.094
M(miR_30d_000420)		_	_	_	_	_	b	-0.910	1.129	-3.125	1.305	0.420
costant	i_1	9.131	0.581	7.991	10.272	<0.001	i_2	157.959	21.401	115.952	199.965	<0.001
		R^2 =0.038						R^2 =0.121				
		F(6,818)=5.445, p<0.001						F(7,817)=16.122, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.202	0.120	-0.034	0.438	0.094
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.015	0.025	-	-	0.438*
		Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.015	0.025	-0.026	0.077	-

Table 58: Simple Mediation Analysis Results for mediator mir_30d_000420 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

mir_92a_000431

							Consequent					
			M(miR_92a_000431)						**Y(Fibrinogen)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
									R^2 = 0.121			
									F(6,818)=18.709, p<0.001			
X(Pm $_{10}$)	*a*	-0.011	0.003	-0.017	-0.005	<0.001	*c'*	0.213	0.120	-0.022	0.448	0.075
M(miR_92a_000431)		_	_	_	_	_	*b*	-0.339	1.409	-3.104	2.426	0.810
costant	i_1	13.512	0.466	12.598	14.426	<0.001	i_2	154.228	26.727	101.766	206.690	<0.001
			R^2 =0.037						**R^2 =0.121**			
			F(6,818)=5.153, p<0.001						**F(7,817)=16.026, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on Fibrinogen	*c*	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	*c'*	0.213	0.120	-0.022	0.448	0.075
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.004	0.020	-	-	0.816*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.004	0.020	-0.032	0.053	-

Table 59: Simple Mediation Analysis Results for mediator mir_92a_000431 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_106a_002169

							Consequent					
			M(miR_106a_002169)						**Y(Fibrinogen)**			
Antecedent		**Coeff.**	**SE**	**95% CI**		**p**		**Coeff.**	**SE**	**95% CI**		**p**
X(PM $_{10}$)		-	-	-	-	-	*c*	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
									R^2 = 0.121			
									F(6,818)=18.709, p<0.001			
X(Pm $_{10}$)	*a*	-0.012	0.003	-0.017	-0.007	<0.001	*c'*	0.197	0.120	-0.039	0.433	0.102
M(miR_106a_002169)		-	-	-	-	-	*b*	-1.627	1.628	-4.823	1.568	0.318
costant	i_1	13.146	0.403	12.356	13.937	<0.001	i_2	171.042	28.454	115.191	226.894	<0.001
			R^2 =0.045						**R^2 =0.122**			
			F(6,818)=6.392, p<0.001						**F(7,817)=16.179, p<0.001**			

		Effect	**SE**	**95% CI**		**p**
Total effect of Pm $_{10}$ on Fibrinogen	*c*	0.217	0.119	-0.016	0.450	0.0678
Direct Effect of Pm $_{10}$ on Fibrinogen	*c'*	0.197	0.120	-0.039	0.433	0.1021
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.020	0.026	-	-	0.337*
		Effect	**Boot SE**	**Boot 95% CI**		**p**
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.020	0.026	-0.024	0.083	-

Table 60: Simple Mediation Analysis Results for mediator mir_106a_002169 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_152_000475

							Consequent					
		M(miR_152_000475)					Y(Fibrinogen)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	a	-0.012	0.003	-0.019	-0.006	<0.001	c'	0.194	0.120	-0.040	0.429	0.105
M(miR_152_000475)		_	_	_	_	_	b	-1.895	1.241	-4.332	0.541	0.127
costant	i_1	7.121	0.528	6.085	8.157	<0.001	i_2	163.145	20.717	122.480	203.811	<0.001
		R^2 =0.0370						R^2 =0.123				
		F(6,818)=5.239, p<0.001						F(7,817)=16.395, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.0678
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.194	0.120	-0.040	0.429	0.1046
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.023	0.023	-	-	0.173*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen	0.023	0.023	-0.011	0.083	-

Table 61: Simple Mediation Analysis Results for mediator mir_152_000475 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_181a_2_002317

							Consequent					
		M(miR_181a_2_002317)					Y(Fibrinogen)					
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
								R^2 = 0.121				
								F(6,818)=18.709, p<0.001				
X(Pm $_{10}$)	a	-0.017	0.004	-0.025	-0.009	<0.001	c'	0.180	0.120	-0.055	0.415	0.133
M(miR_181a_2_002317)		-	-	-	-	-	b	-2.188	1.033	-4.215	-0.161	0.034
costant	i_1	2.382	0.634	1.139	3.626	<0.001	i_2	154.861	18.873	117.816	191.906	<0.001
		R^2 =0.0263						R^2 =0.126				
		F(6,818)=3.689, p<0.001						F(7,817)=16.746, p<0.001				

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.180	0.120	-0.055	0.415	0.133
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.037	0.027	-	-	0.064*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen	0.037	0.027	-0.005	0.106	-

Table 62: Simple Mediation Analysis Results for mediator mir_181a_2_002317 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature.
miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 μg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

mir_218_000521

		Consequent									
		M(miR_218_000521)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
							R^2 = 0.121					
							F(6,818)=18.709, p<0.001					
X(Pm $_{10}$)	a	-0.017	0.004	-0.024	-0.009	<0.001	c'	0.181	0.120	-0.055	0.416	0.132
M(miR_218_000521)		-	-	-	-	-	b	-2.175	1.132	-4.397	0.047	0.055
costant	i_1	4.637	0.578	3.502	5.772	<0.001	i_2	159.732	19.443	121.568	197.896	<0.001
		R^2 =0.034					R^2 =0.013					
		F(6,818)=4.741, p<0.001					F(7,817)=16.115, p<0.001					

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.181	0.120	-0.055	0.416	0.132
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.036	0.027	-	-	0.083*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen	0.0361	0.0273	-0.007	0.103	-

Table 63: Simple Mediation Analysis Results for mediator mir_218_000521 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test

mir_652_002352

		Consequent									
		M(miR_652_002352)					Y(Fibrinogen)				
Antecedent		Coeff.	SE	95% CI		p		Coeff.	SE	95% CI		p
X(PM $_{10}$)		-	-	-	-	-	c	0.217	0.119	-0.0159	0.450	0.068
costant		-	-	-	-	-	i_3	149.649	18.752	112.841	186.456	<0.001
							R^2 = 0.121					
							F(6,818)=18.709, p<0.001					
X(Pm $_{10}$)	a	-0.022	0.005	-0.032	-0.012	<0.001	c'	0.199	0.120	-0.037	0.434	0.098
M(miR_652_002352)		-	-	-	-	-	b	-0.828	0.801	-2.401	0.745	0.302
costant	i_1	8.055	0.818	6.449	9.661	<0.001	i_2	156.314	19.831	117.389	195.240	<0.001
		R^2 =0.040					R^2 =0.122					
		F(6,818)=5.601, p<0.001					F(7,817)=16.190, p<0.001					

		Effect	SE	95% CI		p
Total effect of Pm $_{10}$ on Fibrinogen	c	0.217	0.119	-0.016	0.450	0.068
Direct Effect of Pm $_{10}$ on Fibrinogen	c'	0.199	0.120	-0.037	0.434	0.098
Indirect Effect of Pm $_{10}$ on Fibrinogen		0.018	0.024	-	-	0.328*

	Effect	Boot SE	Boot 95% CI		p
Indirect Effect of Pm $_{10}$ on Fibrinogen	0.018	0.024	-0.020	0.075	-

Table 64: Simple Mediation Analysis Results for mediator mir_652_002352 on log2 scale. Bootstrap standard error and bootstrap 95% were obtained for Indirect effect. Models adjusted for age, sex, bmi, smoking status, glycated haemoglobin, temperature. miRNAs expression was log2 transformed.
Back transforming in model with miRNAs expression as independent variable and PM_{10} as dependent variable it was obtained the %change in miRNAs expression : (2^β -1)*100 for an increase 1 µg/m^3 in PM_{10}.
Back transforming in model with Fibrinogen as independent variable and miRNAs expression as dependent variable it was obtained the change in Fibrinogen: β log2(101/100) associated with 1%change in miRNAs expression.
*Sobel test.

