Acute effects of second-hand smoke on complete blood count

Petros C. Dinas\textsuperscript{a}, Giorgos S. Metsios\textsuperscript{b}, Athanasios Z. Jamurtas\textsuperscript{c}, Manolis N. Tzatzarakis\textsuperscript{d}, A. Wallace Hayes\textsuperscript{e}, Yiannis Koutedakis\textsuperscript{c}, Aristidis M. Tsatsakis\textsuperscript{d} & Andreas D. Flouris\textsuperscript{a}

\textsuperscript{a} FAME Laboratory, Centre for Research and Technology Thessaly, Trikala, Greece.
\textsuperscript{b} School of Sport, Performing Arts and Leisure, University of Wolverhampton, Wolverhampton, UK.
\textsuperscript{c} Department of Exercise Sciences, University of Thessaly, Trikala, Greece.
\textsuperscript{d} Centre of Toxicology Science and Research, School of Medicine, University of Crete, Herakleio, Greece.
\textsuperscript{e} Harvard School of Public Health, Harvard University, Boston, USA.

Published online: 02 Apr 2013.


To link to this article: http://dx.doi.org/10.1080/09603123.2013.782603
Acute effects of second-hand smoke on complete blood count

Petros C. Dinasa, Giorgos S. Metsiosb, Athanasios Z. Jamurtasc, Manolis N. Tzatzarakisd, A. Wallace Hayese, Yiannis Koutedakis, Aristidis M. Tsatsakisd and Andreas D. Flourisa*

aFAME Laboratory, Centre for Research and Technology Thessaly, Trikala, Greece; bSchool of Sport, Performing Arts and Leisure, University of Wolverhampton, Wolverhampton, UK; cDepartment of Exercise Sciences, University of Thessaly, Trikala, Greece; dCentre of Toxicology Science and Research, School of Medicine, University of Crete, Herakleio, Greece; eHarvard School of Public Health, Harvard University, Boston, USA

(Received 8 November 2012; final version received 17 January 2013)

We assessed the acute effects of a 1-h exposure to second-hand smoke (SHS) on complete blood count (CBC) markers in a controlled simulated bar/restaurant environment. Nineteen adult never-smokers completed a 1-h exposure to SHS at bar/restaurant levels, and a 1-h exposure to normal room air. Blood samples were collected at the baseline at 30 min during each exposure, and at 0, 0.5, 1, 2, 3, and 4 h after each exposure. The values of white blood cells (WBC) at 1 h ($p=0.010$), 3 h ($p=0.040$), and 4 h ($p=0.008$) following SHS were significantly increased compared with the baseline values. Also, there was a positive association between the WBC and cotinine levels ($r=0.28$, $p=0.007$). A 1-h exposure to SHS at bar/restaurant levels significantly increased the WBC for at least 4 h following the exposure time. This effect of SHS on WBC has dose–response characteristics and should be considered to prescribing CBC.

Keywords: passive smoking; environmental tobacco smoke; white blood cells; inflammation

1. Introduction

Chronic exposure to second-hand smoke (SHS) causes a host of health problems, such as endothelial dysfunction, atherosclerosis, and increased platelet activation (Metsios et al. 2007). This is because the effects of chronic SHS exposure are on average 80–90% as harmful as those of chronic active smoking (Barnoya & Glantz 2005; Metsios et al. 2007). Interestingly, recent evidence suggests that the acute effects of SHS are also harmful (Metsios et al. 2007; Flouris et al. 2008, 2009; Flouris, Metsios et al. 2010). Indeed, acute exposure to SHS undermines lung function, increases cytokine production, and up-regulates metabolism through changes in thyroid hormone secretion (Flouris et al. 2008, 2009; Flouris, Metsios et al. 2010; Flouris, Vardavas et al. 2010). However, there is a lack of data on more routinely performed haematology tests such as the complete blood count (CBC). The CBC is one of the most commonly ordered tests in medicine and, in particular, for the preoperative assessment of patients (Michota & Frost 2004), providing an overview of an individual’s general health status as well as...
information on infection, inflammation, and inflammatory disease, deficiencies in the immune system, bone marrow disease, and other pathological conditions.

