

**Cycling to work in London and inhaled dose of black carbon**

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**Running Head:** Macrophage carbon and cycling

CN, developed initial study with JG, recruited subjects, performed induced sputum, collated data, and contributed to draft

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## **Abstract**

Modelling studies suggest that urban cycling is associated with an increased inhaled dose of fossil-fuel derived black carbon. Using the amount of black material in airway macrophages as a marker of long-term inhaled black carbon, we sought to compare inhaled black carbon dose in London cyclists and non-cyclists.

Alveolar macrophage carbon was assessed in 28/48 (58%) healthy adults (14 cyclists and 14 non-cyclists) who attended for induced sputum. Short-term (24 h) exposure to black carbon was assessed on a representative working day in 27/28. Serum interleukin (IL)-1 beta, IL-2, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor-alpha (TNF- $\alpha$ ) was assessed 26/28.

Cyclists had increased airway macrophage carbon compared with non-cyclists ( $1.81 \pm 0.21 \mu\text{m}^2$  vs.  $1.11 \pm 0.07 \mu\text{m}^2$ ,  $P < 0.01$ ). Short-term monitoring showed no difference in 24 h black carbon exposure between the 2 groups. However, cyclists were exposed to higher concentrations of black carbon during commuting ( $P < 0.01$ ). Airway macrophage carbon was associated monitored commute black carbon ( $n=28$ ,  $r = 0.47$ ,  $P < 0.05$ ). TNF- $\alpha$ , but not other cytokines, was increased in cyclists ( $P < 0.05$ ).

Commuting to work by bicycle London is associated with increased long-term inhaled dose of BC. Whether cycling *per se* increases inhaled BC dose remains unclear.

*Key Words:* cycling, personal black carbon, airway macrophage carbon, commuting

## Background

Inhalation of particulate matter (PM) from fossil-fuel combustion is associated with adverse health effects, including reduced lung function <sup>1</sup> and increased mortality <sup>2</sup>. Although the mechanism for PM-induced health effects is not fully defined, animal models and *in vitro* studies suggest that pro-inflammatory cytokine release from airway cells is an important factor <sup>3</sup>. Fossil-fuel derived PM in the inhalable size range (<10µm in aerodynamic diameter; PM<sub>10</sub>) is dominated by aggregates of nanoparticles of elemental black carbon (BC) <sup>4</sup>. Variation in BC concentrations within cities therefore mirror local variations in the concentration of fossil-fuel derived PM<sub>10</sub> <sup>5</sup>. For example, zones of high BC and PM<sub>10</sub> occur along urban main roads <sup>6</sup>. Thus the distance of the home to the nearest main road is widely used as a marker of individual exposure to either BC or PM<sub>10</sub> in epidemiological studies <sup>7 8</sup>. However, individuals are also exposed to BC when using roads to travel to work <sup>6</sup>. Indeed, there is concern that commuting to work by bicycle, by combining increased minute ventilation <sup>9</sup> and increased commuting time, with proximity to freshly-generated exhaust fumes, significantly increases the inhaled dose of BC <sup>10 11</sup>.

To date, the effect of commuting on inhaled BC dose is unknown. Recently, we developed a method for assessing the long-term inhaled dose of BC using the capacity of lower airway macrophages (AM) to retain inhaled black carbon in a dose-dependent manner over long periods of time <sup>12</sup>. In adolescents, we previously reported an inverse association between area of carbon in AM (AM carbon) and lung function <sup>13</sup>, and other groups have reported an association between AM carbon and surrogate markers for cardiovascular disease in diabetic adults <sup>14 15</sup>. In this study we hypothesised that, compared with individuals commuting by public transport, regular

cycling to work exposes individuals to prolonged and high concentrations of BC, resulting in an increased lower airway dose of BC. We sought to test this hypothesis using a combination of AM carbon and personal BC monitoring in healthy adults who either exclusively cycled to work in London, or exclusively used public transport and/or walking. We also sought to compare systemic pro-inflammatory cytokines in the 2 groups.