8. APPENDIX 2: SAS MACRO PROCESS FOR MEDIATION ANALYSIS

```
* PROCESS for SAS v2.13 */.
* Copyright 2012-2014 */.
* by Andrew F. Hayes */.
* www.afhayes.com */.

* Documentation available in Appendix A of http://www.guilford.com/p/hayes3;

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%macro bcboot (databcbt=,estmte=9999);
temp=&databcbt;
temp[rank(temp)]=&databcbt;
badlo=0;
badhi=0;
if (&estmte ^= 9999) then;
  do;pv=(temp < &estmte);pv=pv[+]/boot;
  ppv=pv;
  if (pv > 0.5) then;do;ppv=1-pv;end;
  y5=sqrt(-2*log(ppv));

xp=y5+((((y5*p4+p3)*y5+p2)*y5+p1)*y5+p0)/((((y5*q4+q3)*y5+q2)*y5+q1)*y5+q0);
  if (pv <= .5) then;do;xp=-xp;end;
  cilow=round(boot*(probnorm(2*xp+xp2)));
  cihigh=int(boot*(probnorm(2*xp+(-xp2))))+1;
  if (cilow < 1) then;do;cilow=1;booterr=1;badlo=1;end;
  if (cihigh > boot) then;do;cihigh=boot;booterr=1;badhi=1;end;
  llcit=temp[cilow,1];
  ulcit=temp[cihigh,1];
  end;
if (&estmte=9999) then;do;llcit=temp[cilow,1];ulcit=temp[cihigh,1];end;
%mend;

%macro bcci (data=,var=,point=9999,conf=95);
proc iml;
use &data;
```

```
read all var{&var} into perdata;
xx=(perdata = .);xx=xx[,+];
j=1;do i = 1 to nrow(perdata);if xx[i,1]=0
then;do;perdata[j,]=perdata[i,];j=j+1;end;end;
perdata=perdata[1:j-1,];
berror=0;
p0=-.322232431088;
p1 = -1;
p2 = -.342242088547;
p3 = -.0204231210245;
p4 = -.0000453642210148;
q0 = .0993484626060;
q1 = .588581570495;
q2 = .531103462366;
q3 = .103537752850;
q4 = .0038560700634;
conf=&conf;
if ((floor(conf) >= 100) | (floor(conf) <= 50)) then;do;conf=95;end;
alpha2=(1-(conf/100))/2;
y5=sqrt(-2*log(alpha2));
xp2=-
(y5+((((y5*p4+p3)*y5+p2)*y5+p1)*y5+p0)/((((y5*q4+q3)*y5+q2)*y5+q1)*y5+q0));
boot=nrow(perdata);
temp=perdata;
temp[rank(temp)]=perdata;
badlo=0;badhi=0;
if (&point ^= 9999) then;
  do;pv=(temp < &point);pv=pv[+]/boot;
  if ((pv=0) | (pv=1)) then;do;berror=2;end;
  if (berror=0) then;do;
    ppv=pv;
    if (pv > 0.5) then;do;ppv=1-pv;end;
    y5=sqrt(-2*log(ppv));

xp=y5+(((((y5*p4+p3)*y5+p2)*y5+p1)*y5+p0)/((((y5*q4+q3)*y5+q2)*y5+q1)*y5+q0))
;
    if (pv <= .5) then;do;xp=-xp;end;
    cilow=round(boot*(probnorm(2*xp+xp2)));
    cihigh=int(boot*(probnorm(2*xp+(-xp2))))+1;
    if (cilow < 1) then;do;berror=1;end;
    if (cihigh > boot) then;do;berror=1;end;
    if (berror=0) then;do;
      llcit=temp[cilow,1];
      ulcit=temp[cihigh,1];
      nametmp="Conf%"//"LLCI"//"ULCI";
        outp=conf//llcit//ulcit;
      print outp [label="Bias corrected confidence interval:" rowname =
nametmp format = 10.4];
    end;
  end;
end;
if (&point = 9999) then;do;
  cilowp=round(boot*(probnorm(xp2)));
  cihighp=int(boot*(probnorm(-xp2)))+1;
  if (cilowp < 1) then;do;berror=1;end;
  if (cihighp > boot) then;do;berror=1;end;
  if (berror=0) then;do;
    llcitp=temp[cilowp,1];
    ulcitp=temp[cihighp,1];
    nametmp="Conf%"//"LLCI"//"ULCI";
      outp=conf//llcitp//ulcitp;
    print outp [label="Percentile confidence interval:" rowname = nametmp
format = 10.4];
```

```
  end;
end;
if (berror=1) then;do;
  print "Error: Decrease your confidence or increase the number of bootstrap
estimates.";
end;
if (berror=2) then;do;
  print "Error: Impossible point estimate provided";
end;
quit;
%mend;

%macro process (data=,vars=,model=77,y=,m=,x=,w=,z=,v=,q=,conf=95,
  hc3=0,cluster=,wmodval=999,zmodval=999,vmodval=999,qmodval=999,mmodval=999,

xmodval=999,boot=1000,center=0,quantile=0,effsize=0,normal=0,varorder=2,total
=0,
  plot=0,detail=1,iterate=10000,converge=0.00000001,percent=0,jn=0,coeffci=1,

covmy=0,contrast=0,seed=0,save=xxx,mc=0,decimals=10.4,covcoeff=0,olsdichy=0,o
lsdichm=0,ws=0);
options pagesize=32767;
proc iml;
use &data;
read all var{&vars} into dat;
vnames={&vars};
yname={&y};xname={&x};mnames={&m};
if ("&w" = "") then wname="xxx";else wname = "&w";
if ("&z" = "") then zname="xxx";else zname = "&z";
if ("&v" = "") then vname="xxx";else vname = "&v";
if ("&q" = "") then qname="xxx";else qname = "&q";
if ("&save" = "") then saveboot="xxx";else saveboot="&save";
if ("&cluster" = "") then clname="xxx";else clname = "&cluster";

wname=upcase(wname);zname=upcase(zname);vname=upcase(vname);qname=upcase(qnam
e);clname=upcase(clname);
ninit=nrow(dat);
xx=(dat = .);xx=xx[,+];
j=1;do i = 1 to nrow(dat);if xx[i,1]=0 then;do;dat[j,]=dat[i,];j=j+1;end;end;
dat=dat[1:j-1,];
n=nrow(dat);
p0=-.322232431088;
p1 = -1;
p2 = -.342242088547;
p3 = -.0204231210245;
p4 = -.0000453642210148;
q0 = .0993484626060;
q1 = .588581570495;
q2 = .531103462366;
q3 = .103537752850;
q4 = .0038560700634;
badend=0;priorlo=-9999999;priorhi=9999999;
criterr=0;cluster=0;clsdmy=0;jndich=0;booterr=0;
wvdich=0;mod74dic=0;
effsize=(&effsize=1);
covcoeff=(&covcoeff=1);
note=j(10,1,0);notes=1;iterr=0;clsmtch=0;
quantile=(&quantile=1);jn=(&jn=1);contrast=(&contrast=1);
center=(&center=1);detail=(&detail=1);coeffci=(&coeffci=1);
conf=(&conf);bconoff=(&percent ^= 1);
covmy=floor(&covmy);if (covmy < 0 | covmy > 2) then;do;covmy=0;end;
if ((floor(conf) >= 100) | (floor(conf) <= 50)) then;do;
  conf=95;note[notes,1]=1;notes=notes+1;
```

```
  end;
if (n < ninit) then;do;nmiss=ninit-n;note[notes,1]=11;notes=notes+1;end;
errs=0;quantd=j(1,6,0);quantc=j(1,6,0);mcheck=0;ttt=0;
plot=(&plot ^= 0);
runerrs=j(50,1,0);
model=floor(&model);
normal=(&normal);
ws=(&ws);
olsdichm=(&olsdichm=1);olsdichy=(&olsdichy=1);
if (ws=1) then;do;
  if (effsize=1) then;do;
    note[notes,1]=19;notes=notes+1;effsize=0;
  end;
  if (normal=1) then;do;
    note[notes,1]=16;notes=notes+1;normal=0;
  end;
end;
if (model ^= 4) then;do;ws=0;end;
if ((jn = 1) & (model ^= 1) & (model ^= 3)) then;do;
  note[notes,1]=7;notes=notes+1;
end;
if ((model > 76) | (model < 1)) then;do;
  model=77;criterr=1;errs=errs+1;runerrs[errs,1]=19;
end;
toteff=0;toteff=((&total=1)*((model=4) | (model=6)));
varorder=(&varorder);
hc3=(&hc3 ^= 0);
centvar={'xxx'};
modelm=
{0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1,
 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 2,
 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 3,
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 4,
 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 5,
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 6,
 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 7,
 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 8,
 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 9,
 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 10,
 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 11,
 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 12,
 1 1 1 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 13,
 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 14,
 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 15,
 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 16,
 0 0 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 17,
 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 18,
 0 0 0 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 19,
 0 0 0 1 1 1 0 0 0 1 0 0 0 0 0 0 0 0 20,
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 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 25,
 1 1 1 1 0 0 1 1 1 0 0 0 0 0 0 0 0 0 26,
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 1 1 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 30,
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 1 1 1 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 32,
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 1 1 1 1 0 0 1 0 0 1 0 0 0 0 0 0 0 0 34,
```

```
 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 35,
 1 0 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 36,
 1 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 37,
 1 0 0 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 38,
 1 0 0 1 1 1 0 0 0 1 0 0 0 0 0 0 0 0 39,
 1 0 0 1 1 0 1 0 0 0 0 0 0 0 0 0 0 0 40,
 1 0 0 1 1 0 1 0 0 1 1 0 0 0 0 0 0 0 41,
 1 0 0 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 42,
 1 0 0 1 1 1 1 0 0 1 1 1 0 0 0 0 0 0 43,
 1 0 0 1 1 1 1 0 0 1 0 0 0 0 0 0 0 0 44,
 1 1 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 45,
 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 46,
 1 1 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 47,
 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 48,
 1 1 0 1 1 0 1 1 0 0 0 0 0 0 0 0 0 0 49,
 1 1 0 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 50,
 1 1 1 1 1 0 1 1 1 0 0 0 0 0 0 0 0 0 51,
 1 1 1 1 1 0 0 0 0 1 1 0 0 0 0 0 0 0 52,
 1 1 0 1 1 1 1 1 0 0 0 0 0 0 0 0 0 0 53,
 1 1 0 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 54,
 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 0 0 0 55,
 1 1 1 1 1 1 0 0 0 1 1 1 0 0 0 0 0 0 56,
 1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 0 57,
 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 58,
 1 0 0 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 59,
 1 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 60,
 1 1 0 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 61,
 1 1 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 62,
 1 1 0 0 0 0 1 1 0 0 0 0 1 0 0 0 0 0 63,
 1 0 0 1 0 0 0 0 0 0 0 0 1 0 0 0 0 0 64,
 1 0 0 1 0 0 1 0 0 0 0 0 1 0 0 0 0 0 65,
 1 0 0 1 0 0 0 0 0 1 0 0 1 0 0 0 0 0 66,
 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 0 67,
 1 1 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 68,
 1 1 1 0 0 0 1 1 1 0 0 0 1 0 0 0 0 0 69,
 1 0 0 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 70,
 1 0 0 1 0 0 1 0 0 1 0 0 1 1 1 0 0 0 71,
 1 1 1 0 0 0 0 0 0 0 0 0 1 0 0 1 1 0 72,
 1 1 1 0 0 0 1 1 1 0 0 0 1 0 0 1 1 0 73,
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 74,
 1 1 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 75,
 1 1 0 0 0 0 1 1 0 0 0 0 1 0 0 1 0 0 76,
 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 77};

wm=modelm[model,1];
zm=modelm[model,2];
wzm=modelm[model,3];
vy=modelm[model,4];
qy=modelm[model,5];
vqy=modelm[model,6];
wy=modelm[model,7];
zy=modelm[model,8];
wzy=modelm[model,9];
vxy=modelm[model,10];
qxy=modelm[model,11];
vqxy=modelm[model,12];
wmy=modelm[model,13];
wvmy=modelm[model,14];
wvxy=modelm[model,15];
zmy=modelm[model,16];
wzmy=modelm[model,17];
xmy=modelm[model,18];
if (ncol(xname) ^= 1) then;do;errs=errs+1;runerrs[errs,1]=20;criterr=1;end;
```

```
if (ncol(yname) ^= 1) then;do;errs=errs+1;runerrs[errs,1]=21;criterr=1;end;
if ((&mmodval=0) & (&wmodval = 0) & (model=3 | model=2) & (contrast=1))
then;do;
  errs=errs+1;runerrs[errs,1]=31;criterr=1;
end;
xlist =
((wm=1)|(zm=1)|(wzm=1)|(wy=1)|(zy=1)|(wzy=1)|(vxy=1)|(qxy=1)|(vqxy=1)|(wvxy=1
)|(xmy=1));
mlist = ((vy=1)|(qy=1)|(vqy=1)|(zmy=1)|(wmy=1)|(wzmy=1)|(xmy=1)|(model < 4));
bad=0;
if (criterr=0) then;do;
  werr=0;verr=0;qerr=0;zerr=0;yerr=1;xerr=1;

wlist=((wm=1)|(wzm=1)|(wy=1)|(wzy=1)|(wm=1)|(wvmy=1)|(wvmy=1)|(wvxy=1)|(wzmy=
1));
  if ((wlist=1) & (wname = "XXX"))
then;do;werr=1;wlist=0;errs=errs+1;runerrs[errs,1]=4;
  end;
  if ((wlist=1) &
((wname=qname)|(wname=vname)|(wname=zname)|(wname=xname)|(wname=yname)))
then;do;
    werr=4;errs=errs+1;runerrs[errs,1]=12;
  end;
  zlist=((zm=1)|(wzm=1)|(zy=1)|(wzy=1)|(zmy=1)|(wzmy=1));
  if ((zlist=1) & (zname = "XXX"))
then;do;zerr=1;zlist=0;errs=errs+1;runerrs[errs,1]=5;
  end;
  if ((zlist=1) &
((zname=qname)|(zname=vname)|(zname=wname)|(zname=xname)|(zname=yname)))
then;do;
    zerr=4;errs=errs+1;runerrs[errs,1]=13;
  end;
  qlist=((qy=1)|(vqy=1)|(qxy=1)|(vqxy=1));
  if ((qlist=1) & (qname = "XXX"))
then;do;qerr=1;qlist=0;errs=errs+1;runerrs[errs,1]=6;
  end;
  if ((qlist=1) &
((qname=zname)|(qname=vname)|(qname=wname)|(qname=xname)|(qname=yname)))
then;do;
    qerr=4;errs=errs+1;runerrs[errs,1]=14;
  end;
  vlist=((vy=1)|(vqy=1)|(vxy=1)|(vqxy=1)|(wvmy=1)|(wvxy=1));
  if ((vlist=1) & (vname = "XXX"))
then;do;verr=1;vlist=0;errs=errs+1;runerrs[errs,1]=7;
  end;
  if ((vlist=1) &
((vname=zname)|(vname=qname)|(vname=wname)|(vname=xname)|(vname=yname)))
then;do;
    qerr=4;errs=errs+1;runerrs[err,1]=15;
  end;
  if ((wlist=0) & (wname ^= "XXX"))
then;do;werr=2;errs=errs+1;runerrs[errs,1]=8;
  end;
  if ((zlist=0) & (zname ^= "XXX"))
then;do;zerr=2;errs=errs+1;runerrs[errs,1]=9;
  end;
  if ((qlist=0) & (qname ^= "XXX"))
then;do;qerr=2;errs=errs+1;runerrs[errs,1]=10;
  end;
  if ((vlist=0) & (vname ^= "XXX"))
then;do;verr=2;errs=errs+1;runerrs[errs,1]=11;
  end;
  if (hc3=1) then;do;note[notes,1]=3;notes=notes+1;
```

```
  end;
  alpha2=(1-(conf/100))/2;
  y5=sqrt(-2*log(alpha2));
  xp2=-
(y5+((((y5*p4+p3)*y5+p2)*y5+p1)*y5+p0)/((((y5*q4+q3)*y5+q2)*y5+q1)*y5+q0));
  cons=j(n,1,1);
  temp=(n*t(dat)*dat)-(t(dat[+,])*dat[+,]);
  temp=temp/(n*(n-1));temp=sqrt(vecdiag(temp));temp=(temp = 0);temp=temp[+,];
  temp2=1;

  do i = 1 to ncol(vnames);
     if (vnames[,i]=xname) then;do;tmp2=dat[,i];end;
  end;

  if (ws=1) then;do;
    temp2=(n*t(tmp2)*tmp2)-(t(tmp2[+,])*tmp2[+,]);temp2=temp2/(n*(n-1));
  end;
  if ((temp > 0 & ws=0) | ((temp > 0) & (ws=1) & (temp2 ^=0))) then;do;
    criterr=1;errs=errs+1;runerrs[errs,1]=27;
  end;
  nmeds=ncol(mnames);
  mcmats=I(nmeds*2);
  mccoeff=j((nmeds*2),1,0);
  sobel=j(nmeds,4,-999);
  if ((model = 6) & (nmeds > 4)) then;do;errs=errs+1;runerrs[errs,1]=2;
  end;
  if ((model < 4) & (nmeds > 1)) then;do;errs=errs+1;runerrs[errs,1]=3;
  end;
  nmods=(model=74);
  bad=0;intcnt=1;modvals=0;modvalsd=0;
  yintemp={"INT_1" "INT_2" "INT_3" "INT_4" "INT_5" "INT_6" "INT_7" "INT_8"
"INT_9" "INT_10" "INT_11" "INT_12"};
  yintemp=yintemp||{"INT_13" "INT_14" "INT_15" "INT_16" "INT_17" "INT_18"
"INT_19" "INT_20" "INT_21" "INT_22"};
  yintemp=yintemp||{"INT_23" "INT_24" "INT_25" "INT_26" "INT_27" "INT_28"
"INT_29" "INT_30" "INT_31" "INT_32"};
  yintemp=yintemp||{"INT_33" "INT_34" "INT_35" "INT_36" "INT_37" "INT_38"
"INT_39" "INT_40" "INT_41" "INT_42"};
  yintemp=yintemp||{"INT_43" "INT_44" "INT_45" "INT_46" "INT_47" "INT_48"
"INT_49" "INT_50" "INT_51" "INT_52"};
  yintemp=yintemp||{"INT_53" "INT_54" "INT_55" "INT_56" "INT_57" "INT_58"
"INT_59" "INT_60" "INT_61" "INT_62"};

cntname="(C1)"//"(C2)"//"(C3)"//"(C4)"//"(C5)"//"(C6)"//"(C7)"//"(C8)"//"(C9)
"//"(C10)";

cntname=cntname//"(C11)"//"(C12)"//"(C13)"//"(C14)"//"(C15)"//"(C16)"//"(C17)
"//"(C18)"//"(C19)"//"(C20)";

cntname=cntname//"(C21)"//"(C22)"//"(C23)"//"(C24)"//"(C25)"//"(C26)"//"(C27)
"//"(C28)"//"(C29)"//"(C30)";

cntname=cntname//"(C31)"//"(C32)"//"(C33)"//"(C34)"//"(C35)"//"(C36)"//"(C37)
"//"(C38)"//"(C39)"//"(C40)";

cntname=cntname//"(C41)"//"(C42)"//"(C43)"//"(C44)"//"(C45)"//"(C46)"//"(C47)
"//"(C48)"//"(C49)"//"(C50)";

cntname=cntname//"(C51)"//"(C52)"//"(C53)"//"(C54)"//"(C55)"//"(C56)"//"(C57)
"//"(C58)"//"(C59)"//"(C60)";

cntname=cntname//"(C61)"//"(C62)"//"(C63)"//"(C64)"//"(C65)"//"(C66)"//"(C67)
"//"(C68)"//"(C69)"//"(C70)";
```

```
cntname=cntname//"(C71)"//"(C72)"//"(C73)"//"(C74)"//"(C75)"//"(C76)"//"(C77)
"//"(C78)"//"(C79)"//"(C80)";

cntname=cntname//"(C81)"//"(C82)"//"(C83)"//"(C84)"//"(C85)"//"(C86)"//"(C87)
"//"(C88)"//"(C89)"//"(C90)";

cntname=cntname//"(C91)"//"(C92)"//"(C93)"//"(C94)"//"(C95)"//"(C96)"//"(C97)
"//"(C98)"//"(C99)"//"(C100)";
  cntname=cntname//"(C101)"//"(C102)"//"(C103)"//"(C104)"//"(C105)";
  apathnam="a path"//"a1 path"//"a2 path"//"a3 path"//"a4 path"//"a5
path"//"a6 path"//"a7 path"//"a8 path"//"a9 path"//"a10 path";
  bpathnam="b path"//"b1 path"//"b2 path"//"b3 path"//"b4 path"//"b5
path"//"b6 path"//"b7 path"//"b8 path"//"b9 path"//"b10 path";
  modvnm = {"xxxxxxxxxxxxxxx"}||{"xxx"}||{"xxx"}||{"xxx"}||{"xxx"};
  modvnm2 = modvnm;
  mlab="M1    ="//"M2    ="//"M3    ="//"M4    ="//"M5    ="//"M6    ="//"M7
="//"M8    ="//"M9    ="//"M10   =";

m=j(n,nmeds,1);mmat=j(16,nmeds,0);ymat=j(8,nmeds,0);deco=j(10,1,0);modmat=j(5
,5,999);
  modmatv=j(1,5,1);modmatp=j(1,5,0);modprod=modmatv;
  iterate=abs(floor(&iterate));converge=abs(&converge);
  boot=abs(floor(&boot));adjust=0;
  if ((ws=1) & (&mc > 0)) then;do;note[notes,1]=17;notes=notes+1;end;
  mc=(abs(floor(&mc))*(1-ws));
  if ((mc > 0) & (model > 5)) then;do;
    if (boot = 0) then;do;boot=mc;end;
    mc=0;
      note[notes,1]=12;notes=notes+1;
  end;
  if (boot ^= 0) then;do;
    cilow=floor(boot*(1-
(conf/100))/2);cihigh=floor((boot*(conf/100)+(boot*(1-(conf/100))/2)))+1;
    do until ((cilow > 0) & (cihigh <= boot));
      cilow=floor(boot*(1-
(conf/100))/2);cihigh=floor((boot*(conf/100)+(boot*(1-(conf/100))/2)))+1;
      if ((cilow < 1) | (cihigh > boot))
then;do;boot=floor((boot+1000)/1000)*1000;adjust=1;
      end;
    end;
    if (adjust = 1) then;do;note[notes,1]=6;notes=notes+1;end;
  end;
  if ((mc > 0) & ((model > 3) & (model < 6))) then;do;boot=0;bconoff=0;end;
  if ((boot > 0) & (mc > 0)) then;do;mc=0;end;
  savboot=0;
  if ((saveboot ^= "xxx") & (boot > 0) & (model > 3)) then;do;savboot=1;
  end;
  if ((savboot=1) & (ws=1)) then;do;
    note[notes,1]=20;notes=notes+1;savboot=0;
  end;
  if ((boot ^= 0) | (mc ^= 0)) then;do;
    bootsz=boot;
      if (mc > 0) then;do;bootsz=mc;end;
    cilow=round(bootsz*(1-
(conf/100))/2);cihigh=int((bootsz*(conf/100)+(bootsz*(1-(conf/100))/2)))+1;
    do until ((cilow > 0) & (cihigh <= bootsz));
      cilow=floor(bootsz*(1-
(conf/100))/2);cihigh=floor((bootsz*(conf/100)+(bootsz*(1-(conf/100))/2)))+1;
      if ((cilow < 1) | (cihigh > bootsz))
then;do;bootsz=floor((bootsz+1000)/1000)*1000;adjust=1;
      end;
    end;
```

```
      boot=bootsz;
      if (mc > 0) then;do;mc=bootsz;end;
      if ((boot > 0) & (mc > 0) & ((model > 3) & (model < 6)))
then;do;boot=0;end;
    if ((adjust = 1) & (boot > 0)) then;do;note[notes,1]=6;notes=notes+1;end;
      if ((adjust = 1) & (mc > 0)) then;do;note[notes,1]=13;notes=notes+1;end;
  end;
  if ((model = 6) & (nmeds > 1)) then;do;
    mmpaths=j((nmeds+2),(nmeds+2),0);
    if (nmeds = 2) then;do;indboot=j((boot+1),3,999);
    end;
    if (nmeds = 3) then;do;indboot=j((boot+1),7,999);
    end;
    if (nmeds = 4) then;do;indboot=j((boot+1),15,999);
    end;
    indlbl={"Total", "Ind1:", "Ind2:", "Ind3:", "Ind4:", "Ind5:", "Ind6:",
"Ind7:", "Ind8:", "Ind9:"};
    indlbl=indlbl//{"Ind10:", "Ind11:", "Ind12:", "Ind13:", "Ind14:",
"Ind15:"};
    indlbl2={"Ind1", "Ind2", "Ind3", "Ind4", "Ind5", "Ind6", "Ind7", "Ind8",
"Ind9"};
    indlbl2=indlbl2//{"Ind10", "Ind11", "Ind12", "Ind13", "Ind14", "Ind15"};
    indces=j((boot+1),4,999);
  end;
  if (model < 4) then;do;boot=0;cmat=j(10,1,0);zmat=j(10,1,0);
  end;
  nvarch=j(1,ncol(dat),0);wmatch=0;zmatch=0;vmatch=0;qmatch=0;mmatch=0;
  minprobe=0;maxprobe=0;
  do i = 1 to ncol(vnames); *[b];
    if (vnames[,i]=yname) then;do;
      y=dat[,i];nvarch[1,i]=1;yerr=0;
      if
((yname=xname)|(yname=wname)|(yname=zname)|(yname=vname)|(yname=qname))
then;do;
        errs=errs+1;runerrs[errs,1]=17;
      end;
    end;
    if (vnames[,i]=xname) then;do;
      x=dat[,i];nvarch[1,i]=1;xdich=1;xerr=0;
      do jj = 1 to n;
        if ((x[jj,1] ^= max(x)) & (x[jj,1] ^= min(x))) then;do;xdich=0;goto
leave;
        end;
      end;
      leave:
      xmean=x[+,]/n;
      if ((center = 1) & ((model < 4) | (xlist > 0))) then;do;
        meanvec=j(n,1,xmean);x=x-meanvec;centvar=centvar||xname;
      end;
      xmean=x[+,]/n;
      tmp=x-(cons*xmean);
      xsd=sqrt((1/(n-1))*(t(tmp)*tmp));
      if (xdich=0) then;do;
        quantc[1,6]=1;matx=(xmean-xsd)//xmean//(xmean+xsd);
            if ((&xmodval = 999) & (quantile = 0)) then;do;
              if ((matx[1,1] < min(x)) & (model=74))
then;do;matx[1,1]=min(x);minprobe=1;end;
              if ((matx[3,1] > max(x)) & (model=74))
then;do;matx[3,1]=max(x);maxprobe=1;end;
            end;
        if (quantile = 1) then;do;
          quantd[1,6]=1;quantc[1,6]=0;tmp=x;tmp[rank(tmp)]=x;
```

```
matx=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
        end;
      end;
      if (xdich=1) then;do;matx=min(x)//max(x);
          if (model=74) then;do;matx=min(x);mod74dic=1;end;
      end;
      if (&xmodval ^= 999) then;do;matx=&xmodval;quantd[1,6]=0;quantc[1,6]=0;
      end;
    end;
    if ((werr=0) & (wlist=1)) then;do;
      if (vnames[,i]=wname) then;do;
        werr=0;wmatch=1;w=dat[,i];
        if (center = 1) then;do;
          wmean=w[+,]/n;meanvec=j(n,1,wmean);w=w-
meanvec;centvar=centvar||wname;
        end;
        nvarch[1,i]=1;nmods=nmods+1;wmean=w[+,]/n;tmp=w-(cons*wmean);
        wsd=sqrt((1/(n-1))*(t(tmp)*tmp));
        wdich=1;
        do jj=1 to n;
          if ((w[jj,1] ^= max(w)) & (w[jj,1] ^= min(w))) then;do;wdich=0;goto
leave2;
          end;
        end;
        leave2:
        if (model = 3) then;do;jndich=wdich;jnmin=min(w);jnmax=max(w);
        end;
        if (wdich = 0) then;do;
          matw=(wmean-wsd)//wmean//(wmean+wsd);quantc[1,1]=1;
              if ((&wmodval=999) & (quantile=0)) then;do;
                if (matw[1,1] < min(w))
then;do;matw[1,1]=min(w);minprobe=1;end;
                if (matw[3,1] > max(w))
then;do;matw[3,1]=max(w);maxprobe=1;end;
              end;
          if (quantile = 1) then;do;
            quantd[1,1]=1;quantc[1,1]=0;tmp=w;tmp[rank(tmp)]=w;

matw=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
          end;
        end;
        if (wdich=1)
then;do;matw=min(w)//max(w);wvdich=1;cmaxw=max(w);cminw=min(w);
        end;
        if (&wmodval ^= 999)
then;do;matw=&wmodval;quantd[1,1]=0;quantc[1,1]=0;
        end;
        modmatv[1,1]=nrow(matw);
        modmat[(1:nrow(matw)),1]=matw;
        modvnm[1,1]=wname;
        modmatp[1,1]=1;
      end;
    end;
    if ((zerr=0) & (zlist=1)) then;do;
      if (vnames[,i]=zname) then;do;
        zerr=0;zmatch=1;z=dat[,i];
        if (center = 1) then;do;
          zmean=z[+,]/n;meanvec=j(n,1,zmean);z=z-
meanvec;centvar=centvar||zname;
        end;
```

```
        nvarch[1,i]=1;nmods=nmods+1;zmean=z[+,]/n;tmp=z-(cons*zmean);
        zsd=sqrt((1/(n-1))*(t(tmp)*tmp));
        zdich=1;
        do jj=1 to n;
          if ((z[jj,1] ^= max(z)) & (z[jj,1] ^= min(z))) then;do;zdich=0;goto
leave3;
          end;
        end;
        leave3:
        if (zdich = 0) then;do;
          matz=(zmean-zsd)//zmean//(zmean+zsd);quantc[1,2]=1;
              if ((&zmodval=999) & (quantile=0)) then;do;
                if (matz[1,1] < min(z))
then;do;matz[1,1]=min(z);minprobe=1;end;
                if (matz[3,1] > max(z))
then;do;matz[3,1]=max(z);maxprobe=1;end;
              end;
          if (quantile = 1) then;do;
            quantd[1,2]=1;quantc[1,2]=0;tmp=z;tmp[rank(tmp)]=z;

matz=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
          end;
        end;
        if (zdich=1) then;do;matz=min(z)//max(z);
        end;
        if (&zmodval ^= 999)
then;do;matz=&zmodval;quantd[1,2]=0;quantc[1,2]=0;
        end;
        modmatv[1,2]=nrow(matz);
        modmat[(1:nrow(matz)),2]=matz;
        modvnm[1,2]=zname;
        modmatp[1,2]=1;
      end;
    end;
    if ((verr=0) & (vlist=1)) then;do;
      if (vnames[,i]=vname) then;do;
        verr=0;vmatch=1;v=dat[,i];
        if (center = 1) then;do;
          vmean=v[+,]/n;meanvec=j(n,1,vmean);v=v-
meanvec;centvar=centvar||vname;
        end;
        nvarch[1,i]=1;nmods=nmods+1;vmean=v[+,]/n;tmp=v-(cons*vmean);
        vsd=sqrt((1/(n-1))*(t(tmp)*tmp));
        vdich=1;
        do jj=1 to n;
          if ((v[jj,1] ^= max(v)) & (v[jj,1] ^= min(v))) then;do;vdich=0;goto
leave4;
          end;
        end;
        leave4:
        if (vdich = 0) then;do;
          matv=(vmean-vsd)//vmean//(vmean+vsd);quantc[1,3]=1;
              if (&vmodval=999) then;do;
                if (matv[1,1] < min(v))
then;do;matv[1,1]=min(v);minprobe=1;end;
                if (matv[3,1] > max(v))
then;do;matv[3,1]=max(v);maxprobe=1;end;
              end;
          if (quantile = 1) then;do;
            quantd[1,3]=1;quantc[1,3]=0;tmp=v;tmp[rank(tmp)]=v;
```

```
matv=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
          end;
        end;
        if (vdich=1)
then;do;matv=min(v)//max(v);wvdich=1;cmaxv=max(v);cminv=min(v);
        end;
        if (&vmodval ^= 999)
then;do;matv=&vmodval;quantd[1,3]=0;quantc[1,3]=0;
        end;
        modmatv[1,3]=nrow(matv);
        modmat[(1:nrow(matv)),3]=matv;
        modvnm[1,3]=vname;
        modmatp[1,3]=1;
      end;
    end;
    if ((qerr=0) & (qlist=1)) then;do;
      if (vnames[,i]=qname) then;do;
        qerr=0;qmatch=1;q=dat[,i];
        if (center = 1) then;do;
          qmean=q[+,]/n;meanvec=j(n,1,qmean);q=q-
meanvec;centvar=centvar||qname;
        end;
        nvarch[1,i]=1;nmods=nmods+1;qmean=q[+,]/n;tmp=q-(cons*qmean);
        qsd=sqrt((1/(n-1))*(t(tmp)*tmp));
        qdich=1;
        do jj=1 to n;
          if ((q[jj,1] ^= max(q)) & (q[jj,1] ^= min(q))) then;do;qdich=0;goto
leave5;
          end;
        end;
        leave5:
        if (qdich = 0) then;do;
          matq=(qmean-qsd)//qmean//(qmean+qsd);quantc[1,4]=1;
              if ((&qmodval=999) & (quantile = 0)) then;do;
                if (matq[1,1] < min(q))
then;do;matq[1,1]=min(q);minprobe=1;end;
                if (matq[3,1] > max(q))
then;do;matq[3,1]=max(q);maxprobe=1;end;
              end;
          if (quantile = 1) then;do;
            quantd[1,4]=1;quantc[1,4]=0;tmp=q;tmp[rank(tmp)]=q;

matq=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
          end;
        end;
        if (qdich=1) then;do;matq=min(q)//max(q);
        end;
        if (&qmodval ^= 999)
then;do;matq=&qmodval;quantd[1,4]=0;quantc[1,4]=0;
        end;
        modmatv[1,4]=nrow(matq);
        modmat[(1:nrow(matq)),4]=matq;
        modvnm[1,4]=qname;
        modmatp[1,4]=1;
      end;
    end;
    if (vnames[,i]=clname) then;do;
      cld=dat[,i];cvname=vnames[,i];nvarch[1,i]=1;clsmtch=1;
    end;
    do j = 1 to ncol(mnames);
```

```
      if (vnames[,i]=mnames[1,j]) then;do;
        mmatch=mmatch+1;m[,j]=dat[,i];
        if ((center=1) & (nvarch[1,i]=0) & (mlist > 0)) then;do;
          tmp=m[,j];
          meanvec=j(n,1,(tmp[+,]/n));m[,j]=m[,j]-meanvec;mmmm=m[,j];
          centvar=centvar||mnames[1,j];
        end;
        nvarch[1,i]=1;
        dichm=1;
        do jj=1 to n;
          if ((m[jj,j] ^= max(m[,j])) & (m[jj,j] ^= min(m[,j])))
then;do;dichm=0;goto leave6;
          end;
        end;
        leave6:
            if ((dichm=1) & (olsdichm =1))
then;do;note[notes,1]=18;notes=notes+1;end;
        if ((dichm=1) & (model > 3) & (mcheck = 0) & (olsdichm=0))
then;do;errs=errs+1;runerrs[errs,1]=1;mcheck=1;
        end;
        if ((model <=3) & (ncol(mnames) = 1)) then;do;
          tmp=m[,j];
          nmods=nmods+1;mmean=tmp[+,]/n;tmp=m[,j]-(cons*mmean);
          msd=sqrt((1/(n-1))*(t(tmp)*tmp));
          mdich=1;
          do jj=1 to n;
            if ((m[jj,j] ^= max(m[,j])) & (m[jj,j] ^= min(m[,j])))
then;do;mdich=0;goto leave7;
            end;
          end;
          leave7:
          if (model = 1)
then;do;jndich=mdich;jnmin=min(m[,j]);jnmax=max(m[,j]);
          end;
          if (mdich=0) then;do;