Some epidemiological studies have assessed the effects of chronic exposure to SHS on CBC, indicating that white blood cell (WBC) count is the parameter most often affected (Ronchetti et al. 1990; Panagiotakos et al. 2004). However, the findings are controversial regarding the chronic effects of SHS on CBC. Indeed, some authors reported that WBC is increased in individuals commonly exposed to SHS (Ronchetti et al. 1990; Panagiotakos et al. 2004), while others report no such changes (Husgafvel-Pursiainen et al. 1987; Venn & Britton 2007; Sochaczewska et al. 2010), or even reduced levels of WBC (Menzies et al. 2006). A possible reason for these differences is the fact that the level of SHS exposure has been assessed primarily through questionnaires – which provide information on average/chronic SHS exposure – yet CBC indicators, such as WBC are heavily influenced by the previous 2–4 days (McGill et al. 2008). Given that CBC is one of the most commonly ordered blood tests, potential effects of SHS on its results could have a considerable impact on clinical decisions, especially in cases where rapid illness progression is at risk, or where the result could either rule out serious infection, or influence a decision on hospital admission or treatment with antibiotics. Therefore, the purpose of this study was to assess the acute effects of a 1-h exposure to SHS on CBC markers of healthy adult never-smokers in a controlled simulated bar/restaurant environment.

2. Methods

2.1. Participants and procedures

In a randomized controlled cross-over trial (EudraCT: 2009–013545–28), 19 healthy adult never-smokers (10 men; 9 women; age: 32.8 ± 5.9 years; body mass index: 23.5 ± 3.1 kg/m²) from the general population volunteered and gave a written informed consent. The exclusion criteria (assessed via medical history) were comprised of: smoking, pregnancy, evidence of cardiac or pulmonary disease, current infection and use of medications, and previous chronic disease and medications. All women participants were premenopausal with regular menstruation and were tested during the late luteal phase of their menstrual cycle. The experimental protocol was approved by the ethical review board at the University of Thessaly (Approval No 164/2009) and adhered to the standards set by the Declaration of Helsinki.

2.2. Experimental design

Participants visited the laboratory on two separate days at 9 am to complete in a random order, either the experimental or the control trial (Figure 1(a)) following a 10-h fast and a 72-h abstinence from strenuous physical activity. The experimental trial included a 1-h exposure to SHS, while the control trial included a 1-h exposure to normal room air. In each trial, blood samples were collected at the baseline, at 30 min during the exposure, as well as at 0, 0.5, 1, 2, 3, and 4 h after each exposure (Figure 1(a)). Given the prolonged nature of the experiment, the subjects were given a small sandwich consisting of 30% fat, 55% carbohydrate, and 15% protein, 1 h following the exposure in each trial.

2.3. Exposure setting

A 6 × 5 × 4 m environmentally controlled chamber (air temperature: 24 °C; air velocity: 0.05 m/s; humidity: 45%) was used in which the participants remained seated, quietly
reading a book or a magazine. In the exposure trial, the air inside the chamber was polluted with 23 ppm CO – which is considered as moderate concentration (Scherer et al. 1990) – to simulate a bar/restaurant smoking environment (Metsios et al. 2007; Flouris et al. 2008, 2009; Flouris, Metsios et al. 2010). Previous studies evaluating the acute health effects of SHS used CO concentrations between 30 and 40 ppm (Kato et al. 1999; Giannini et al. 2007; Leone & Balbarini 2008), while 33 ppm have been recently reported at bars (Goniewicz et al. 2009) and 29 ppm have been reported in workplace environments (White & Froeb 1980). The CO concentration was achieved via cigarette combustion from a variety of popular brands and was continuously monitored using a CO analyzer (Martindale Electric, Watford, England). In the control trial, participants breathed normal room air which was continuously renewed during the 4-h of the trial. The air inside the chamber was continuously mixed throughout each exposure to ensure equal distribution.