## **Materials and Methods**

### **Subjects**

The study's eligibility criteria were; aged 18 to 40 yr, regularly commuting to work in either the east-end of London, or in central London for a least 3 yr, no history of personal smoking or domestic second-hand smoke exposure within the last 3 yr, and no acute or chronic medical conditions. Exclusion criteria were; clinical respiratory infection within the previous 3 months, use of candles, wood, open fires in the home, and commuting to work by car. Car drivers were excluded since the majority of Londoners do not drive to work. Subjects were classified as cyclists if they habitually cycled from home to work. Non-cyclists were classified as individuals who commuted to work by either walking or public transport, or a combination of both. Subjects were studied November 2010 through March 2011. The local research ethics committee approved this study, and all subjects provided written consent.

### **Questionnaire**

Subjects completed a questionnaire on smoking history, and usual mode of commute. Subjects' home postcode was recorded and the distance of the home to the nearest main road distance (m) calculated using Free Map Tools website <sup>16</sup>. Briefly, the postcode of each subject's home was entered into the "quick-find" box on the website, and identified using navigation tool. The nearest point on closest major road (in UK; "A"-road) was then indentified, and the home-main road distance read from the "total distance" box.

### **Lung function**

Lung function was measured using a MicroMedical MicroLab 3500 Spirometer Mk 8 (Cardinal Health UK, Basingstoke, UK) with a data management system compliant with American Thoracic Society guideline<sup>17</sup>. Flow volume loops were displayed for immediate quality control. Post-bronchodilator FEV<sub>1</sub>, FVC, FEF<sub>25-75</sub>, and FEV<sub>1</sub>/FVC were recorded.

### **Airway macrophage carbon**

Airway macrophages were obtained using sputum induction. Induced sputum was processed according to a standard technique<sup>13</sup>. After 0.1% dithiothreitol treatment to remove mucus (Sigma-Aldrich, Poole, Dorset), plugs of airway cells were identified and removed. Cells were cytocentrifuged (Cytospin, Shandon Scientific) onto microscope slides and stained with Diff-Quik (Dade Behring). Slides were analysed AM carbon, as previously reported<sup>13</sup>. Briefly, digital colour images of AM from random microscope fields were obtained from each subject using a Nikon digital camera and a Nikon Eclipse 80i microscope (Nikon Instruments, Amstelveen, The Netherlands) at either x100 magnification. PictureFrame (Optronics, Goleta, CA) was used to acquire the images. An image of a stage micrometer graticule (S-12S stage micrometer, 0.1 mm/50 division; Pyser-SGI, Kent, UK) was also obtained at the same magnification. Image J (NIH, Maryland,) was used to analyze the AM images, with scaling calibrated using the image of the stage micrometer graticule. Each AM image was initially processed using Adobe Photoshop Elements (Adobe Systems Inc., San Jose, CA). First, each AM image was “cut and pasted,” from a wide field image and the nucleus removed. The Image J software was used to measure the area of black carbon ( $\mu\text{m}^2$ ) within AM. The ‘threshold’ command was adjusted to obtain the best fit of the black areas visible on the color image. The area of carbon in each AM was

calculated, and mean AM carbon ( $\mu\text{m}^2$ ) calculated from 50 randomly selected AM per subject, by an operator blinded to commuting status.

### **Monitored black carbon**

Personal exposure to BC was monitored using a portable aethalometer (Magee Scientific AE51, Berkeley, CA) worn on representative working day in subjects in whom sputum induction had produced sufficient AM for carbon analysis (i.e. some days after sputum induction). A representative working day was defined as one during a normal working week when the subject had a return journey between their normal place of work and their usual residence deemed to be typical of their habitual commuting behaviour. The aethalometer was carried on the waist during the day and when subjects moved around indoors. At night, the monitor was placed next to the bed. Black carbon exposure was determined using the microAethCOM PC-based software application. The software output describes the mean concentration of BC ( $\eta\text{g}/\text{m}^3$ ) for a pre-defined sampling period. In this study, the sampling time was set to 5 min and individual exposure was the sum of each 5 min BC concentration. This exposure metric is directly proportional to the area under the concentration time curve. BC exposure for the commute, at home, and during other activities was then calculated using a time-activity diary data completed on the day of monitoring. Background  $\text{PM}_{10}$  concentrations were obtained from a fixed air pollution monitoring station in a central London location (North Kensington)<sup>18</sup>. Meteorological data were obtained from a weather station in Greater London<sup>19</sup>.