            matm=(mmean-msd)//mmean//(mmean+msd);quantc[1,5]=1;
                  if ((&mmodval=999) & (quantile=0)) then;do;
                    if (matm[1,1] < min(m[,j]))
then;do;matm[1,1]=min(m[,j]);minprobe=1;end;
                  if (matm[3,1] > max(m[,j]))
then;do;matm[3,1]=max(m[,j]);maxprobe=1;end;
                  end;
            if (quantile = 1) then;do;
              quantd[1,5]=1;quantc[1,5]=0;tmp=m[,j];tmp[rank(tmp)]=m[,j];

matm=tmp[floor(n*0.10),1]//tmp[floor(n*0.25),1]//tmp[floor(n*0.5),1]//tmp[flo
or(n*0.75),1]//tmp[floor(n*0.9),1];
            end;
          end;
          if (mdich = 1) then;do;matm=min(m)//max(m);
          end;
          if (&mmodval ^= 999) then;do;
            matm=&mmodval;quantd[1,5]=0;quantc[1,5]=0;
          end;
          modmatv[1,5]=nrow(matm);
          modmat[(1:nrow(matm)),5]=matm;
          modvnm[1,5]=mnames[1,j];
          modmatp[1,5]=1;
        end;
      end;
    end;
  end;
  if (minprobe=1)then;do;note[notes,1]=14;notes=notes+1;end;
```

```
  if (maxprobe=1)then;do;note[notes,1]=15;notes=notes+1;end;
  if ((clname ^= "XXX") & (clsmtch = 0))
then;do;errs=errs+1;runerrs[errs,1]=23;
  end;
  if (clname ^= "XXX") then;do;
    if ((clname=zname) | (clname=vname) | (clname=wname) | (clname=xname) |
(clname=yname) | (clname=qname)) then;do;
      errs=errs+1;runerrs[errs,1]=24;
    end;
  end;
  if ((wlist=1) & (werr=0) & (wmatch=0))
then;do;werr=3;errs=errs+1;runerrs[errs,1]=4;
  end;
  if ((zlist=1) & (zerr=0) & (zmatch=0))
then;do;zerr=3;errs=errs+1;runerrs[errs,1]=5;
  end;
  if ((qlist=1) & (qerr=0) & (qmatch=0))
then;do;qerr=3;errs=errs+1;runerrs[errs,1]=6;
  end;
  if ((vlist=1) & (verr=0) & (vmatch=0))
then;do;verr=3;errs=errs+1;runerrs[errs,1]=7;
  end;
  if (yerr = 1) then;do;errs=errs+1;runerrs[errs,1]=16;
  end;
  if (xerr = 1) then;do;errs=errs+1;runerrs[errs,1]=32;
  end;
  if ((model=6) & (nmeds < 2)) then;do;errs=errs+1;runerrs[errs,1]=18;
  end;
  if (mmatch < ncol(mnames)) then;do;errs=errs+1;runerrs[errs,1]=25;
  end;
end;



if (clname ^= "XXX") then;do;
  cld=design(cld);cluster=ncol(cld);cld=cld[,2:ncol(cld)];clsdmy=ncol(cld);
  if (clsdmy > 19) then;do;errs=errs+1;runerrs[err,1]=26;
  end;
end;
dichy=1;
do jj=1 to n;
  if ((y[jj,1] ^= max(y)) & (y[jj,1] ^= min(y))) then;do;dichy=0;goto leave8;
  end;
  leave8:
end;
if ((dichy=1) & (olsdichy = 1)) then;do;note[notes,1]=18;notes=notes+1;end;
if (dichy = 1) then;do;jncrit=xp2*xp2;
  if (ws=1) then;do;critterr=1;errs=errs+1;runerrs[errs,1]=28;end;
end;
ncovs = ncol(dat)-nvarch[,+];
if ((effsize=1) & (covmy ^= 0) & (ncovs > 0) & (model > 3) & (model < 7))
then;do;
  note[notes,1]=22;notes=notes+1;effsize=0;
end;
if (ws=1 & ncovs < nmeds)
then;do;critterr=1;errs=errs+1;runerrs[errs,1]=29;end;
if (ws=1 & ncovs > nmeds)
then;do;critterr=1;errs=errs+1;runerrs[errs,1]=30;end;
if (errs = 0) then;do;  *[cccc];
  tmp1=quantd[,+];tmp2=quantc[,+];
  if (tmp1 > 0) then;do;note[notes,1]=4;notes=notes+1;
  end;
  if (tmp2 > 0) then;do;note[notes,1]=5;notes=notes+1;
```

```
  end;
  if (ncovs > 0) then;do;
    c=j(n,ncovs,0);cnames={"x"};j=1;
    do i = 1 to ncol(vnames);
      if (nvarch[1,i]=0) then;do;
        c[,j]=dat[,i];nvarch[1,i]=1;j=j+1;cnames=cnames||vnames[,i];
      end;
    end;
    cnames=cnames[1,2:ncol(cnames)];
    if (ws=1) then;do;
         covmean=c[+,]/n;
         do i = 1 to ncovs;
           meanvec=j(n,1,covmean[1,i]);
             c[,i]=c[,i]-meanvec;
         end;
         centvar=centvar||cnames;
       x=c[,1:ncovs];
         if (ncovs=nmeds) then;do;ncovs=0;end;
      end;
  end;
  names=yname||xname||mnames||wname||zname||vname||qname;
  if (ncovs > 0) then;do;
    names=names||cnames;
  end;
  if ((dichy=1) & (effsize=1)) then;do;
    note[notes,1]=2;notes=notes+1;
  end;
  if ((model > 3) & (model < 6)) then;do;
    indeff=j(nmeds,1,0);indboot=j((boot+1),nmeds,999);
      if (mc > 0) then;do;indboot=j((mc+1),nmeds,999);end;
    if ((effsize=1) & (dichy=0)) then;do;
      rmeff=j((boot+1),(nmeds+1),999);
      abpseff=j((boot+1),(nmeds+1),999);
      abcseff=j((boot+1),(nmeds+1),999);
      pmeff=j((boot+1),(nmeds+1),999);
      r245=j((boot+1),1,999);
      kappa2=j((boot+1),1,999);
    end;
  end;
  if ((model = 6) & (effsize=1) & (dichy=0)) then;do;
    rmeff=j((boot+1),ncol(indboot),999);
    abpseff=j((boot+1),ncol(indboot),999);
    abcseff=j((boot+1),ncol(indboot),999);
    pmeff=j((boot+1),ncol(indboot),999);
  end;
  if (nmods > 0) then;do;
    tmp=1;
    do i = 1 to 5;
      if (modmatp[1,i]=1) then;do;

modmat[,tmp]=modmat[,i];modvnm[1,tmp]=modvnm[1,i];modmatv[1,tmp]=modmatv[1,i]
;
        tmp=tmp+1;
      end;
    end;

modmat=modmat[,(1:nmods)];modvnm=modvnm[,(1:nmods)];modmatv=modmatv[,(1:nmods
)];
    do i = 1 to (ncol(modmatv)-1);
      tmp=1;
      do j = (i+1) to ncol(modmatv);
        tmp=tmp*modmatv[1,j];
      end;
```

```
      modprod[1,i]=tmp;
    end;
    modvals=j((modmatv[1,1]*modprod[1,1]),nmods,0);
    do i = 1 to nmods;
      strt=1;fnsh=0;
      do while (fnsh < nrow(modvals));
        do j = 1 to modmatv[1,i];
          tmp=j(modprod[1,i],1,modmat[j,i]);
          fnsh=fnsh+nrow(tmp);
          modvals[(strt:fnsh),i]=tmp;
          strt=fnsh+1;
        end;
      end;
    end;
    if (model = 74) then;do;modvals=matx;modvnm=xname;
    end;
    vmat=j(8,nrow(modvals),0);
    vmat[1,1:nrow(modvals)]=j(1,nrow(modvals),1);
    vmat[5,1:nrow(modvals)]=j(1,nrow(modvals),1);
      indeff=j(nrow(modvals),1,0);
    if (model ^= 5) then;do;
      indboot=j(((boot+1)*nmeds),nrow(modvals),-99999999);
      indbootp=j((boot+1),nmeds,-99999999);
    end;
  end;
  if (nmods > 0) then;do;
    do i = 1 to ncol(modvals);
      if (modvnm[1,i]=wname) then;do;wcol=i;
      end;
      if (modvnm[1,i]=zname) then;do;zcol=i;
      end;
      if (modvnm[1,i]=vname) then;do;vcol=i;
      end;
      if (modvnm[1,i]=qname) then;do;qcol=i;
      end;
    end;
  end;
  if (dichy=1) then;do;
    omx=max(y);omn=min(y);y=(y=omx);rcd=omn||0;rcd1=omx||1;rcd=rcd//rcd1;
  end;
  data=cons||y||m||x;
  datamed=data;datayed=data;
  datanm={"CONSTANT"}//yname//t(mnames)//xname;
  datanmm={"CONSTANT"}//yname//t(mnames)//xname;
  datanmy={"CONSTANT"}//yname//t(mnames)//xname;
  yintkey={" "}||{" "}||{" "}||{" "}||{" "}||{" "};
  if ((model < 4) & (errs = 0)) then;do;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||mnames||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datayed=datayed||(x#m);
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
    do i = 1 to nrow(modvals);
      vmat[1,i]=1;
      vmat[2,i]=modvals[i,1];
    end;
  end;
  if ((model = 2) | (model = 3)) then;do;
    int1=x#w;
    datayed=datayed||w||int1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//wname//yintemp[1,intcnt];
```

```
    intcnt=intcnt+1;
    do i = 1 to nrow(modvals);
      vmat[2,i]=modvals[i,2];
      vmat[3,i]=modvals[i,1];
      vmat[4,i]=modvals[i,1]#modvals[i,2];
    end;
  end;
  if (model = 3) then;do;
    yintkeyt=yintemp[1,intcnt]||mnames||{"  X"}||wname||{"  "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
    int1=w#m;int2=x#w#m;
    datayed=datayed||int1||int2;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||mnames||{"
X"}||wname;yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
  end;
  if ((model = 4) | (model = 5)) then;do;
    vmat=j(8,1,1);
  end;
  yintkey2=yintkey;
  if (wm = 1) then;do;
    int1=x#w;
    datamed=datamed||w||int1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmm=datanmm//wname//yintemp[1,intcnt];
    intcnt=intcnt+1;
    do i = 1 to nrow(modvals);
      vmat[2,i]=modvals[i,wcol];
    end;
    if (zm = 1) then;do;
      int1=x#z;
      datamed=datamed||z||int1;
      yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
      datanmm=datanmm//zname//yintemp[1,intcnt];
      intcnt=intcnt+1;
      do i = 1 to nrow(modvals);
        vmat[3,i]=modvals[i,zcol];
      end;
    end;
    if (wzm = 1) then;do;
      yintkeyt=yintemp[1,intcnt]||wname||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
      datanmm=datanmm//yintemp[1,intcnt];
      intcnt=intcnt+1;
      yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{"
X"}||zname;yintkey=yintkey//yintkeyt;
      datanmm=datanmm//yintemp[1,intcnt];
      intcnt=intcnt+1;
      int1=w#z;
      int2=x#w#z;
      datamed=datamed||int1||int2;
      do i = 1 to nrow(modvals);
        vmat[4,i]=(modvals[i,wcol])#(modvals[i,zcol]);
      end;
    end;
  end;
  mdatcol=ncol(datamed);
  mintkey=yintkey;
```

```
  yintkey={" "}||{" "}||{" "}||{" "}||{" "}||{" "};
  medints=intcnt-1;
  if ((vy=1) | (xmy=1)) then;do;
    mp=1;
    do i = 1 to nrow(modvals);
      vmat[6,i]=modvals[i,1];
    end;
    if (vy=1) then;do;
      datayed=datayed||v;
      datanmy=datanmy//vname;
      mmods=1;
      do i = 1 to nrow(modvals);
        vmat[6,i]=modvals[i,vcol];
      end;
      if (qy=1) then;do;
        mp=2;datayed=datayed||q;
        datanmy=datanmy//qname;
        mmods=2;
        do i = 1 to nrow(modvals);
          vmat[7,i]=modvals[i,qcol];
        end;
      end;
      if (vqy=1) then;do;
        mp=3;datayed=datayed||(v#q);
        mmodls=3;
        do i = 1 to nrow(modvals);
          vmat[8,i]=modvals[i,vcol]#modvals[i,qcol];
        end;
      end;
    end;
    mints=j(n,(nmeds*mp),0);
    do i = 0 to (nmeds-1);
      if ((i = 0) & (vqy=1)) then;do;
        yintkeyt=yintemp[1,intcnt]||vname||{"  X"}||qname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
      if (vy=1) then;do;
        mints[,((i*mp)+1)]=m[,(i+1)]#v;
        yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||vname||{"
"}||{" "};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
      if (xmy=1) then;do;
        mints[,((i*mp)+1)]=m[,(i+1)]#x;
        yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||xname||{"
"}||{" "};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
      if (qy=1) then;do;
        mints[,((i*mp)+2)]=m[,(i+1)]#q;
        yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||qname||{"
"}||{" "};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
        if (vqy=1) then;do;
          mints[,((i*mp)+3)]=m[,(i+1)]#v#q;
          yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||vname||{"
X"}||qname;yintkey=yintkey//yintkeyt;
          datanmy=datanmy//yintemp[1,intcnt];
```

```
          intcnt=intcnt+1;
        end;
      end;
    end;
    datayed=datayed||mints;
  end;
  mp=1;
  if (wvmy=1) then;do;
    mp=2;
    do i = 1 to nrow(modvals);
      vmat[8,i]=modvals[i,wcol]#modvals[i,vcol];
    end;
  end;
  mints2=j(n,(nmeds*mp),0);
  if (wmy=1) then;do;
    do i = 1 to nrow(modvals);
      vmat[7,i]=modvals[i,wcol];
    end;
    if ((wy=0) & (model > 3)) then;do;
      datayed=datayed||w;
      datanmy=datanmy//wname;
    end;
    do i = 0 to (nmeds-1);
      if ((i = 0) & (wvmy = 1)) then;do;
        datayed=datayed||(w#v);
        yintkeyt=yintemp[1,intcnt]||wname||{"  X"}||vname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
      mints2[,((i*mp)+1)]=m[,(i+1)]#w;
      yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||wname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
      datanmy=datanmy//yintemp[1,intcnt];
      intcnt=intcnt+1;
      if (wvmy=1) then;do;
        mints2[,((i*mp)+2)]=m[,(i+1)]#w#v;
        yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||wname||{"
X"}||vname;yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
    end;
    datayed=datayed||mints2;
  end;
  mp=1;
  if (zmy=1) then;do;
    do i = 1 to nrow(modvals);
      vmat[6,i]=modvals[i,zcol];
    end;
    if (wzmy=1) then;do;
      mp=2;
      do i = 1 to nrow(modvals);
        vmat[8,i]=modvals[i,zcol]#modvals[i,wcol];
      end;
    end;
  end;
  if (zmy=1) then;do;
    mints3=j(n,(nmeds*mp),0);
    if (zy=0) then;do;
      datayed=datayed||z;
      datanmy=datanmy//zname;
    end;
```

```
    do i = 0 to (nmeds-1);
      if ((i=0) & (wzmy = 1) & (wzy=0)) then;do;
        datayed=datayed||(w#z);
        yintkeyt=yintemp[1,intcnt]||wname||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
      mints3[,((i*mp)+1)]=m[,(i+1)]#z;
      yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
      datanmy=datanmy//yintemp[1,intcnt];
      intcnt=intcnt+1;
      if (wzmy = 1) then;do;
        mints3[,((i*mp)+2)]=m[,(i+1)]#w#z;
        yintkeyt=yintemp[1,intcnt]||mnames[1,(i+1)]||{"  X"}||wname||{"
X"}||zname;yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
    end;
    datayed=datayed||mints3;
  end;
  decoc=1;
  modmat=j(5,5,999);modmatv=j(1,5,1);modmatp=j(1,5,0);modprod=modmatv;
  if ((wy = 1) & (model > 3)) then;do;
    datayed=datayed||w||x#w;
    decoc=decoc+1;
    deco[decoc,1]=ncol(datayed)-1;
    modmatv[1,1]=nrow(matw);
    modmat[(1:nrow(matw)),1]=matw;
    modvnm2[1,1]=wname;
    modmatp[1,1]=1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//wname//yintemp[1,intcnt];
    intcnt=intcnt+1;
  end;
  if (zy = 1) then;do;
    datayed=datayed||z||x#z;
    decoc=decoc+1;
    deco[decoc,1]=ncol(datayed)-1;
    modmatv[1,2]=nrow(matz);
    modmat[(1:nrow(matz)),2]=matz;
    modvnm2[1,2]=zname;
    modmatp[1,2]=1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//zname//yintemp[1,intcnt];
    intcnt=intcnt+1;
  end;
  if (wzy = 1) then;do;
    datayed=datayed||w#z||x#w#z;
    decoc=decoc+1;
    deco[decoc,1]=ncol(datayed)-1;
    yintkeyt=yintemp[1,intcnt]||wname||{"  X"}||zname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{"
X"}||zname;yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
```

```
  end;
  if (vxy = 1) then;do;
    datayed=datayed||x#v;
    decoc=decoc+1;
    deco[decoc,1]=ncol(datayed)-1;
    modmatv[1,3]=nrow(matv);
    modmat[(1:nrow(matv)),3]=matv;
    modvnm2[1,3]=vname;
    modmatp[1,3]=1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||vname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
    if (qxy = 1) then;do;
      datayed=datayed||x#q;
      decoc=decoc+1;
      deco[decoc,1]=ncol(datayed)-1;
      modmatv[1,4]=nrow(matq);
      modmat[(1:nrow(matq)),4]=matq;
      modvnm2[1,4]=qname;
      modmatp[1,4]=1;
      yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||qname||{" "}||{"
"};yintkey=yintkey//yintkeyt;
      datanmy=datanmy//yintemp[1,intcnt];
      intcnt=intcnt+1;
      if (vqxy = 1) then;do;
        datayed=datayed||x#v#q;
        decoc=decoc+1;
        deco[decoc,1]=ncol(datayed)-1;
        yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||vname||{"
X"}||qname;yintkey=yintkey//yintkeyt;
        datanmy=datanmy//yintemp[1,intcnt];
        intcnt=intcnt+1;
      end;
    end;
  end;
  if (wvxy = 1) then;do;
    datayed=datayed||x#w#v;
    decoc=decoc+1;
    deco[decoc,1]=ncol(datayed)-1;
    yintkeyt=yintemp[1,intcnt]||xname||{"  X"}||wname||{"
X"}||vname;yintkey=yintkey//yintkeyt;
    datanmy=datanmy//yintemp[1,intcnt];
    intcnt=intcnt+1;
  end;
  modvalsd=0;ttt=modmatp[,+];ssss=modmatp[,+];
  if (ssss > 0) then;do;
    tmp=1;
    do i = 1 to 5;
      if (modmatp[1,i]=1) then;do;
        modmat[,tmp]=modmat[,i];
        modvnm2[1,tmp]=modvnm2[1,i];
        modmatv[1,tmp]=modmatv[1,i];
        tmp=tmp+1;
      end;
    end;
    modmat=modmat[,(1:ttt)];
    modvnm2=modvnm2[,(1:ttt)];
    modmatv=modmatv[,(1:ttt)];
    do i = 1 to (ncol(modmatv)-1);
      tmp=1;
      do j = (i+1) to ncol(modmatv);
        tmp=tmp*modmatv[1,j];
```

```
    end;
    modprod[1,i]=tmp;
  end;
  modvalsd=j((modmatv[1,1]*modprod[1,1]),ttt,0);
  do i = 1 to ttt;
    strt=1;fnsh=0;
    do while (fnsh < nrow(modvalsd));
      do j = 1 to modmatv[1,i];
        tmp=j(modprod[1,i],1,modmat[j,i]);
        fnsh=fnsh+nrow(tmp);
        modvalsd[(strt:fnsh),i]=tmp;
        strt=fnsh+1;
      end;
    end;
  end;
end;
if (ttt > 0) then;do;
  do i = 1 to ncol(modvalsd);
    if (modvnm2[1,i] = wname) then;do;wcol=i;
    end;
    if (modvnm2[1,i] = zname) then;do;zcol=i;
    end;
    if (modvnm2[1,i] = vname) then;do;vcol=i;
    end;
    if (modvnm2[1,i] = qname) then;do;qcol=i;
    end;
  end;
  directv=j(nrow(modvalsd),1,1);
  if (wy=1) then;do;directv=directv||modvalsd[,wcol];
  end;
  if (zy=1) then;do;directv=directv||modvalsd[,zcol];
  end;
  if (wzy=1) then;do;directv=directv||(modvalsd[,wcol])#(modvalsd[,zcol]);
  end;
  if (vxy=1) then;do;directv=directv||modvalsd[,vcol];
  end;
  if (qxy=1) then;do;directv=directv||modvalsd[,qcol];
  end;
  if (vqxy=1) then;do;directv=directv||(modvalsd[,vcol])#(modvalsd[,qcol]);
  end;
  if (wvxy=1) then;do;directv=directv||(modvalsd[,vcol])#(modvalsd[,wcol]);
  end;
end;
ydatacol=ncol(datayed);
if (ncovs > 0) then;do;
  if (covmy ^= 2) then;do;
    datamed=datamed||c;
  end;
  if (covmy ^= 1) then;do;
    datayed=datayed||c;
  end;
  covmeans=c[+,]/n;
end;
if (cluster > 0) then;do;
  datamed=datamed||cld;
  datayed=datayed||cld;
  cldmeans=cld[+,]/n;
end;
mst=3;
mnd=mst+nmeds-1;
ydatacol=ncol(datayed);
mdatacol=ncol(datamed);
if (ncovs > 0) then;do;
```

```
  datanmy=datanmy//(cnames`);
  if (model > 3) then;do;
    datanmm=datanmm//(cnames`);
  end;
end;
datanmy={"constant"}//datanmy[3:nrow(datanmy),1];
if (model > 3) then;do;
  datanmm={"constant"}//datanmm[3:nrow(datanmm),1];
end;
amm=j(2,1,0);abmm=j(2,1,0);mnv=datayed[,2]/n;mnv=mnv[+,];mnv=j(n,1,mnv);
ssty=(datayed[,2]-mnv)##2;ssty=ssty[+,];
sigma=(n*t(datayed)*datayed)-(t(datayed[+,])*datayed[+,]);
sigma=sigma/(n*(n-1));
if (ws=1) then;do;
  stddevm=vecdiag(sigma);stddevm=sqrt(stddevm[3:(nmeds+2),1]);
end;
stddevy=sqrt(sigma[2,2]);
stddevx=sqrt(sigma[(3+nmeds),(3+nmeds)]);
r2xy=(sigma[2,(3+nmeds)]/(stddevy*stddevx))##2;
r2my=(sigma[2,3]/(stddevy*sqrt(sigma[3,3])))##2;
ctot=sigma[2,(3+nmeds)]/sigma[(3+nmeds),(3+nmeds)];
if ((model = 4) & (nmeds = 1) & (cluster = 0) & (ncovs = 0)) then;do;
  kappaa=sigma[2,3]*sigma[2,4];
  kappab=sqrt((sigma[3,3]*sigma[2,2])-(sigma[2,3]*sigma[2,3]));
  kappac=sqrt((sigma[4,4]*sigma[2,2])-(sigma[2,4]*sigma[2,4]));
  kappad=sigma[4,4]*sigma[2,2];
  kappae=sqrt((sigma[4,4]*sigma[3,3])-(sigma[3,4]*sigma[3,4]));
  amm[1,1]=(kappaa+(kappab*kappac))/kappad;
  amm[2,1]=(kappaa-(kappab*kappac))/kappad;
  if (sigma[3,4] < 0) then;do;
    amma=min(amm);
  end;
  if (sigma[3,4] > 0) then;do;
    amma=max(amm);
  end;
  abmm[1,1]=-amma*(kappac/kappae);
  abmm[2,1]=amma*(kappac/kappae);
end;
datatm=datamed;
dataty=datayed;
mdlnms2=compress(char(model))//yname//xname;
mdlnms={"Model ="}//{"Y     = "}//{"X     = "};
if (ws=1) then;do;
  mdlnms2=compress(char(model))//yname;
  mdlnms={"Model ="}//{"Y     = "};
end;
do i = 1 to ncol(mnames);
  mdlnms2=mdlnms2//mnames[1,i];
  if ((i=1) & (ncol(mnames)=1)) then;
    mdlnms=mdlnms//{"M     = "};
    else mdlnms=mdlnms//mlab[i,1];
end;
if (wname ^= "XXX") then;do;
  mdlnms2=mdlnms2//wname;
  mdlnms=mdlnms//{"W     = "};
end;
if (zname ^= "XXX") then;do;
  mdlnms2=mdlnms2//zname;
  mdlnms=mdlnms//{"Z     = "};
end;
if (vname ^= "XXX") then;do;
  mdlnms2=mdlnms2//vname;
  mdlnms=mdlnms//{"V     = "};
```

```
  end;
  if (qname ^= "XXX") then;do;
    mdlnms2=mdlnms2//qname;
    mdlnms=mdlnms//{"Q     = "};
  end;
  if ((jn = 1) & (model = 1) & (jndich = 1)) then;do;
    note[notes,1]=8;
    notes=notes+1;
  end;
  if ((jn = 1) & (model = 3) & (jndich = 1)) then;do;
    note[notes,1]=8;
    notes=notes+1;
  end;
  yes=((nrow(modvals)=1) & (contrast=1) & (model = 3 | model = 2));
  yes2=((nrow(modvals)=1) & (contrast=1) & (model = 2));

  print "************************ PROCESS Procedure for SAS Release 2.13
*************************";
  print "Written by Andrew F. Hayes, Ph.D.  http://www.afhayes.com";
  print "Documentation available in Hayes (2013).
www.guilford.com/p/hayes3";
  print
"**************************************************************************
**************";
  print mdlnms2 [rowname = mdlnms label = "Model and Variables"];
  if (ncovs > 0) then;do;
    print cnames [label = "Statistical controls:"];
  end;
  print n [label = "Sample size:" format = 7.0];
  if (cluster > 0) then;do;
    print cluster [label = "Clustering variable and number of clusters:"
rowname = cvname];
  end;
  if ((model > 3) & (&seed ^= 0)) then;do;
    seedt=&seed;
      print seedt [label = "Custom seed:"];
  end;
  do bt = 1 to (boot+1); *[b];
    if ((bt = 2) & (savboot = 1)) then;do;
         bootstrp=j(boot,ncol(bootcoef),-999);
      end;
      bootcoef=0;
    if (bt > 1) then;do;
      rk=1;
      do while (rk = 1);
        v=floor((ranuni(j(n,1,&seed)))*n)+1;
        datayed=datay[v,];
        detcheck=det(t(datayed)*datayed);
            rk=(detcheck=0);
        if (model > 3) then;do;
              datamed=datatm[v,];
              detcheck=det(t(datamed)*datamed);
              if (rk=1) then;do;rk=(detcheck=0);end;
        end;
            sigma=(n*t(datayed)*datayed)-(t(datayed[+,])*datayed[+,]);
        sigma=sigma/(n*(n-1));
        temp=vecdiag(sigma);
        * rk=temp[2:nrow(temp),1];*rk=(rk = 0);*rk=rk[+,];
            bad=bad+rk;
            false=1;
      end;
      stddevy=sqrt(sigma[2,2]);
        stddevx=sqrt(sigma[(3+nmeds),(3+nmeds)]);
```

```
    ctot=sigma[2,(3+nmeds)]/sigma[(3+nmeds),(3+nmeds)];
  if ((model = 4) & (nmeds = 1) & (cluster = 0) & (ncovs = 0)) then;do;
      r2xy=(sigma[2,4]/(stddevy*stddevx))##2;
    r2my=(sigma[2,3]/(stddevy*sqrt(sigma[3,3])))##2;
    sstot=sigma[2,2]*(n-1);
    kappaa=sigma[2,3]*sigma[2,4];
    kappab=sqrt((sigma[3,3]*sigma[2,2])-(sigma[2,3]*sigma[2,3]));
    kappac=sqrt((sigma[4,4]*sigma[2,2])-(sigma[2,4]*sigma[2,4]));
    kappad=sigma[4,4]*sigma[2,2];
    kappae=sqrt((sigma[4,4]*sigma[3,3])-(sigma[3,4]*sigma[3,4]));
    amm[1,1]=(kappaa+(kappab*kappac))/kappad;
    amm[2,1]=(kappaa-(kappab*kappac))/kappad;
    if (sigma[3,4] < 0) then;do;
      amma=min(amm);
    end;
    if (sigma[3,4] > 0) then;do;
      amma=max(amm);
    end;
    abmm[1,1]=-amma*(kappac/kappae);
    abmm[2,1]=amma*(kappac/kappae);
  end;
  end;
  * mediator model;
if (model > 3) then;do;
  do im = 1 to nmeds;
      xm=cons||datamed[,((mnd+1):mdatacol)];
        xmnm={"Constant"}//datanmm[((2+nmeds):nrow(datanmm)),1];
        invxtx=inv(xm`*xm);
        coeff=invxtx*xm`*datamed[,(2+im)];
    if (model = 6) then;do;
          if (im = 1) then;do;
            xm=cons||datamed[,((mnd+1):mdatacol)];
        invxtx=inv(xm`*xm);
              coeff=invxtx*xm`*datamed[,(2+im)];
          end;
          if (im > 1) then;do;
            xm=cons||datamed[,(3:(im+1))]||datamed[,((mnd+1):mdatacol)];

  xmnm={"Constant"}//datanmm[(2:im),1]//datanmm[(mnd:nrow(datanmm)),1];
              invxtx=inv(xm`*xm);
              coeff=invxtx*xm`*datamed[,(2+im)];
              mmpaths[(im+1),(2:im)]=(coeff[(2:im),1])`;
          end;
        end;
        if (ws=1) then;do;
          coeff[1,1]=datamed[+,(2+im)]/n;
        end;
        bootcoef=bootcoef||(coeff[1:(nrow(coeff)-clsdmy),1])`;
    if (bt = 1) then;do;
          resid=datamed[,(2+im)]-xm*coeff;
          sse=ssq(resid);
      mse=sse/(n-ncol(xm));
          mnv=data[,(2+im)];mnv=mnv[+,]/n;mnv=j(n,1,mnv);
      sstm=(data[,(2+im)]-mnv)##2;sstm=sstm[+,];
          k3=nrow(coeff);
          if (hc3 = 1) then;do;
            h=xm[,1];
              do i3 = 1 to n;
                h[i3,1]=xm[i3,]*invxtx*(xm[i3,])`;
              end;
              do i3 = 1 to k3;
                xm[,i3]=(resid[,ncol(resid)]/(1-h))#(xm[,i3]);
              end;
```

```
            end;
            if (hc3 ^= 1) then;do;
              do i3 = 1 to k3;
                  xm[,i3]=sqrt(mse)#xm[,i3];
                end;
            end;
            lmat=I(nrow(coeff));
            lmat=lmat[,2:ncol(lmat)];
            hccov=invxtx*xm`*xm*invxtx;
            mcmats[im,im]=hccov[2,2];
          dfnum=nrow(coeff)-1;
            dfden=n-dfnum-1;

fratio=(((lmat`)*coeff)`*inv(lmat`*hccov*lmat)*(lmat`*coeff))/dfnum;
          coeff=coeff[1:(nrow(coeff)-clsdmy),1];
            mccoeff[im,1]=coeff[2,1];
            standerr=sqrt(vecdiag(invxtx*xm`*xm*invxtx));
            standerr=standerr[1:(nrow(standerr)-clsdmy),1];
            if (ws=1) then;do;
              standerr[1,1]=stddevm[im,1]/sqrt(n);
            end;
          tratio=coeff/standerr;
            p=2*(1-probt(abs(tratio),(n-ncol(xm))));
            temp=(n-ncol(xm));
            if (ws=1) then;do;
              p=2*(1-probt(abs(tratio),(n-1)));
                temp=n-1;
          end;
            xd=abs(xp2);
            temp=(temp*(exp((temp-(5/6))*((xd/(temp-
(2/3)+(.11/temp)))*(xd/(temp-(2/3)+(.11/temp)))))-1));
          temp1=coeff-sqrt(abs(temp))*standerr;
            temp2=coeff+sqrt(abs(temp))*standerr;
            op=coeff||standerr||tratio||p||temp1||temp2;
            if (ws=1) then;do;
              op=op[1,];
                xmnm=apathnam[((nmeds>1)+1+(im-1)),1];
            end;
            sobel[im,1]=coeff[2,1];
            sobel[im,2]=standerr[2,1];
          temp=mnames[1,im];
            r2full=1-(sse/sstm);
            pfr=1-probf(fratio,dfnum,dfden);
            summ=sqrt(r2full)||r2full||mse||fratio||dfnum||dfden||pfr;
          if (detail = 1) then;do;
                print
"*************************************************************************
*************";
                print temp [rowname = "Outcome:" label = " "];
                  clnm = {"R" "R-sq" "MSE" "F" "df1" "df2" "p"};
             if (ws ^= 1) then;do;
                print summ [label = "Model Summary" colname = clnm
format=&decimals];
                  end;
                  if (coeffci=0) then;do;
                    op=op[,1:(ncol(op)-2)];
             end;
                  clnm = {"coeff" "se" "t" "p" "LLCI" "ULCI"};
                  print op [label = "Model" colname = clnm rowname = xmnm
format=&decimals];
                  if (covcoeff=1) then;do;
              hccovtmp=hccov[1:nrow(op),1:nrow(op)];
                    cnamestp=xmnm`;
```

```
                print hccovtmp [label = "Covariance matrix of regression
parameter estimates" rowname=xmnm colname=cnamestp format=&decimals];
              end;
              if ((nmods > 0) & (nrow(mintkey) > 1)) then;do;
                print mintkey [label = "Interactions:"];
              end;
              if (ws = 1) then;do;
                wsdf=n-1;
                tmpnamb={"df = "};
                print wsdf [label = " " rowname=tmpnamb];
              end;
            end;
        end;
      ymat[1,im]=coeff[2,1];
          if (ws=1) then;do;
            ymat[1,im]=coeff[1,1];
          end;
          if (wm = 1) then;do;
            ymat[2,im]=coeff[4,1];
            if (zm = 1) then;do;
              ymat[3,im]=coeff[6,1];
                if (wzm = 1) then;do;
                  ymat[4,im]=coeff[8,1];
                end;
            end;
          end;
      if (model = 6) then;do;
            mmpaths[(im+1),1]=coeff[(im+1),1];
          end;
      end;
    end;

  * estimate model of outcome;
  do totlp = 1 to (1+(toteff*(bt=1))); *[d];
      xy=cons||datayed[,3:ydatacol];
    if ((toteff=1) & (totlp = 2)) then;do;
      xy=cons||datayed[,(3+nmeds):ydatacol];
    end;
      if (dichy = 1) then;do;
        meany=datayed[,2];meany=meany[+,]/n;
        pt2=j(nrow(datayed[,2]),1,meany);
        LL3 = datayed[,2]#log(pt2)+(1-datayed[,2])#log(1-pt2);
        LL3 = -2*LL3[+,];
        pt1=j(n,1,0.5);
        bt1=j(ncol(xy),1,0);
        LL1=0;
        LL2=LL3;
        xy22=xy;
      do jjj= 1 to iterate UNTIL (abs(LL1-LL2) < converge);
          LL1=LL2;
          do ijk = 1 to ncol(xy);
              xy22[,ijk]=xy[,ijk]#pt1#(1-pt1);
          end;
          coeff=bt1+inv(xy22`*xy)*xy`*(datayed[,2]-pt1);
          pt1 = 1/(1+exp(-(xy*coeff)));
      temp1=((pt1 < .00000000000001) | (pt1 > .9999999999999));
          itprob=temp1[+,];
          if (itprob = 0) then;do;
            LL=datayed[,2]#log(pt1)+(1-datayed[,2])#log(1-pt1);
            LL2=-2*LL[+,];
          end;
          bt1=coeff;
      end;
```

```
          if ((jjj >= iterate) & (iterr = 0)) then;do;
            errs=errs+1;runerrs[err,1]=22;iter=1;
          end;
          do ijk = 1 to ncol(xy);
          xy22[,ijk]=xy[,ijk]#pt1#(1-pt1);
          end;
          covmat=inv(xy22`*xy);
            if (totlp ^= 2) then;do;
              bootcoef=bootcoef||(coeff[1:(nrow(coeff)-clsdmy),1])`;
            end;
        end;
        if (dichy = 0) then;do;
          invxtx=inv(xy`*xy);
            coeff=invxtx*xy`*datayed[,2];
        if (effsize=1 & model > 3 & model < 7 & (ncovs > 0 | clsdmy > 0))
then;do;
          xysdy=cons||datayed[,(4+nmeds):ydatacol];
              coeffsd=inv(xysdy`*xysdy)*xysdy`*datayed[,2];
          resid=datayed[,2]-(xysdy*coeffsd);
          sdycov=sqrt(ssq(resid)/(n-ncol(xysdy)));
              xvaron=datayed[,(3+nmeds)];
              coeffx2=inv(xysdy`*xysdy)*xysdy`*xvaron;
          residx2=xvaron-(xysdy*coeffx2);
          sdxcov=sqrt(ssq(residx2)/(n-ncol(xysdy)));
        end;
            if (totlp ^= 2) then;do;
              bootcoef=bootcoef||(coeff[1:(nrow(coeff)-clsdmy),1])`;
            end;
            if ((nmeds = 1) & (ncovs = 0) & (cluster = 0) & (model = 4) & (bt
> 1)) then;do;
              resid=datayed[,2]-xy*coeff;sse=ssq(resid);r2full=1-(sse/sstot);
            end;
            if (bt = 1) then;do;
              resid=data[,2]-xy*coeff;
              k3=nrow(coeff);
              sse=ssq(resid);mse=sse/(n-ncol(xy));
              if (hc3 = 1) then;do;
                h=xy[,1];
                  do i3 = 1 to n;
                    h[i3,1]=xy[i3,]*invxtx*xy[i3,]`;
                  end;
                  do i3 = 1 to k3;
                    xy[,i3]=(resid[,ncol(resid)]/(1-h))#(xy[,i3]);
                  end;
              end;
          if (hc3 ^= 1) then;do;
                do i3 = 1 to k3;
                  xy[,i3]=sqrt(mse)#xy[,i3];
                end;
              end;
              covmat=invxtx*xy`*xy*invxtx;
          end;
        end;
        if (bt = 1) then;do; *[f];
          if (model=2) then;do;
              xy2=cons||datayed[,3:ydatacol];
              temp=ncol(xy2);
              if (temp > 6) then;do;
                xy3=xy2[,7:temp];
              end;
              xy2=xy2[,1:3]||xy2[,5];
          if (temp > 6) then;do;
                xy2=xy2||xy3;
```

```
            end;
            invxtx=inv(xy2`*xy2);
            coeff2=invxtx*xy2`*datayed[,2];
            ssem2=ssq(datayed[,2]-xy2*coeff2);
          end;
          standerr=sqrt(vecdiag(covmat));
          if (totlp=1) then;do;

mcmats[(nmeds+1):ncol(mcmats),(nmeds+1):ncol(mcmats)]=covmat[2:(1+nmeds),2:(1
+nmeds)];
          end;
          standerr=standerr[1:(nrow(standerr)-clsdmy),1];
          if (ws=1 & totlp=2) then;do;
            standerr[1,1]=stddevy/sqrt(n);
          end;
          coeffplt=coeff;
          lmat=I(nrow(coeff));
          lmat=lmat[,2:ncol(lmat)];
          dfnum=nrow(coeff)-1;
          dfden=n-dfnum-1;
        fratio=(((lmat`)*coeff)`*inv(lmat`*covmat*lmat)*(lmat`*coeff))/dfnum;
          coeff=coeff[1:(nrow(coeff)-clsdmy),1];
          if (totlp=1) then;do;
            mccoeff[(nmeds+1):nrow(mccoeff)]=coeff[2:(1+nmeds),1];
          end;
          bbbb=coeff[2,1];
        if (totlp = 1) then;do;
            deco[1,1]=2+nmeds;
            deco=deco[1:decoc,1];
            covdirt=j((nrow(covmat)-clsdmy),(ncol(covmat)-clsdmy),0);
            covdirt=covmat[deco,];
            covdir=j(nrow(covdirt),nrow(covdirt),0);
            covdir=covdirt[,deco`];
            deco=coeff[deco,1];
            if (ttt > 0) then;do;
              sedir=sqrt(vecdiag(directv*covdir*directv`));
                directv=directv*deco;
            end;
            sobel[,3]=coeff[2:(1+nmeds),1];
            sobel[,4]=standerr[2:(1+nmeds),1];
            sobel2=sobel#sobel;
            if (varorder ^= 2) then;do;
              sobel[,2]=sqrt(sobel2[,1]#sobel2[,4]+sobel2[,3]#sobel2[,2]);
            end;
            if (varorder = 2) then;do;

sobel[,2]=sqrt(sobel2[,1]#sobel2[,4]+sobel2[,3]#sobel2[,2]+sobel2[,2]#sobel2[
,4]);
            end;
            sobel[,1]=sobel[,1]#sobel[,3];
            sobel[,3]=sobel[,1]/sobel[,2];
            sobel[,4]=2*(1-probnorm(abs(sobel[,3])));
          end;
          if (dichy=0) then;do;
            tratio=coeff/standerr;
            p=2*(1-probt(abs(tratio),(n-ncol(xy))));
            if ((ws=1) & (totlp=2)) then;do;
              p=2*(1-probt(abs(tratio),(n-1)));
                dfden=n-1;
            end;
         cnms="coeff"||"se"||"t"||"p"||"LLCI"||"ULCI";
            op=coeff||standerr|tratio||p;
          end;
```

```
        if(dichy=1) then;do;
              tratio=coeff/standerr;
          p=2*(1-probnorm(abs(tratio)));
              wald=tratio#tratio;
              cnms="coeff"||"se"||"Z"||"p"||"LLCI"||"ULCI";
              temp=coeff-abs(xp2)*standerr;
              op=coeff||standerr||tratio||p||temp;
              temp=coeff+abs(xp2)*standerr;
              op=op||temp;
            end;
        if (detail = 1) then;do;
              if (totlp=2) then;do;
                print "******************************** TOTAL EFFECT MODEL
*******************************";
                print yname [rowname = "Outcome:" label = " "];
              end;
              if (totlp ^= 2) then;do;
                print
"*****************************************************************************
*************";
                print yname [rowname = "Outcome:" label = " "];
              end;
            end;
            if ((dichy=1) & (bt=1) & (totlp=1)) then;do;
              nmsd=yname||"Analysis";
              print rcd [label = "Coding of binary DV for analysis" colname =
nmsd format = 9.2];
            end;
        if (dichy = 0) then;do;
              r2full=1-(sse/ssty);
              pfr=1-probf(fratio,dfnum,dfden);
              jndf=dfden;
              if (ws=1) then;do;
                wsdf=dfden;
              end;
              xd=abs(xp2);
              jncrit=(dfden*(exp((dfden-(5/6))*((xd/(dfden-
(2/3)+(.11/dfden)))*(xd/(dfden-(2/3)+(.11/dfden)))))-1));
              summ=sqrt(r2full)||r2full||mse||fratio||dfnum||dfden||pfr;
              temp1=coeff-sqrt(jncrit)*standerr;
              temp2=coeff+sqrt(jncrit)*standerr;
              op=coeff||standerr||tratio||p||temp1||temp2;
              if (detail = 1) then;do;
                if (ws ^= 1) then;do;
                    clnm = {"R" "R-sq" "MSE" "F" "df1" "df2" "p"};
                  print summ [label = "Model Summary" colname = clnm format =
&decimals];
                  end;
                end;
            end;
            if (dichy = 1) then;do;
              LLdiff=LL3-LL2;
              mcF = LLdiff/LL3;
              cox=1-exp(-LLdiff/n);
              nagel=cox/(1-exp(-(LL3)/n));
              pf=LL2||LLdiff||mcF||cox||nagel||n;
              if (detail = 1) then;do;
                clnm = {"-2LL" "Model LL" "McFadden" "CoxSnell" "Nagelkrk"
"n"};
                print pf [label = "Logistic Regression Summary" colname = clnm
format = &decimals];
              end;
            end;
```

```
            if (totlp=2) then;do;
              datanmy="constant"//datanmy[(nmeds+2):nrow(datanmy),1];
            end;
            if (detail = 1) then;do;
              if (coeffci = 0) then;do;
                op=op[,1:(ncol(op)-2)];
              end;
              if (ws = 1) then;do;
                op2=op[1:(1+nmeds),];
                  datanmy2="c'
path"//bpathnam[(1+(nmeds>1)):((1+(nmeds>1))+(nmeds-1)),1];
                 if (totlp=2) then;do;
                   datanmy2="c path";op2=op[1,];wsdf=n-1;
                 end;
                 print op2 [label = "Model" rowname=datanmy2 colname=cnms
format = &decimals];
                 tmpnamb={"df = "};
                print wsdf [label = " " rowname=tmpnamb];
          end;
              if (ws ^= 1) then;do;
                print op [label = "Model" rowname = datanmy colname=cnms
format = &decimals];
                 if (covcoeff=1) then;do;
               covmattp=covmat[1:nrow(op),1:nrow(op)];
                     cnamestp=datanmy`;
                     print covmattp [label="Covariance matrix of regression
parameter estimates" rowname=datanmy colname=cnamestp format=&decimals];
                 end;
              end;
            end;
            if ((ttt = 0) & (totlp = 1)) then;do;
          deco=op[(nmeds+2),];
              if (ws=1) then;do;
                deco=op[1,];
              end;
            end;
            if ((ttt = 0) & (totlp = 2)) then;do;
              decotot = op[2,];
              if (ws=1) then;do;
                decotot=op[1,];
              end;
            end;
            if ((nmods > 0) & (model > 4) & (detail = 1) & (nrow(yintkey) >
1)) then;do;
              print yintkey [label = "Interactions:"];
            end;
            if ((nmods > 0) & (model < 4) & (detail = 1)) then;do;
              print yintkey2 [label = "Interactions:"];
              if (((model = 1) | (model = 2)) & (dichy = 0) & (hc3 = 0))
then;do;
             temp = (((op[4,3]#op[4,3])*(1-
r2full))/dfden)||(op[4,3]#op[4,3])||1||dfden||op[4,4];
                 rnms=yintkey2[2,1];
                 if (model = 2) then;do;
                   temp2=(((op[6,3]#op[6,3])*(1-
r2full))/dfden)||(op[6,3]#op[6,3])||1||dfden||op[6,4];
               temp = temp//temp2;
                     frat2=(dfden*(r2full-(1-(ssem2/ssty))))/(2*(1-r2full));
                     temp2=(r2full-(1-(ssem2/ssty)))||frat2||2||dfden||(1-
probf(frat2,2,dfden));
                   temp = temp//temp2;
                   rnms=rnms//yintkey2[3,1]//"Both";
                 end;
```

```
                clnm = {"R2-chng" "F" "df1" "df2" "p"};
                  print temp [label = "R-square increase due to
interaction(s):" rowname=rnms colname=clnm format = &decimals];
              end;
           if ((model = 3) & (dichy = 0) & (hc3 = 0)) then;do;
                 temp = (((op[8,3]#op[8,3])*(1-
r2full))/dfden)||(op[8,3]#op[8,3])||dfden||op[8,4];
                 rnms=yintkey2[5,1];
                 clnm = {"R2-chng" "F(1,df2)" "df2" "p"};
                 print temp [label = "R-square increase due to three-way
interaction:" rowname = rnms colname = clnm format = &decimals];
           end;
             end;
         end; *[f];
       if ((model = 6) & (totlp = 1)) then;do;
         mmpaths[nrow(mmpaths),1]=coeff[nrow(mmpaths),1];
           mmpaths[nrow(mmpaths),(2:(nmeds+1))]=coeff[2:(nmeds+1),1]`;
         end;
         if (totlp = 1) then;do; *[g];
           do im = 1 to nmeds; *[h];
               if (model < 4) then;do;
                 ymat[1,im]=coeff[3,1]*(1-
yes);ymat[2,im]=coeff[4,1];cmat[1,im]=covmat[3,3];cmat[2,im]=covmat[4,4];

cmat[5,im]=covmat[3,4];jnb1=coeff[3,1];jnb3=coeff[4,1];jnsb1=covmat[3,3];jnsb
3=covmat[4,4];
                 jnsb1b3=covmat[3,4];
                 if ((model = 2) | (model = 3)) then;do;

ymat[3,im]=coeff[6,1];cmat[3,im]=covmat[6,6];cmat[6,im]=covmat[3,6];cmat[8,im
]=covmat[4,6];
                   end;
                   if (model = 3) then;do;

ymat[4,im]=coeff[8,1];cmat[4,im]=covmat[8,8];cmat[7,im]=covmat[3,8];cmat[9,im
]=covmat[4,8];

cmat[10,im]=covmat[6,8];jnb1=coeff[4,1];jnb3=coeff[8,1];jnsb1=covmat[4,4];jns
b3=covmat[8,8];
                     jnsb1b3=covmat[4,8];
                   end;
               end;
           if (model > 3) then;do;
                 ymat[5,im]=coeff[(1+im),1];
               end;
               if (xmy = 1) then;do;
                 ymat[6,im]=coeff[(2+nmeds+im),1];
               end;
               if (vy = 1) then;do;
                 ymat[6,im]=coeff[(3+nmeds+im),1];
               end;
               if ((qy = 1) & (vy = 1)) then;do;
                 ymat[6,im]=coeff[(5+nmeds+((im-1)*2)),1];
                   ymat[7,im]=coeff[(6+nmeds+((im-1)*2)),1];
               end;
           if (vqy = 1) then;do;
                 ymat[6,im]=coeff[(6+nmeds+((im-1)*3)),1];
                   ymat[7,im]=coeff[(7+nmeds+((im-1)*3)),1];
                   ymat[8,im]=coeff[(8+nmeds+((im-1)*3)),1];
               end;
               if (wmy = 1) then;do;
                 ymat[7,im]=coeff[(3+nmeds+im-wy),1];
               end;
```

```
              if ((wmy = 1) & (vy = 1)) then;do;
            ymat[7,im]=coeff[(4+(nmeds*2)+im-wy),1];
              end;
          if ((wmy = 1) & (vy = 1) & (wvmy = 1)) then;do;
                ymat[7,im]=coeff[(6+(nmeds*2)+((im-1)*2)-wy),1];
                  ymat[8,im]=coeff[(7+(nmeds*2)+((im-1)*2)-wy),1];
              end;
          if ((wmy = 1) & (zmy = 1)) then;do;
                ymat[6,im]=coeff[((6-(wzy*3)+(wzm-1)+(nmeds*2)+((im-
1)*2)*wzm)+((im-1)*(1-wzm))-((zy-wzy)*2)),1];
            ymat[7,im]=coeff[(3-zy+im+nmeds),1];
                  if (wzmy = 1) then;do;
                    ymat[8,im]=coeff[(7-(wzy*3)+(nmeds*2)+((im-1)*2)),1];
                  end;
              end;
          if ((nmods > 0) & (model ^= 5)) then;do;
                do indlp = 1 to nrow(modvals);
                    temp1=ymat[1:4,im]#vmat[1:4,indlp];temp1=temp1[+,];
                    indeff[indlp,1]=temp1;
              if (model > 6) then;do;
                      temp2=ymat[5:8,im]#vmat[5:8,indlp];temp2=temp2[+,];
                indeff[indlp,1]=temp1*temp2;
              end;
                  end;
                  indboot[(bt+(im-1)*(boot+1)),]=indeff`;
                  if ((model = 8) | (model = 7)) then;do;
                    indbootp[bt,im]=ymat[2,im]*ymat[5,im];
                    if (wdich = 1) then;do;
                      indbootp[bt,im]=indbootp[bt,im]*(cmaxw-cminw);
                    end;
                  end;
                  if ((model = 14) | (model = 15) | (model = 74)) then;do;
                    indbootp[bt,im]=ymat[1,im]*ymat[6,im];
                    if (wvdich = 1) then;do;
                      indbootp[bt,im]=indbootp[bt,im]*(cmaxv-cminv);
                    end;
                  end;
                  if (model = 12) then;do;
                    indbootp[bt,im]=ymat[4,im]*ymat[5,im];
                  end;
            if (((model = 58) | (model = 59)) & (wvdich=1)) then;do;
              indbootp[bt,im]=(ymat[1,im]*ymat[7,im]*(cmaxw-
cminw))+(ymat[2,im]*ymat[5,im]*(cmaxw-cminw));

indbootp[bt,im]=indbootp[bt,im]+(ymat[2,im]*ymat[7,im]*((cmaxw*cmaxw)-
(cminw*cminw)));
            end;
              end;
          if ((model = 4) | (model = 5)) then;do;
                  temp1=ymat[1:4,im]#vmat[1:4,1];temp1=temp1[+,];
                  temp2=ymat[5:8,im]#vmat[5:8,1];temp2=temp2[+,];
                indboot[bt,im]=temp1*temp2;
            if ((effsize=1) & (dichy=0)) then;do;
                    if (ctot = 0) then;do;ctot=.00000000000001;end;
              sumind=indboot[bt,+];
              if (im = nmeds)
then;do;pmeff[bt,2:(im+1)]=indboot[bt,]/(sumind+coeff[(2+nmeds),1]);end;
                  rmeff[bt,(im+1)]=indboot[bt,im]/coeff[(2+nmeds),1];
                    abpseff[bt,(im+1)]=indboot[bt,im]/stddevy;
                    abcseff[bt,(im+1)]=abpseff[bt,(im+1)]*stddevx;
              if (ncovs >  0 | clsdmy > 0) then;do;
                abpseff[bt,(im+1)]=indboot[bt,im]/sdycov;
                        abcseff[bt,(im+1)]=abpseff[bt,(im+1)]*sdxcov;
```

```
                end;
            if ((nmeds = 1) & (ncovs = 0) & (cluster = 0) & (model = 4))
then;do;
              r245[bt,1]=r2my-(r2full-r2xy);
                   abmmr=1;
                   if (indboot[bt,im] < 0) then;do;
                     abmmr=min(abmm);
                   end;
                   if (indboot[bt,im] > 0) then;do;
                     abmmr=max(abmm);
                   end;
                   kappa2[bt,1]=indboot[bt,im]/abmmr;
                   tmp=indboot[bt,im]/abmmr;
                 end;
               end;
             end;
          if (model = 6) then;do;
                if (nmeds = 2) then;do;
                    indboot[bt,1]=mmpaths[2,1]*mmpaths[4,2];
                    indboot[bt,2]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[4,3];
                    indboot[bt,3]=mmpaths[3,1]*mmpaths[4,3];
                  end;
                  if (nmeds = 3) then;do;
                    indboot[bt,1]=mmpaths[2,1]*mmpaths[5,2];
                    indboot[bt,2]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[5,3];
                    indboot[bt,3]=mmpaths[2,1]*mmpaths[4,2]*mmpaths[5,4];

indboot[bt,4]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[4,3]*mmpaths[5,4];
                    indboot[bt,5]=mmpaths[3,1]*mmpaths[5,3];
                    indboot[bt,6]=mmpaths[3,1]*mmpaths[4,3]*mmpaths[5,4];
                    indboot[bt,7]=mmpaths[4,1]*mmpaths[5,4];
                  end;
                  if (nmeds = 4) then;do;
                    indboot[bt,1]=mmpaths[2,1]*mmpaths[6,2];
                    indboot[bt,2]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[6,3];
                    indboot[bt,3]=mmpaths[2,1]*mmpaths[4,2]*mmpaths[6,4];
                    indboot[bt,4]=mmpaths[2,1]*mmpaths[5,2]*mmpaths[6,5];

indboot[bt,5]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[4,3]*mmpaths[6,4];

indboot[bt,6]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[5,3]*mmpaths[6,5];

indboot[bt,7]=mmpaths[2,1]*mmpaths[4,2]*mmpaths[5,4]*mmpaths[6,5];

indboot[bt,8]=mmpaths[2,1]*mmpaths[3,2]*mmpaths[4,3]*mmpaths[5,4]*mmpaths[6,5
];
                    indboot[bt,9]=mmpaths[3,1]*mmpaths[6,3];
                    indboot[bt,10]=mmpaths[3,1]*mmpaths[4,3]*mmpaths[6,4];
                    indboot[bt,11]=mmpaths[3,1]*mmpaths[5,3]*mmpaths[6,5];

indboot[bt,12]=mmpaths[3,1]*mmpaths[4,3]*mmpaths[5,4]*mmpaths[6,5];
                    indboot[bt,13]=mmpaths[4,1]*mmpaths[6,4];
                    indboot[bt,14]=mmpaths[4,1]*mmpaths[5,4]*mmpaths[6,5];
                    indboot[bt,15]=mmpaths[5,1]*mmpaths[6,5];
              end;
              if ((effsize=1) & (dichy=0)) then;do;
                    if (ctot = 0) then;do;ctot=.00000000000001;end;

pmeff[bt,]=indboot[bt,]/(indboot[bt,+]+mmpaths[nrow(mmpaths),1]);
                    rmeff[bt,]=indboot[bt,]/mmpaths[nrow(mmpaths),1];
                    abpseff[bt,]=indboot[bt,]/stddevy;
                    abcseff[bt,]=(stddevx*indboot[bt,])/stddevy;
              if (ncovs > 0 | clsdmy > 0) then;do;
```

```
                abpseff[bt,]=indboot[bt,]/sdycov;
                        abcseff[bt,]=(sdxcov*indboot[bt,]/sdycov);
                    end;
                    if ((nmeds = 1) & (ncovs = 0) & (cluster = 0) & (model =
4)) then;do;
                r245[bt,]=r2my-(r2full-r2xy);
                    end;
                  end;
              end;
            end; *[h];
        end; *[g];
      end; *[d];
      if ((savboot = 1) & (bt > 1)) then;do;bootstrp[(bt-1),]=bootcoef;
      end;
  end; *[b];
  if (savboot=1) then;do;bootstrp=bootstrp[,2:ncol(bootstrp)];
    create &save from bootstrp;
    append from bootstrp;
  end;

  if (mc > 0) then;do;
    x1=sqrt(-
2*log(ranuni(j(mc,nrow(mcmats),&seed))))#cos((2*3.14159265358979)*(ranuni(j(m
c,nrow(mcmats),&seed))));
      x1=x1*root(mcmats);
      do i = 1 to nrow(x1);
        x1[i,]=x1[i,]+mccoeff`;
      end;
      do i = 1 to nmeds;
        indboot[2:nrow(indboot),i]=x1[,i]#x1[,(i+nmeds)];
      end;
  end;


  if ((ttt = 0) & (model > 3)) then;do;
    if (toteff = 0) then;do;
        print "***************************** DIRECT AND INDIRECT EFFECTS
*****************************";
    end;
      if (toteff ^= 0) then;do;
        print "************************** TOTAL, DIRECT AND INDIRECT EFFECTS
**************************";
      end;
    if (model ^= 74) then;do;
      if (dichy = 0) then;do;
          clnm = {"Effect" "SE" "t" "p" "LLCI" "ULCI"};
          if (toteff = 1) then;do;
          print decotot [label = "Total effect of X on Y" colname = clnm
format = &decimals];
        end;
        print deco [label = "Direct effect of X on Y" colname = clnm format =
&decimals];
      end;
      if (dichy = 1) then;do;
        clnm = {"Effect" "SE" "Z" "p" "LLCI" "ULCI"};
          if (toteff = 1) then;do;
            print decotot [label = "Total effect of X on Y" colname = clnm
format = &decimals];
        end;
        print deco [label = "Direct effect of X on Y" colname = clnm format =
&decimals];
      end;
    end;
```

```
  end;
  if (ttt > 0) then;do;
    print "***************************** DIRECT AND INDIRECT EFFECTS
*****************************";
    clbs=modvnm2||"Effect"||"SE"||"t"||"p"||"LLCI"||"ULCI";
      tratio=directv/sedir;
      p=2*(1-probt(abs(tratio),(n-ncol(xy))));
      outp=modvalsd||directv||sedir||tratio||p;
      if (dichy = 0) then;do;
        temp1=directv-sqrt(jncrit)*sedir;
        temp2=directv+sqrt(jncrit)*sedir;
        outp=outp||temp1||temp2;
      end;
      if (dichy = 1) then;do;
        p=2*(1-probnorm(abs(tratio)));
        temp=directv-abs(xp2)*sedir;
        outp=outp||temp;
        temp=directv+abs(xp2)*sedir;
        outp=outp||temp;
        clbs=modvnm2||"Effect"||"SE"||"Z"||"p"||"LLCI"||"ULCI";
      end;
      if (coeffci=0) then;do;
        outp=outp[,1:(ncol(outp)-2)];
      end;
      print outp [label = "Conditional direct effect(s) of X on Y at values of
the moderator(s)"
              format = &decimals colname = clbs rowname = " "];
  end;
  if ((nmods > 0) & (model ^= 5)) then;do; *[xx];
    if (model < 4) then;do;
       print
"*************************************************************************
*************";
      zmat[1,1]=1*(1-yes);
        cfse=j(nrow(modvals),1,0);
        do mmm = 1 to nrow(modvals);
          if (model = 1) then;do;
              zmat[2,1]=modvals[mmm,1]##2;
              zmat[5,1]=2*modvals[mmm,1];
            end;
        if ((model = 2) | (model = 3)) then;do;
              zmat[2,1]=modvals[mmm,2]##2;
              zmat[3,1]=modvals[mmm,1]##2;
              zmat[4,1]=(modvals[mmm,1]##2)*(modvals[mmm,2]##2)*(1-yes2);
              zmat[5,1]=2*modvals[mmm,2]*(1-yes);
              zmat[6,1]=2*modvals[mmm,1]*(1-yes);
              zmat[7,1]=2*modvals[mmm,1]*modvals[mmm,2]*(1-yes);
              zmat[8,1]=2*modvals[mmm,1]*modvals[mmm,2];
              zmat[9,1]=2*modvals[mmm,1]*(modvals[mmm,2]##2)*(1-yes2);
              zmat[10,1]=2*(modvals[mmm,1]##2)*modvals[mmm,2]*(1-yes2);
            end;
            temp=zmat#cmat;cfse[mmm,1]=sqrt(temp[+,]);
        end;
    end;
    if (nmods > 0) then;do; *[bbb];
        clbs=modvnm||"Effect";
        do im = 1 to nmeds;
          obs=indboot[(1+(im-1)*(boot+1)),]`;
            outp=modvals||obs;
            if (model < 4) then;do;
              tstat=obs/cfse;
              if (dichy=0) then;do;
                pval=2*(1-probt(abs(tstat),(n-ncol(xy))));
```

```
                temp=obs-sqrt(jncrit)*cfse;
                outp=outp||cfse||tstat||pval||temp;
                temp=obs+sqrt(jncrit)*cfse;
                outp=outp||temp;
                clbs = clbs||"se"||"t"||"p"||"LLCI"||"ULCI";
                jnclbs=clbs;
            end;
            if (dichy=1) then;do;
              pval=2*(1-probnorm(abs(tstat)));
                temp=obs-abs(xp2)*cfse;
                outp=outp||cfse||tstat||pval||temp;
                temp=obs+abs(xp2)*cfse;
                outp=outp||temp;
                clbs=clbs||"se"||"Z"||"p"||"LLCI"||"ULCI";
                jnclbs=clbs;
            end;
          end;
        if (boot > 0) then;do;
            ones=j(boot,1,1);
            estmte=indboot[(1+(im-1)*(boot+1)),];
          indboot2=indboot[(2+(im-1)*(boot+1)):(1+(im-1)*(boot+1)+boot),];
            mnind=indboot2[+,]/boot;mnind=mnind`;
            tmp=indboot2##2;tmp=tmp[+,];
          stdind=(sqrt((tmp-((indboot2[+,]##2)/boot))/(boot-1)))`;
          llci=j(1,ncol(indboot2),-999);
            ulci=j(1,ncol(indboot2),-999);
          do eee = 1 to ncol(indboot2);
              inpt=indboot2[,eee];inpt2=(estmte[1,eee]*bconoff)+(9999*(1-
bconoff));
              %bcboot (databcbt=inpt,estmte=inpt2);
                llci[1,eee]=llcit;
                ulci[1,eee]=ulcit;
                if ((badlo=1) & (llcit ^= priorlo)) then;do;
                  badend=badend||llcit;
                  priorlo=llcit;
                end;
                if ((badhi=1) & (ulcit ^= priorhi)) then;do;
                  badend=badend||ulcit;
                  priorhi=ulcit;
                end;
            end;
            outp=modvals||obs||stdind||llci`||ulci`;
            clbs=modvnm||"Effect"||"Boot SE"||"BootLLCI"||"BootULCI";
          end;
        mtemp=mnames[1,im];
            rlbs=j(nrow(modvals),1,mnames[1,im]);
        if (model < 4) then;do;
            if (coeffci = 0) then;do;
              outp=outp[,1:(ncol(outp)-2)];
            end;
            if (yes=0) then;do;
          print outp [label = "Conditional effect of X on Y at values of the
moderator(s)" colname = clbs
            format=&decimals rowname = " "];
            end;
            if (yes=1) then;do;
           outp=outp[,3:ncol(outp)];
                clbs=clbs[,3:ncol(clbs)];
                clbs[1,1]={"Contrast"};
                print outp [label = "Contrast of conditional effects of X on
Y" colname = clbs format=&decimals rowname = " "];
            end;
          end;
```

```
            if ((model > 5) & (mod74dic ^= 1)) then;do;
              if (im = 1) then;do;
                print "Conditional indirect effect(s) of X on Y at values of
the moderator(s)";
              end;
              print outp [label = " " rowname = rlbs colname = clbs format =
&decimals];
            end;
            if ((model = 74) & (mod74dic = 1)) then;do;