### **Systemic cytokines**

Subjects underwent optional blood sampling after sputum induction. Blood was centrifuged and serum frozen at  $-70^{\circ}$  C. Cytokines were defined *a priori* by their reported ability to be released by lung cells stimulated by  $PM_{10}$  *in vivo* ; i.e. Interleukin (IL)-1 beta<sup>20 21</sup>, IL-2<sup>22</sup>, IL-6<sup>21</sup>, IL-8<sup>20 21</sup>, Granulocyte-macrophage colony-stimulating factor (GM-CSF)<sup>20</sup>, and tumor necrosis factor-alpha (TNF- $\alpha$ )<sup>23</sup>. Serum cytokines were determined using a 96-Well Multi-Spot MS2400 Human ProInflammatory-9 plex Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, Maryland). The plate was processed according to the manufacture's instructions and read on the MSD SECTOR imager. Data were analyzed using the MSD Discovery Workbench Software.

### **Statistical analysis**

Data were analysed using IBM SPSS version 18.0 (Armonk, NY). Data are summarized as mean (standard error of the mean). Comparisons were done using unpaired t test and expressed as the 95% confidence interval (CI) for the difference and correlations done using Pearson correlation. A *P* value of  $<0.05$  was considered significant.

## Results

48 adults attended for sputum induction. Twenty-eight (58%) produced sufficient number of AM ( $n \geq 50$ ) for assessment of carbon loading (14 cyclists and 14 non-cyclists). 26/28 subjects agreed to blood sampling. Other than a small difference in weight, there were no significant demographic differences between subjects analysed ( $n=28$ ) and non-analysed for AM carbon ( $n=20$ ) (Online Table). Although a greater proportion of cycling subjects were male ( $P < 0.05$ , Table 1), there were no significant differences in age, height, weight, home-main road distance, and post-bronchodilator lung function between cyclists and non-cyclists (Table 1). Personal BC monitoring was done in 27/28 subjects at a mean interval of  $79.2 \pm 8.8$  days after induced sputum. For the day of monitoring, there was no difference in background  $PM_{10}$  or meteorological variables between cyclists and non-cyclists (Table 2).

The mean exposure/time plots for the 2 groups show highest BC exposure occurs during the day and lowest exposure occurs at night (Fig. 2). There was no difference in either 24 hr BC, or home BC, or exposure to BC during other periods, between cyclists and non-cyclists (Table 2). However, commute BC was 2.6 fold higher in cyclists ( $P < 0.01$ , Table 2), and a higher fraction of 24h BC exposure was associated with commuting in cyclists ( $41 \pm 3.4\%$  vs.  $18 \pm 4.2\%$ ,  $P < 0.001$ , Figure 3A, B). This increased commute BC in cyclists is due to a combination of increased time commuting ( $P < 0.01$ , Table 2), and higher BC on the roads used for commuting ( $11681 \pm 1375$  vs.  $7135 \pm 1714$ ,  $\eta g/m^3/5$  min,  $P < 0.05$ ). Maximum peak BC always occurred during commuting in both groups, but the maximum peak concentration was higher in cyclists ( $P < 0.05$ , Table 2).

Compatible with our previous data from healthy children<sup>13</sup>, there was marked intra-subject variation in carbon loading in AM carbon loading in both cyclists and non-cyclists (Fig. 4). Cyclists had 1.6 fold higher AM carbon compared with non-cyclists ( $1.81 \pm 0.21 \mu\text{m}^2$  vs.  $1.11 \pm 0.07 \mu\text{m}^2$ , 95% CI; 0.22 to 1.17,  $P < 0.01$ , Figure 5). AM carbon was associated with commute BC ( $r = 0.47$ ,  $P < 0.05$ ,  $n=27$ ), but was not associated with either BC exposure at home, or BC exposure during non-commuting activity (“other” BC). There was no association between AM carbon and age ( $r=0.16$ ,  $P=NS$ ).

There was no difference in systemic GM-CSF, IL-1 $\beta$ , IL-2, IL-6, and IL-8 between cyclists and non-cyclists (Table 3). However, cyclists ( $n=14$ ) had higher TNF- $\alpha$  compared with non-cyclists ( $n=12$ ,  $P < 0.05$ , Table 3).

## Discussion

In this study of healthy Londoners who regularly commute to work by either bicycle, or public transport/walking, we sought to test the hypothesis that commuting by bicycle is associated with increased long-term inhaled dose of BC. Using the area of black carbon in AM as a marker of the long-term “internal” dose of BC, we found that cyclists had a 1.6 fold higher AM carbon compared with commuters using public transport. Short-term personal monitoring of “external” exposure to BC performed after AM carbon analysis showed no difference in 24 h BC between cyclists and non-cyclists. However, cyclists spent longer commuting and therefore had 2.6 fold higher commute BC. These results suggest that; i) exposure to BC during commuting has a disproportionate influence on AM carbon, and ii) short term monitoring provides insights into long-term inhaled BC dose when behavior is habitual. In a recent study on the effect of mode of commute on exposure to air pollution, Zuurbier *et al*<sup>6</sup> reported that cyclists using commuting routes in Arnhem (the Netherlands) were not exposed to increased levels of BC (soot) compared with subjects using public transport, but when the effect of minute ventilation was considered, cycling was associated with increased modelled inhaled BC dose<sup>6</sup>. The increased AM carbon in cyclists is compatible with the hypothesis that exercise increases inhaled BC dose. However, cyclists in our study had free choice of commuting route, and therefore commuted for longer and were therefore exposed to higher levels of BC on their commute. We cannot therefore assess the independent contribution of cycling/increased minute ventilation *per se* on AM carbon. An alternative explanation why exposure to BC during commuting, but not at other times, is associated with AM carbon, is that high peaks of BC associated freshly generated exhaust fumes evade the

filtering mechanisms of the upper airway. Indeed the individual plots of BC exposure of cyclists show very high BC peaks during the commute (Online Figure). Further studies are required to assess the effect of BC peaks on AM carbon - since this may also be relevant to the inhaled dose of BC from indoor environmental cigarette smoke, and indoor biomass smoke.

Epidemiological studies suggest that individual exposure to fossil-fuel derived BC should be as low as practicable. For example, a meta-regression analysis of triggers of myocardial infarction, found that the highest population attributable fraction was for exposure to traffic emissions - followed by physical exercise<sup>24</sup>. In addition, a population-based study found that BC concentration over a 5 yr period at the home address was associated with increased hospitalisation for coronary heart disease (CHD), and increased CHD mortality after adjusting for age, sex, preexisting co-morbidity, neighborhood socioeconomic status, and PM<sub>2.5</sub> and NO<sub>2</sub><sup>7</sup>. Exposure to BC is also associated with surrogates for health effects in healthy adults. For example, McCracken *et al*<sup>25</sup> studied telomere length, a marker of cellular ageing<sup>26</sup>, and found that increased annual BC exposure was associated with decreased telomere length in healthy non-smoking men. Whether increased levels of AM carbon in healthy London cyclists is a risk factor for future health effects is unknown, but in non-smoking diabetic adults, Jacobs *et al*<sup>14</sup> reported that a 7.2 U/L increase in plasma oxidised low-density lipoprotein (a risk factor for coronary artery disease<sup>27</sup>), was associated with each 0.25  $\mu\text{m}^2$  increase in AM carbon. We did not measure lipoproteins, but it is interesting to note that the 95% CI for the difference in AM carbon between cyclists and non-cyclists in the present study is between 0.22 and 1.17  $\mu\text{m}^2$ . The moderate increase in serum TNF- $\alpha$  in cyclists in the present study does provide preliminary