              if (im = 1) then;do;
                print "Indirect effect(s) of X on Y:";
                clbs3=clbs[1,2:ncol(clbs)];
              end;
              outp3=outp[1,2:ncol(outp)];
              print outp3 [label = " " rowname = rlbs colname=clbs3 format =
&decimals];
            end;
        end;
      do i = notes to 1 by -1;
          if ((note[i,1]=4) & (yes=0)) then;do;
              print "Values for quantitative moderators are the 10th, 25th,
50th, 75th, and 90th percentiles.";
              print "Values for dichotomous moderators are the two values of
the moderator.";
            end;
            if ((note[i,1]=5) & (yes=0)) then;do;
              print "Values for quantitative moderators are the mean and
plus/minus one SD from mean.";
              print "Values for dichotomous moderators are the two values of
the moderator.";
            end;
            if ((note[i,1]=14) & (yes=0)) then;do;
          print "NOTE: For at least one moderator in the conditional effect
table above, one SD";
              print "      below the mean was replaced with the minimum
because one SD below the mean";
              print "      is outside of the range of the data.";
        end;
          if (note[i,1]=15) then;do;
          print "NOTE: For at least one moderator in the conditional effect
table above, one SD";
              print "      below the mean was replaced with the maximum
because one SD above the mean";
              print "      is outside of the range of the data.";
        end;
        end;
        if ((model = 3) & (yes=0)) then;do;
          jnvals=j(nrow(matw),7,0);
            jnvals[,1]=matw;
            jnvals[,2]=jnb1+jnb3*jnvals[,1];
            jnvals[,3]=sqrt(jnsb1+2*jnvals[,1]*jnsb1b3+(jnvals[,1]##2)*jnsb3);
            jnvals[,4]=jnvals[,2]/jnvals[,3];
            if (dichy=0) then;do;
              jnvals[,5]=2*(1-probt(abs(jnvals[,4]),jndf));
            end;
            if (dichy=1) then;do;
              jnvals[,5]=2*(1-probnorm(abs(jnvals[,4])));
            end;
            jnvals[,6]=jnvals[,2]-sqrt(jncrit)#jnvals[,3];
            jnvals[,7]=jnvals[,2]+sqrt(jncrit)#jnvals[,3];
            clbs=clbs[,1]||clbs[,3:ncol(clbs)];
            if (coeffci = 0) then;do;
              jnvals=jnvals[,1:(ncol(jnvals)-2)];
```

```
            end;
            print jnvals [label = "Conditional effect of X*M interaction at
values of W" colname = clbs rowname = " " format = &decimals];
         end;
       if ((jn = 1) & ((model = 1) | (model = 3)) & (jndich=0) & (yes=0))
then;do;
           ajn=(jncrit*jnsb3)-(jnb3*jnb3);bjn=2*((jncrit*jnsb1b3)-(jnb1*jnb3));
             cjn=(jncrit*jnsb1)-(jnb1*jnb1);radarg=(bjn*bjn)-(4*ajn*cjn);
             den=2*ajn;nrts=0;
             print "******************************** JOHNSON-NEYMAN TECHNIQUE
********************************";
          if ((radarg >= 0) & (den ^= 0)) then;do;
               x21=(-bjn+sqrt(radarg))/den;
               x22=(-bjn-sqrt(radarg))/den;
               roots = 0;
               if ((x21 >= jnmin) & (x21 <= jnmax)) then;do;
                 nrts=1;roots=roots//x21;
               end;
               if ((x22 >= jnmin) & (x22 <= jnmax)) then;do;
                 nrts=nrts+1;roots=roots//x22;
               end;
               roots=roots||j(nrow(roots),2,0);
            modtemp=m;
               if (model=3) then;do;modtemp=w;end;
            if (nrts > 0) then;do;
                 roots = roots[2:nrow(roots),1:3];
                   rootsum=(modtemp <
roots[1,1]);roots[1,2]=(rootsum[+]/n)*100;
              rootsum=(modtemp > roots[1,1]);roots[1,3]=(rootsum[+]/n)*100;
              if (nrow(roots)=2) then;do;
                rootsum=(modtemp < roots[2,1]);roots[2,2]=(rootsum[+]/n)*100;
                rootsum=(modtemp > roots[2,1]);roots[2,3]=(rootsum[+]/n)*100;
              end;
                   lohilbs="Value"||"% below"||"% above";
                   print roots [label = "Moderator values(s) defining Johnson-
Neyman significance region(s)" colname=lohilbs format = &decimals];
                  jnvals=j((21+nrts),7,0);
                  do i = 0 to 20;
                    jnvals[(i+1),1]=jnmin+(i*((jnmax-jnmin)/20));
                  end;
                  do i = 1 to nrts;
                    do j = 2 to nrow(jnvals);
                 if ((roots[i,1] > jnvals[(j-1),1]) & (roots[i,1] <
jnvals[j,1])) then;do;
                          jnvals[(j+1):(21+i),1]=jnvals[j:(20+i),1];
                          jnvals[j,1]=roots[i,1];
                        end;
                    end;
                  end;
                do i = 1 to nrow(jnvals);
                    jnvals[i,2]=jnb1+jnb3*jnvals[i,1];

jnvals[i,3]=sqrt(jnsb1+2*jnvals[i,1]*jnsb1b3+(jnvals[i,1]##2)*jnsb3);
                    jnvals[i,4]=jnvals[i,2]/jnvals[i,3];
                    if (dichy = 0) then;do;
                      jnvals[i,5]=2*(1-probt(abs(jnvals[i,4]),jndf));
                    end;
                    if (dichy = 1) then;do;
                      jnvals[i,5]=2*(1-probnorm(abs(jnvals[i,4])));
                    end;
                    jnvals[i,6]=jnvals[i,2]-sqrt(jncrit)*jnvals[i,3];
                    jnvals[i,7]=jnvals[i,2]+sqrt(jncrit)*jnvals[i,3];
                  end;
```

```
                if (model = 1) then;do;
                  print jnvals [label = "Conditional effect of X on Y at
values of the moderator (M)"
              colname=jnclbs rowname = " " format = &decimals];
            end;
                if (model = 3) then;do;
                  jnclbs=jnclbs[,1]||jnclbs[,3:ncol(jnclbs)];
                  print jnvals [label = "Conditional effect of X*M on Y at
values of the moderator (W)"
              colname=jnclbs rowname = " " format=&decimals];
                  end;
              end;
        end;
        if (nrts = 0) then;do;
             print "There are no statistical significance transition points
within the observed range of the moderator";
        end;
        end;
    end; *[bbb];
    if ((model < 4) & (plot = 1)) then;do;
      dataplot=j((nrow(modvals)*nrow(matx)),(ncol(modvals)+1),0);
        tmp=1;
        do i = 1 to nrow(modvals);
          do j = 1 to nrow(matx);
              dataplot[tmp,]=matx[j,1]||modvals[i,];
              tmp=tmp+1;
          end;
        end;
        dataplot=dataplot||j(nrow(dataplot),(1+dichy),0);
        dataplo2=j(nrow(dataplot),1,1);
        if (model = 1) then;do;

dataplo2=dataplo2||dataplot[,2]||dataplot[,1]||(dataplot[,1]#dataplot[,2]);
        end;
        if ((model = 2) | (model = 3)) then;do;

dataplo2=dataplo2||dataplot[,3]||dataplot[,1]||(dataplot[,1]#dataplot[,3])||d
ataplot[,2]||(dataplot[,1]#dataplot[,2]);
        if (model = 3) then;do;

dataplo2=dataplo2||(dataplot[,2]#dataplot[,3])||(dataplot[,1]#dataplot[,2]#da
taplot[,3]);
            end;
      end;
      do i = 1 to nrow(dataplot);
          tmp=dataplo2[i,];
            if (ncovs > 0) then;do;
              tmp=tmp||covmeans;
            end;
            if (cluster > 0) then;do;
              tmp=tmp||cldmeans;
            end;
            dataplot[i,(ncol(dataplot)-(dichy))]=tmp*coeffplt;
            if (dichy=1) then;do;

dataplot[i,(ncol(dataplot))]=exp(tmp*coeffplt)/(1+exp(tmp*coeffplt));
            end;
        end;
      clbs=xname||modvnm||"yhat";
        if (dichy = 1) then;do;
          clbs=xname||modvnm||"ln(odds)"||"prob";
        end;
```

```
        print
"*************************************************************************
*************";
        print dataplot [label = "Data for visualizing conditional effect of X
on Y" colname = clbs rowname = " " format = &decimals];
      if (ncovs > 0) then;do;
          print "Estimates in this table are based on setting covariates to
their sample means";
        end;
      end;
  end; *[xx];
  if (((model = 8) | (model = 12) | (model = 7) | (model=14) | (model=15) |
((model=74) & (mod74dic=0))) | (((model = 58) | (model = 59)) & (wvdich =
1))) then;do;
      obsprod=indbootp[1,]`;
      if (boot > 0) then;do;
        ones=j(boot,1,1);
        estmte=indbootp[1,];
        indbootp=indbootp[2:(boot+1),];
        mnindp=indbootp[+,]/boot;mnindp=mnindp`;
        tmp=indbootp##2;tmp=tmp[+,];
      stdindp=(sqrt((tmp-((indbootp[+,]##2)/boot))/(boot-1)))`;
      llcip=j(1,ncol(indbootp),-999);
      ulcip=j(1,ncol(indbootp),-999);
      do eee = 1 to ncol(indbootp);
            inpt=indbootp[,eee];inpt2=(estmte[1,eee]*bconoff)+(9999*(1-
bconoff));
            %bcboot (databcbt=inpt,estmte=inpt2);
            llcip[1,eee]=llcit;
            ulcip[1,eee]=ulcit;
            if ((badlo=1) & (llcit ^= priorlo)) then;do;
              badend=badend||llcit;
              priorlo=llcit;
            end;
            if ((badhi=1) & (ulcit ^= priorhi)) then;do;
              badend=badend||ulcit;
              priorhi=ulcit;
            end;
        end;
        outp=obsprod||stdindp||llcip`||ulcip`;
        clbs="Effect"||"Boot SE"||"BootLLCI"||"BootULCI";
        if ((model=8) | (model=12)) then;do;
          print outp [label = "Indirect effect of highest order interaction"
colname = clbs rowname = mnames format = &decimals];
        end;
        if (model ^= 12) then;do;
          print "****************************** INDEX OF MODERATED MEDIATION
******************************";
        clbs="Index"||"Boot SE"||"BootLLCI"||"BootULCI";
        print outp [label = " " colname = clbs rowname= mnames format =
&decimals];
            if (wvdich=1) then;do;
          print "When the moderator is dichotomous, this is a test of
equality of the indirect effects in the two groups";
        end;
      end;
      end;
      if (boot = 0) then;do;
        if ((model = 8) | (model = 12)) then;do;

            clnm43="Effect";
          print obsprod [label = "Indirect effect of highest order
interaction" colname = clnm43 rowname = mnames format = &decimals];
```

```
        end;
      if (model ^= 12) then;do;
          print "*****************************INDEX OF MODERATED MEDIATION
*******************************";
            clnm43="Index";
          print obsprod [label = " " colname = clnm43 rowname = mnames format
= &decimals];
        end;
      end;
  end;
  conmake=0;concols=0;
  if (((model > 3) & (model < 7)) & (contrast=1) & (nmods = 0) & (nmeds > 1))
then;do;
    concols=(ncol(indboot)*(ncol(indboot)-1))/2;
      indcon=j(nrow(indboot),concols,-999);
      conkey=" "||" "||" ";
    temp=1;
      conmake=1;
    do i = 1 to (ncol(indboot)-1);
        do j = (i+1) to (ncol(indboot));
          indcon[,temp]=indboot[,i]-indboot[,j];
            if (model ^= 6) then;do;

conkeyt=mnames[1,i]||"minus"||mnames[1,j];conkey=conkey//conkeyt;
            end;
            if (model = 6) then;do;

conkeyt=indlbl2[i,1]||"minus"||indlbl2[j,1];conkey=conkey//conkeyt;
            end;
            temp=temp+1;
        end;
      end;
  end;
  if ((model = 4 ) | (model = 5)) then;do;  *[ddd];
    clbs="Effect";rlbs="TOTAL"//mnames`;
      obs=indboot[1,]`;obs=obs[+,]//obs;
      if (conmake=1) then;do;
        obs=obs//indcon[1,]`;
        rlbs=rlbs//cntname[1:ncol(indcon),1];
      end;
      outp=obs;
      outp2=outp;
      if ((effsize=1) & (dichy=0)) then;do;
      tmp=pmeff[,2:ncol(pmeff)];pmeff[,1]=tmp[,+];
        tmp=rmeff[,2:ncol(rmeff)];rmeff[,1]=tmp[,+];
      tmp=abpseff[,2:ncol(abpseff)];abpseff[,1]=tmp[,+];
      tmp=abcseff[,2:ncol(abcseff)];abcseff[,1]=tmp[,+];
        eff=pmeff||rmeff||abpseff||abcseff;
        if ((nmeds=1) & (ncovs = 0) & (cluster = 0) & (model = 4)) then;do;
          eff=eff||r245||kappa2;
            r245obs=r245[1,1]//r245[1,1];
            kappa2ob=kappa2[1,1];
        end;
        pmobs=pmeff[1,1:(nmeds+1)]`;
      rmobs=rmeff[1,1:(nmeds+1)]`;
      psobs=abpseff[1,1:(nmeds+1)]`;
      csobs=abcseff[1,1:(nmeds+1)]`;
        if (contrast = 0) then;do;
          outp2=obs||psobs||csobs||pmobs||rmobs;
        end;
        if (contrast = 1) then;do;
          obs2=obs[1:nrow(psobs),];
            outp2=obs2||psobs||csobs||pmobs||rmobs;
```

```
        end;
        clbs="ab"||"ab_ps"||"ab_cs"||"ab/c"||"ab/c'";
        if ((nmeds = 1) & (ncovs = 0) & (cluster = 0) & (model = 4)) then;do;
          outp2=outp2||r245obs||(obs/abmmr);
            clbs=clbs||"R-sq_med"||"kappa2";
        end;
      end;
    if ((boot = 0) & (mc = 0)) then;do;
        if (nmeds = 1) then;do;
          outp2=outp2[2,];
            rlbs=rlbs[2,1];
        end;
        if (model=5) then;do;
          outp2=outp2[,1:3];
        end;
        print outp2 [label = "Indirect effect(s) of X on Y" rowname = rlbs
colname = clbs format = &decimals];
      if ((contrast=1) & (effsize=1) & (nmeds > 1)) then;do;
        outp2=indcon[1,]`;
          rlbs2=cntname[1:ncol(indcon),1];
          print outp2 [label = "Contrast(s) between indirect effects" rowname
= rlbs2 colname = clbs format = &decimals];
      end;
    end;
    if ((boot > 0) | (mc > 0)) then;do;
      temp=indboot[,+];indboot=temp||indboot;
        bootsz=boot;
        if (mc > 0) then;do;bootsz=mc;end;
        ones=j(bootsz,1,1);
        if (conmake=1) then;do;
          indboot=indboot||indcon;
        end;
        estmte=indboot[1,];
        indboot=indboot[2:(bootsz+1),];
        mnind=(indboot[+,]/bootsz)`;
        tmp=indboot##2;tmp=tmp[+,];
      stdind=(sqrt((tmp-((indboot[+,]##2)/bootsz))/(bootsz-1)))`;
      llci=j(1,ncol(indboot),-999);
        ulci=j(1,ncol(indboot),-999);
      do eee = 1 to ncol(indboot);
            inpt=indboot[,eee];inpt2=(estmte[1,eee]*bconoff)+(9999*(1-
bconoff));
            %bcboot (databcbt=inpt,estmte=inpt2);
            llci[1,eee]=llcit;
            ulci[1,eee]=ulcit;
            if ((badlo=1) & (llcit ^= priorlo)) then;do;
              badend=badend||llcit;
              priorlo=llcit;
            end;
            if ((badhi=1) & (ulcit ^= priorhi)) then;do;
              badend=badend||ulcit;
              priorhi=ulcit;
            end;
        end;
        if ((effsize=1) & (dichy=0)) then;do;
          estmte=eff[1,];
            eff=eff[2:nrow(eff),];
            tmp=eff##2;tmp=tmp[+,];
        stdindf=(sqrt((tmp-((eff[+,]##2)/boot))/(boot-1)))`;
        llcif=j(1,ncol(eff),-999);
          ulcif=j(1,ncol(eff),-999);
        do eee = 1 to ncol(eff);
              inpt=eff[,eee];inpt2=(estmte[1,eee]*bconoff)+(9999*(1-bconoff));
```

```
            %bcboot (databcbt=inpt,estmte=inpt2);
            llcif[1,eee]=llcit;
            ulcif[1,eee]=ulcit;
            if ((badlo=1) & (llcit ^= priorlo)) then;do;
              badend=badend||llcit;
              priorlo=llcit;
            end;
            if ((badhi=1) & (ulcit ^= priorhi)) then;do;
              badend=badend||ulcit;
              priorhi=ulcit;
            end;
          end;
      end;
      end;
    if ((boot > 0) | (mc > 0)) then;do;
        outp=obs||stdind||llci`||ulci`;
        if (nmeds = 1) then;do;
          outp=outp[2,];rlbs=rlbs[2,1];
        end;
        clbs="Effect"||"Boot SE"||"BootLLCI"||"BootULCI";
        if (mc > 0) then;do;
        clbs="Effect"||"MC SE"||"MC LLCI"||"MC ULCI";
        end;
        print outp [label = "Indirect effect of X on Y" rowname = rlbs colname
= clbs format = &decimals];
      if ((dichy=0) & (effsize = 1)) then;do;

outp=psobs||stdindf[(2*(nmeds+1)+1):(3*(nmeds+1)),1]||(llcif[1,(2*(nmeds+1)+1
):(3*(nmeds+1))])`||(ulcif[1,(2*(nmeds+1)+1):(3*(nmeds+1))])`;
        if (nmeds = 1) then;do;
              outp=outp[2,];
            end;
          print outp [label = "Partially standardized indirect effect of X on
Y" rowname = rlbs colname = clbs format = &decimals];

outp=csobs||stdindf[(3*(nmeds+1)+1):(4*(nmeds+1)),1]||(llcif[1,(3*(nmeds+1)+1
):(4*(nmeds+1))])`||(ulcif[1,(3*(nmeds+1)+1):(4*(nmeds+1))])`;
        if (nmeds = 1) then;do;
              outp=outp[2,];
            end;
          print outp [label = "Completely standardized indirect effect of X on
Y" rowname = rlbs colname = clbs format = &decimals];

outp=pmobs||stdindf[1:(nmeds+1),1]||(llcif[1,1:(nmeds+1)])`||(ulcif[1,1:(nmed
s+1)])`;
            if (model = 4) then;do;
            if (nmeds = 1) then;do;
                outp=outp[2,];
              end;
            print outp [label = "Ratio of indirect to total effect of X on Y"
rowname = rlbs colname = clbs format = &decimals];

outp=rmobs||stdindf[((nmeds+1)+1):(2*(nmeds+1)),1]||(llcif[1,((nmeds+1)+1):(2
*(nmeds+1))])`||(ulcif[1,((nmeds+1)+1):(2*(nmeds+1))])`;
            if (nmeds = 1) then;do;
                outp=outp[2,];
              end;
            print outp [label = "Ratio of indirect to direct effect of X on Y"
rowname = rlbs colname = clbs format = &decimals];
          if ((nmeds = 1) & (cluster = 0) & (ncovs = 0) & (model = 4))
then;do;
              r245obs=r245obs[1,1];
```

```
outp=r245obs||stdindf[(4*(nmeds+1)+1):(4*(nmeds+1)+1),1]||(llcif[1,(4*(nmeds+
1)+1):(4*(nmeds+1)+1)])`||(ulcif[1,(4*(nmeds+1)+1):(4*(nmeds+1)+1)])`;
                print outp [label = "R-squared mediation effect size" rowname
= rlbs colname = clbs format = &decimals];

outp=kappa2ob||stdindf[nrow(stdindf),1]||llcif[1,ncol(llcif)]||ulcif[1,ncol(u
lcif)];
                print outp [label = "Preacher and Kelley (2011) Kappa-squared"
rowname = rlbs colname = clbs format = &decimals];
           end;
             end;
      end;
    end;
    if (normal = 1) then;do;
        clbs2="Effect"||"se"||"Z"||"p";
        if (nmeds = 1) then;do;
          print sobel [label = "Normal theory test for indirect effect"
colname = clbs2 format = &decimals];
      end;
      if (nmeds > 1) then;do;
          rlbs2=rlbs[2:nrow(rlbs),1];
          print sobel [label = "Normal theory tests for specific indirect
effects" rowname = rlbs2 colname = clbs2 format = &decimals];
      end;
    end;
      if (conmake = 1) then;do;
         conkey=conkey[2:nrow(conkey),];
         conlbs=cntname[1:ncol(indcon),1];
         print conkey [label = "Specific indirect effect contrast definitions"
rowname = conlbs];
      end;
  end; *[dddd];
  if (model = 6) then;do;
    clbs="Effect";
      rlbs="TOTAL"//mnames`;
      obs=indboot[1,]`;
      obsfsum=obs[+,];
      obs=obsfsum//obs;
      indlbl=indlbl[1:nrow(obs),1];
      if (conmake = 1) then;do;
        obs=obs//indcon[1,]`;
      indlbl=indlbl//cntname[1:ncol(indcon),1];
      end;
      obs2=obs;
      if (boot = 0) then;do;
        if ((dichy=0) & (effsize = 1)) then;do;
          clbs="eff"||"eff_ps"||"eff_cs"||"eff/c"||"eff/c'";
            if (ncovs=0) then;do;

obs=obs||obs/stddevy||obs*stddevx/stddevy||obs/ctot||obs/mmpaths[nrow(mmpaths
),1];
        end;
            if ((ncovs > 0) | (clsdmy >  0)) then;do;

obs=obs||obs/sdycov||obs*sdxcov/sdycov||obs/(obsfsum+mmpaths[nrow(mmpaths),1]
)||obs/mmpaths[nrow(mmpaths),1];
        end;
            obs2=obs;
            if (contrast=1) then;do;
              obs2=obs[1:(nrow(obs)-concols),];
            end;
        end;
```

```
        print obs2 [label = "Indirect effect(s) of X on Y" rowname = indlbl
colname = clbs format = &decimals];
        if ((contrast=1) & (effsize=1)) then;do;
          outp2=indcon[1,]`;
            rlbs2=cntname[1:ncol(indcon),1];
            print outp2 [label = "Contrast(s) between indirect effects"
rowname=rlbs2 colname=clbs format=&decimals];
        end;
      end;
      if (boot > 0) then;do;
        ones=j(boot,1,1);
        indboot=indboot[,+]||indboot;
        if (conmake=1) then;do;
          indboot=indboot||indcon;
        end;
        estmte=indboot[1,];
      /* here it is */;
        indboot=indboot[2:(boot+1),];
        mnind=indboot[+,]/boot;mnind=mnind`;
        tmp=indboot##2;tmp=tmp[+,];
      stdind=(sqrt((tmp-((indboot[+,]##2)/boot))/(boot-1)))`;
        temp=nrow(indboot);
        llci=j(1,ncol(indboot),-999);
        ulci=j(1,ncol(indboot),-999);
      do eee = 1 to ncol(indboot);
           inpt=indboot[,eee];inpt2=(estmte[1,eee]*bconoff)+(9999*(1-
bconoff));
           %bcboot (databcbt=inpt,estmte=inpt2);
           llci[1,eee]=llcit;
           ulci[1,eee]=ulcit;
           if ((badlo=1) & (llcit ^= priorlo)) then;do;
             badend=badend||llcit;
             priorlo=llcit;
           end;
           if ((badhi=1) & (ulcit ^= priorhi)) then;do;
             badend=badend||ulcit;
             priorhi=ulcit;
           end;
        end;
      obs=obs||stdind||llci`||ulci`;
        clbs="Effect"||"Boot SE"||"BootLLCI"||"BootULCI";
      print obs [label = "Indirect effect(s) of X on Y" rowname = indlbl
colname = clbs format = &decimals];
      if ((effsize=1) & (dichy=0)) then;do;
          indboot=indboot[,1:(ncol(indboot)-concols)];

eff=abpseff[,+]||abpseff||abcseff[,+]||abcseff||pmeff[,+]||pmeff||rmeff[,+]||
rmeff;
        effobs=eff[1,];
          /* here it is */;
          eff=eff[2:nrow(eff),];
        tmp=eff##2;tmp=tmp[+,];
        stdindf=(sqrt((tmp-((eff[+,]##2)/boot))/(boot-1)))`;
        llcif=j(1,ncol(eff),-999);
          ulcif=j(1,ncol(eff),-999);
        do eee = 1 to ncol(eff);
           inpt=eff[,eee];inpt2=(effobs[1,eee]*bconoff)+(9999*(1-bconoff));
           %bcboot (databcbt=inpt,estmte=inpt2);
           llcif[1,eee]=llcit;
           ulcif[1,eee]=ulcit;
           if ((badlo=1) & (llcit ^= priorlo)) then;do;
             badend=badend||llcit;
             priorlo=llcit;
```

```
            end;
            if ((badhi=1) & (ulcit ^= priorhi)) then;do;
              badend=badend||ulcit;
              priorhi=ulcit;
            end;
          end;
        temp2=stdindf[1:ncol(indboot),1];
            temp3=effobs[,1:ncol(indboot)];
            templow=llcif[1,1:ncol(indboot)];
            temphi=ulcif[1,1:ncol(indboot)];
            outp=temp3`||temp2||templow`||temphi`;
            print outp [label = "Partially standardized indirect effect of X
on Y" colname = clbs rowname = indlbl format = &decimals];
        temp2=stdindf[(ncol(indboot)+1):(2*ncol(indboot)),1];
            temp3=effobs[,(ncol(indboot)+1):(2*ncol(indboot))];
            templow=llcif[1,(ncol(indboot)+1):(2*ncol(indboot))];
            temphi=ulcif[1,(ncol(indboot)+1):(2*ncol(indboot))];
            outp=temp3`||temp2||templow`||temphi`;
            print outp [label = "Completely standardized indirect effect of X
on Y" colname = clbs rowname = indlbl format = &decimals];
        temp2=stdindf[(2*(ncol(indboot))+1):(3*ncol(indboot)),1];
        temp3=effobs[,(2*(ncol(indboot))+1):(3*ncol(indboot))];
        templow=llcif[1,(2*(ncol(indboot))+1):(3*ncol(indboot))];
            temphi=ulcif[1,(2*(ncol(indboot))+1):(3*ncol(indboot))];
            outp=temp3`||temp2||templow`||temphi`;
            print outp [label = "Ratio of indirect to total effect of X on Y"
colname = clbs rowname = indlbl format = &decimals];
        temp=eff[,(3*(ncol(indboot))+1):(4*(ncol(indboot)))];
            temp2=stdindf[(3*(ncol(indboot))+1):(4*ncol(indboot)),1];
            temp3=effobs[,(3*(ncol(indboot))+1):(4*ncol(indboot))];
            templow=llcif[1,(3*(ncol(indboot))+1):(4*ncol(indboot))];
            temphi=ulcif[1,(3*(ncol(indboot))+1):(4*ncol(indboot))];
            outp=temp3`||temp2||templow`||temphi`;
            print outp [label = "Ratio of indirect to direct effect of X on Y"
colname = clbs rowname = indlbl format = &decimals];
        end;
    end;
    if (nmeds = 2) then;do;
        effkey=xname||"->"||mnames[1,1]||"->"||yname||" "||" ";
        tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"-
>"||yname;effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,2]||"->"||yname||" "||"
";effkey=effkey//tempkey;
        effkey=indlbl[2:4,1]||effkey;
      end;
      if (nmeds = 3) then;do;
        effkey=xname||"->"||mnames[1,1]||"->"||yname||" "||" "||" "||" ";
        tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"->"||yname||"
"||" ";effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,3]||"->"||yname||"
"||" ";effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"-
>"||mnames[1,3]||"->"||yname;effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,2]||"->"||yname||" "||" "||" "||"
";effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,2]||"->"||mnames[1,3]||"->"||yname||"
"||" ";effkey=effkey//tempkey;
        tempkey=xname||"->"||mnames[1,3]||"->"||yname||" "||" "||" "||"
";effkey=effkey//tempkey;
        effkey=indlbl[2:8,1]||effkey;
      end;
      if (nmeds = 4) then;do;
```

```
      effkey=xname||"->"||mnames[1,1]||"->"||yname||" "||" "||" "||" "||
"||" ";
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,3]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,4]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"-
>"||mnames[1,3]||"->"||yname||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"-
>"||mnames[1,4]||"->"||yname||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,3]||"-
>"||mnames[1,4]||"->"||yname||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,1]||"->"||mnames[1,2]||"-
>"||mnames[1,3]||"->"||mnames[1,4]||"->"||yname;effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,2]||"->"||yname||" "||" "||" "||" "||
"||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,2]||"->"||mnames[1,3]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,2]||"->"||mnames[1,4]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,2]||"->"||mnames[1,3]||"-
>"||mnames[1,4]||"->"||yname||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,3]||"->"||yname||" "||" "||" "||" "||
"||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,3]||"->"||mnames[1,4]||"->"||yname||"
"||" "||" "||" ";effkey=effkey//tempkey;
      tempkey=xname||"->"||mnames[1,4]||"->"||yname||" "||" "||" "||" "||
"||" ";effkey=effkey//tempkey;
        effkey=indlbl[2:16,1]||effkey;
      end;
    print effkey [label = "Indirect effect key"];
      if (conmake = 1) then;do;
        conkey=conkey[2:nrow(conkey),];
        conlbs=cntname[1:ncol(indcon),1];
        print conkey [label = "Specific indirect effect contrast definitions"
rowname = conlbs];
      end;
  end;
end; *[cccc];
if (bad > 0) then;do;note[notes,1]=9;notes=notes+1;
end;
print "***************************** ANALYSIS NOTES AND WARNINGS
*****************************";
do i = 1 to errs;
  if (runerrs[i,1]=1) then;do;
    print "ERROR: One of your declared mediators is dichotomous. This
procedure cannot be used.";
  end;
  if (runerrs[i,1]=2) then;do;
    print "ERROR: For model 6, this procedure limits the number of mediators
to four.";
  end;
  if (runerrs[i,1]=3) then;do;
    print "ERROR: For models 1, 2, and 3, only a single variable can be
listed in the M list.";
  end;
  if (runerrs[i,1]=4) then;do;
    print "ERROR: You requested a model involving W but did not provide a
valid W variable name.";
  end;
  if (runerrs[i,1]=5) then;do;
```

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    print "ERROR: You requested a model involving Z but did not provide a
valid Z variable name.";
  end;
  if (runerrs[i,1]=6) then;do;
    print "ERROR: You requested a model involving Q but did not provide a
valid Q variable name.";
  end;
  if (runerrs[i,1]=7) then;do;
    print "ERROR: You requested a model involving V but did not provide a
valid V variable name.";
  end;
  if (runerrs[i,1]=8) then;do;
    print "ERROR: You specified a W variable for a model that does not need
it.";
  end;
  if (runerrs[i,1]=9) then;do;
    print "ERROR: You specified a Z variable for a model that does not need
it.";
  end;
  if (runerrs[i,1]=10) then;do;
    print "ERROR: You specified a Q variable for a model that does not need
it.";
  end;
  if (runerrs[i,1]=11) then;do;
    print "ERROR: You specified a V variable for a model that does not need
it.";
  end;
  if (runerrs[i,1]=12) then;do;
    print "ERROR: The variable specified for W has already been assigned.";
  end;
  if (runerrs[i,1]=13) then;do;
    print "ERROR: The variable specified for Z has already been assigned.";
  end;
  if (runerrs[i,1]=14) then;do;
    print "ERROR: The variable specified for Q has already been assigned.";
  end;
  if (runerrs[i,1]=15) then;do;
    print "ERROR: The variable specified for V has already been assigned.";
  end;
  if (runerrs[i,1]=16) then;do;
    print "ERROR: You did not provide a valid Y variable name.";
  end;
  if (runerrs[i,1]=17) then;do;
    print "ERROR: The variable specified for Y has already been assigned.";
  end;
  if (runerrs[i,1]=18) then;do;
    print "ERROR: Model 6 requires more than one mediator.";
  end;
  if (runerrs[i,1]=19) then;do;
    print "ERROR: You have not specified a valid model number.";
  end;
  if (runerrs[i,1]=20) then;do;
    print "ERROR: At least one and only one variable must be listed for X.";
  end;
  if (runerrs[i,1]=21) then;do;
    print "ERROR: At least one and only one variable must be listed for Y.";
  end;
  if (runerrs[i,1]=22) then;do;
    print "ERROR: Iteration didn't converge to a solution. Interpret results
with caution.";
  end;
  if (runerrs[i,1]=23) then;do;
```