evidence of a downstream systemic effect of inhaled BC. These data are compatible with Törnqvist *et al*<sup>28</sup>, who reported increased serum TNF- $\alpha$  in healthy adults 24 h after inhalation of 300  $\mu\text{g}/\text{m}^3$  diesel exhaust PM - but our TNF- $\alpha$  data should be interpreted with caution. First, multiple cytokine testing in a small number of subjects increases the possibility of type I error. Second, we found no significant association between serum TNF- $\alpha$  and either commute BC, or 24 h BC, or AM carbon.

There are limitations to our study. For example, it is unclear whether AM carbon reflects the dose of BC inhaled over weeks, months, or even years. It is, however, unlikely that AM carbon exclusively reflects short-term inhaled BC dose. First, in rats there is a strong association between AM carbon and long-term inhaled BC dose<sup>12</sup>. Second, in Malawian adults we found that AM carbon tracks closely with the BC-generating potential of the fuel regularly used in the home for cooking<sup>29</sup>. Third, the lifespan of AM in the lower airway, and therefore its ability to acquire inhaled BC, is up to several months<sup>30</sup>. Finally, there was a gender imbalance between cyclists and non-cyclists in the present study. This imbalance reflects cycling behavior in London, where male cyclists make twice as many trips than women in the 25-44 age range<sup>31</sup>. An effect of sex on AM carbon is unlikely since i) we previously found no association between sex and AM carbon in healthy adolescents<sup>13</sup>, ii) Jacobs *et al*<sup>15</sup> found no association between AM carbon and sex in a large study of adult diabetic patients, and iii) there was no significant difference in AM carbon between males and females (unpaired t test).

In summary, cycling to work in London is associated with increased AM carbon compared with travelling to work by public transport and/or walking. Increased AM

carbon in cyclists is due, in part, to the longer time taken to travel to work and exposure to high local concentrations of BC. Personal BC monitoring is a promising method for planning personal commute routes, but interpreting these data is not straightforward. For example, cycling to work by a low traffic-density road will certainly reduce commute BC *per unit time*, but if travel time increases, total commute BC exposure may remain the same. In conclusion, cycling to work in London is associated with increased long-term inhaled dose of BC. Whether increased minute ventilation associated with cycling is an independent risk factor for increased inhaled BC dose, remains unclear.

## Legend for Figures

**Figure 1.** A cycle lane (shaded line painted on side of road) in the east end of London. Cyclists commute close to vehicles.

**Figure 2.** Personal black carbon (BC; ( $\eta\text{g}/\text{m}^3/5 \text{ min}$ ) monitored using a portable aethalometer in (blue line) cyclists ( $n=14$ ) and (red line) non-cyclists ( $n=13$ ). Data are represented as the mean BC exposure for each group. Time is on the horizontal axis and mean BC on the vertical axis. Exposure to BC is low in both groups when subjects are at home.

**Figure 3** The proportion of 24 h black carbon (BC) exposure at i) home, ii) during commuting, and iii) during non-commuting (“other”) activities in **A**) cyclists ( $n=14$ ) and **B**) non-cyclists ( $n=13$ ). BC exposure was the sum of mean BC ( $\eta\text{g}/\text{m}^3$ ) for each 5 min period. Activity was determined from a diary completed on the day of monitoring. A greater proportion of 24 h BC exposure in cyclists is from the commute ( $P < 0.001$  vs. non-cyclists).

**Figure 4.** Airway macrophages (AM) from a cyclist. There is marked heterogeneity in uptake of carbon by AM (black areas in the cytoplasm). AM were imaged under light microscopy (x100).