```
    print "ERROR: You specified a clustering variable that does not exist in
your variable list.";
  end;
  if (runerrs[i,1]=24) then;do;
    print "ERROR: You specified a clustering variable that has already been
assigned.";
  end;
  if (runerrs[i,1]=25) then;do;
    print "ERROR: One of more of your M variables is not listed in the
variables list.";
  end;
  if (runerrs[i,1]=26) then;do;
    print "ERROR: A maximum of 20 cluster units is allowed. Use multilevel
modeling instead.";
  end;
  if (runerrs[i,1]=27) then;do;
    print "ERROR: One of the variables in your model is a constant.";
  end;
  if (runerrs[i,1]=28) then;do;
    print "ERROR: Dichotomous Y is not permitted with WS option.";
  end;
  if (runerrs[i,1]=29) then;do;
    print "ERROR: Insufficient number of variables in vars= list when using
WS option.";
  end;
  if (runerrs[i,1]=30) then;do;
    print "ERROR: Too many number of variables in vars= list when using WS
option.  Covariates not allowed.";
  end;
  if (runerrs[i,1]=31) then;do;
    print "ERROR: mmodval and wmodval cannot both be set to zero with
contrast option.";
  end;
  if (runerrs[i,1]=32) then;do;
    print "ERROR: You did not provide a valid X variable name.";
  end;
end;
if (errs = 0) then;do;
  if ((boot > 1) | (mc > 0)) then;do;
    if ((bconoff = 1) & (boot > 0)) then;do;
      print boot [label = "Number of bootstrap samples for bias corrected
bootstrap confidence intervals:" format = 10.0];
    end;
    if ((bconoff = 0) & (boot > 0)) then;do;
      print boot [label = "Number of bootstrap samples for percentile
bootstrap confidence intervals:" format = 10.0];
    end;
    if (mc > 1) then;do;
      print mc [label = "Number of samples for Monte Carlo confidence
intervals:" format = 10.0];
    end;
    if (booterr = 1) then;do;
      badend=badend[1,2:(ncol(badend))];
        badend=badend`;
      print badend [label = "WARNING: Bootstrap CI endpoints below not
trustworthy. Decrease confidence or increase bootstraps" format = &decimals];
    end;
  end;
  print conf [label = "Level of confidence for all confidence intervals in
output:" format = 10.4];
  if (((center = 1) | (ws=1)) & (ncol(centvar) > 0)) then;do;
    centvar=centvar[1,2:ncol(centvar)];
```

```
      print centvar [label = "NOTE: The following variables were mean centered
prior to analysis:"];
  end;
  warnrep=0;
  do i = 1 to notes;
    if (note[i,1]=1) then;do;
      print "NOTE: Confidence level restricted to between 50 and 99.9999%.
95% confidence is provided.";
    end;
    if (note[i,1]=2) then;do;
      print "NOTE: Effect size measures not available for models with
dichotomous outcomes.";
    end;
    if (note[i,1]=3) then;do;
      print "NOTE: All standard errors for continuous outcome models are
based on the HC3 estimator.";
    end;
    if (note[i,1]=6) then;do;
      print "NOTE: The number of bootstrap samples was adjusted upward given
your desired confidence.";
    end;
    if (note[i,1]=7) then;do;
      print "NOTE: The Johnson-Neyman method is available only for models 1
and 3.";
    end;
    if (note[i,1]=8) then;do;
      print "NOTE: The Johnson-Neyman method cannot be used with a
dichotomous moderator.";
    end;
    if (note[i,1]=9) then;do;
      print bad [label = "NOTE: Some bootstrap samples had to be replaced.
The number of such replacements was:" format = 10.0];
    end;
    if (note[i,1]=11) then;do;
      print nmiss [label = "NOTE: Some cases were deleted due to missing
data. The number of such cases was:" format = 10.0];
    end;
      if (note[i,1]=12) then;do;
      print "NOTE: Monte Carlo method available only for models 4 and 5.
Bootstrapping was used instead.";
    end;
    if (note[i,1]=13) then;do;
      print "NOTE: The number of Monte Carlo samples was adjusted upward
given your desired confidence.";
    end;
      if (note[i,1]=19) then;do;
      print "NOTE: Effect sizes not available for within-subject analyses.";
    end;
    if (note[i,1]=16) then;do;
      print "NOTE: Normal theory tests not available for within-subject
analyses.";
    end;
    if (note[i,1]=17) then;do;
      print "NOTE: Monte Carlo confidence intervals not available for within-
subject analyses.";
    end;
      if (note[i,1]=18 & warnrep=0) then;do;
      print "WARNING: You have requested OLS estimation with a dichotomous
criterion.";
      print "Interpret model coefficients and inferential statistics with
caution.";
      warnrep=1;
    end;
```

```
    if (note[i,1]=20) then;do;
      print "NOTE: Saving of bootstrap estimates not available for within-
subject analyses.";
    end;
      if (note[i,1]=22) then;do;
      print "NOTE: Effect size option with covariates requires covariates in
models of M and Y.";
    end;
  end;
end;
quit;
options pagesize=54;
%mend;
```

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