**Figure 5.** Alveolar macrophage (AM) carbon in cyclists and non-cyclists. Bar represents mean. For each subject, the mean AM carbon ( $\mu\text{m}^2$ ) was determined from 50 randomly imaged cells \* $P < 0.01$ .

**Table 1.** Demographics and lung function in the 28 subjects who had airway macrophage carbon determined.

	Cyclists (n=14)	Non-Cyclists (n=14)	95% confidence interval of the difference
Male/Female	9/5	2/12*	
Age (yr)	27 $\pm$ 1.7	23 $\pm$ 1.4	-0.49 to 8.92
Height (cm)	174.2 $\pm$ 1.7	169.1 $\pm$ 3.06	-2.9 to 13.1
Weight (kg)	71.2 $\pm$ 3.0	64.4 $\pm$ 3.0	-2.0 to 15.6
Distance of home to nearest main road (m)	100 $\pm$ 22.0	209 $\pm$ 52.3	-226 to 7.4
FEV <sub>1</sub> (% predicted)	101 $\pm$ 2.8	100.3 $\pm$ 2.3	-6.8 to 8.2
FVC (% predicted)	96.5 $\pm$ 3.0	94.2 $\pm$ 2.8	-6.1 to 10.7
FEV <sub>1</sub> /FVC (% predicted)	104.5 $\pm$ 1.8	107.5 $\pm$ 1.9	-8.5 to 2.5
FEF <sub>25-75</sub> (% predicted)	114.1 $\pm$ 8.4	112.7 $\pm$ 3.7	-17.5 to 20.4

FEV<sub>1</sub> = forced expiratory volume in 1 s, FVC = forced vital capacity, FEF<sub>25-75</sub> =

forced expiratory flow 25-75%. Lung function was done 10 min post inhaled

bronchodilator (salbutamol 400  $\mu$ g). \*P<0.05 by Chi-Square test. Data are described

as mean (standard error of the mean) and compared by unpaired t test.

**Table 2** Short-term personal black carbon exposure data from cycling- and non-cycling adults. Black carbon monitoring was done on a representative working day (24 h) after assessment of alveolar macrophage carbon. Black carbon exposure during specific activities was determined from a time/activity diary completed on the day of monitoring.

	Cyclists (n=14)	Non-cyclists (n=13) <sup>#</sup>	95% confidence interval of difference
Interval from sputum induction to personal monitoring (days)	70.3 ± 9.5	84.4 ± 15.5	-51.1 to 22.9
Mean background PM <sub>10</sub> (µg/m <sup>3</sup> ) <sup>¶</sup>	27.9 ± 3.5	33.5 ± 4.6	-17.4 to 6.3
Mean background temperature (°C) <sup>¶</sup>	9.7 ± 1.1	6.7 ± 0.8	-0.07 to 6.0
Mean background precipitation (mm) <sup>¶</sup>	0.44 ± 0.24	0.71 ± 0.34	-1.1 to 0.58
Mean background humidity (%) <sup>¶</sup>	72.6 ± 2.7	80.0 ± 2.3	-14.9 to 0.2
Mean background wind speed (km/h) <sup>¶</sup>	13.0 ± 0.9	12.0 ± 1.6	-2.7 to 4.7
Proportion of time			
at home (%)	40.6 ± 3.4	64.1 ± 4.1	-21 to 0.5
commuting (%)	10.3 ± 1.2	5.7 ± 0.4	1.5 to 7.7
other (%)	26.8 ± 3.8	20.9 ± 3.5	-5.0 to 16.7
Peak BC (ηg/m <sup>3</sup> )	47370 ± 9960	21338 ± 5150	2396 to 49666
24 h total BC (ηg/m <sup>3</sup> ) <sup>§</sup>	7.58 x10 <sup>5</sup>	6.50 x10 <sup>5</sup>	-9.99 x 10 <sup>4</sup> to 3.14 x 10 <sup>5</sup>

home BC ( $\eta\text{g}/\text{m}^3$ )	$\pm 7.0 \times 10^4$ $2.88 \times 10^5$	$\pm 7.1 \times 10^4$ $4.18 \times 10^5$	$-2.8 \times 10^5$ to $2.4 \times 10^4$
commute BC ( $\eta\text{g}/\text{m}^3$ )	$\pm 3.8 \times 10^4$ $3.25 \times 10^5$	$\pm 6.5 \times 10^4$ $1.20 \times 10^5$	$8.2 \times 10^4$ to $3.2 \times 10^5$
other BC ( $\eta\text{g}/\text{m}^3$ )	$\pm 4.8 \times 10^4$ $1.44 \times 10^5$	$\pm 3.2 \times 10^4$ $1.11 \times 10^5$	$-4.9 \times 10^4$ to $1.1 \times 10^5$
	$\pm 3.4 \times 10^4$	$\pm 1.8 \times 10^4$	

PM<sub>10</sub> = particulate matter less than 10 microns in aerodynamic diameter. BC= black carbon. <sup>#</sup>Personal monitoring was not done in one subject with airway macrophage carbon. <sup>¶</sup>Background PM<sub>10</sub> and meteorological data are expressed as 24 h mean for each subject's day of monitoring. <sup>§</sup>Black carbon (BC) is the sum of BC concentrations for each 5 min period monitored by aethalometer on a representative working day. Data are described as mean (standard error of the mean) and compared by t test.

**Table 3.** Airway macrophage carbon and serum cytokines in cyclists and non-cyclists.

	Cyclists (n=14)	Non-Cyclists (n=14)	95% Confidence interval of difference
Airway macrophage carbon ( $\mu\text{m}^2$ )	$1.81 \pm 0.21$	$1.11 \pm 0.07$	0.22 to 1.17
GM-CSF ( $\rho\text{g/mL}$ )	$1.04 \pm 0.65$	$0.59 \pm 0.19$ (n=12)	-1.05 to 1.94
IL-1 $\beta$ ( $\rho\text{g/mL}$ )	$0.68 \pm 0.12$	$1.11 \pm 0.22$ (n=12)	-0.93 to 0.69
IL-2 ( $\rho\text{g/mL}$ )	$2.07 \pm 0.52$	$0.74 \pm 0.46$ (n=12)	-0.14 to 2.78
IL-6 ( $\rho\text{g/mL}$ )	$0.84 \pm 0.06$	$0.98 \pm 0.22$ (n=12)	-0.59 to 0.30
IL-8 ( $\rho\text{g/mL}$ )	$3.73 \pm 0.22$	$4.56 \pm 0.69$ (n=12)	-2.25 to 0.59
TNF- $\alpha$ ( $\rho\text{g/mL}$ )	$7.18 \pm 0.39$	$5.99 \pm 0.29$ (n=12)	0.14 to 2.23

GM-CSF = Granulocyte-macrophage colony-stimulating factor; IL= interleukin;

TNF- $\alpha$  = tumor necrosis factor alpha. Data are described as mean (standard error of the mean) and compared by unpaired t test.

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Fig 1



Fig 2

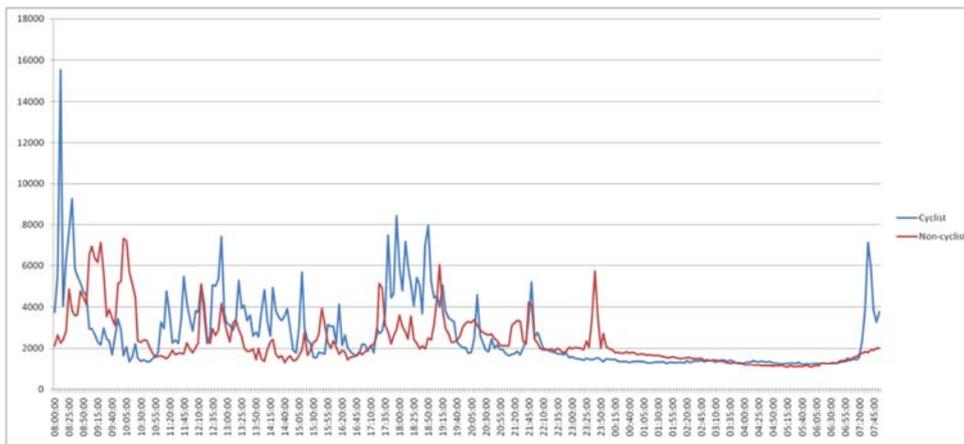


Fig 3 a

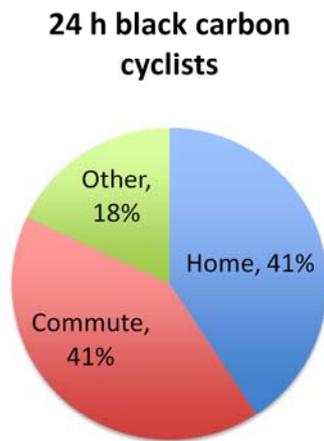


Fig 3b

**24 h black carbon  
non-cyclists**

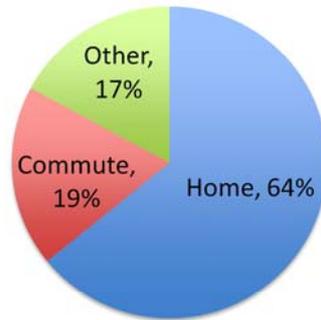


Fig 4

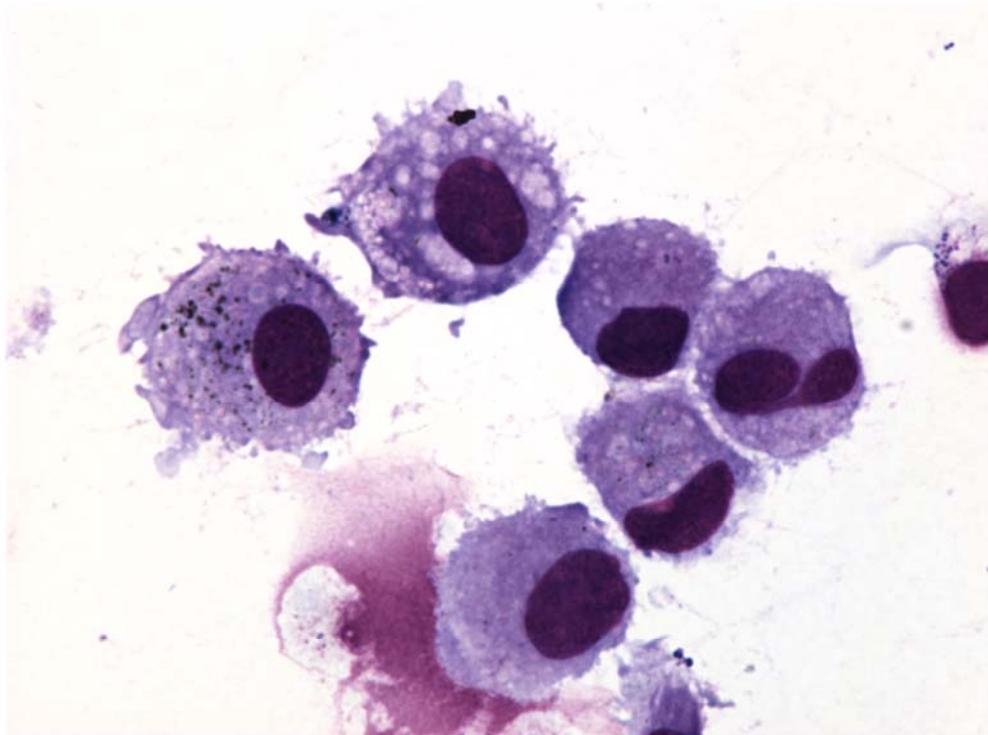


Fig 5