2.4. CBC and cotinine measurements

Blood samples were collected by a certified phlebotomist from an antecubital vein into plain evacuated test tubes. A total of 3 ml of whole blood was used to assess: WBC, red blood cells (RBC), hemoglobin (HGB), hematocrit (HGT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT). All blood samples were tested using a Sysmex K-1000 (TOA Electronics, Japan) autoanalyzer.

Blood samples for cotinine measurements were immediately centrifuged and aliquots were stored at −80 °C. Serum (1 ml) was mixed with phosphate buffer solution (pH = 6.88) and the solid phase extraction was performed using SPE C18 (100 mg) columns. Following previously published procedures (Metsios et al. 2007; Flouris et al. 2008, 2009; Flouris, Metsios et al. 2010), cotinine was eluted with dichloromethane-

Figure 1. Experimental design (a) and change in WBC count (exposure trial minus control trial) (b). Shaded area represents SHS. *p < 0.05.
2.5. Statistical analyses

Sample size calculations were conducted based on previously published values of interleukin five before and after (37.2 ± 6.8 vs. 69.7 ± 7.5 in men and 35.1 ± 1.4 vs. 56.0 ± 8.0 in women) a similar 1-h SHS exposure (Flouris et al. 2009). The resulting minimum required sample size was eight participants for two-tailed type 1 and type 2 errors of 5%. In order to focus the analyses entirely on the effect of SHS while removing any diurnal variation, values from the control trial data were subtracted from the values of the exposure trial for both CBC indices and cotinine. One-way analysis of variance (ANOVA) was performed on CBC indices and cotinine using time (eight data collection time points) as an independent variable. Post-hoc t-tests incorporating a Bonferroni adjustment were used to detect statistically significant differences between specific time points. For any CBC index that demonstrated changes across time, Pearson’s correlation coefficient was used to assess its linear association with cotinine levels in order to detect potential dose–response effects with SHS exposure. The level of significance was set at $p < 0.05$, except where the Bonferroni adjustment was applied.

3. Results

No significant effects of SHS were detected in RBC, HGB, HCT, MCV, MCH, MCHC, and PLT indices ($p > 0.05$). In contrast, as illustrated in Figure 1(b), one-way ANOVA detected that WBC increased across time [main effect of time ($F_{(7,86)} = 3.355$, $p = 0.003$)]. The post-hoc $t$-tests revealed that the values of WBC at 1 h ($p = 0.010$), 3 h ($p = 0.040$), and 4 h ($p = 0.008$) following SHS were significantly increased compared with the baseline values (Figure 1(b)). One-way ANOVA also detected that cotinine levels increased across time [main effect of time ($F_{(7144)} = 94.576$, $p = 0.001$). The post-hoc $t$-tests showed significantly increased cotinine values immediately after the exposure and at 30 min, 1, 2, 3, and 4 h ($p < 0.001$) after the exposure time compared with the baseline values. Pearson’s correlation coefficient demonstrated a statistically significant positive association between WBC and cotinine levels ($r = 0.028$, $p = 0.007$; Figure 2).

![Figure 2. The relationship between the change in WBC count and the change in cotinine levels.](image-url)
4. Discussion

The purpose of this study was to assess the acute effects of a 1-h exposure to SHS on CBC markers of healthy adult never-smokers in a controlled simulated bar/restaurant environment. We found that a 1-h exposure to SHS was associated with a significant increase in WBC. In contrast, acute SHS did not influence other CBC indices including RBC, HGB, HCT, MCV, MCH, MCHC, and PLT. This information is in line with some epidemiological studies reporting that adults (Panagiotakos et al. 2004) and children (Ronchetti et al. 1990) who are chronically exposed to SHS reveal increased WBC. The increased WBC observed in our study for at least 4 h following the SHS exposure alludes to chronic low-grade systemic inflammation in individuals exposed to SHS on a daily basis and/or at higher smoke concentrations for shorter periods. Moreover, the linear association detected between WBC and cotinine levels suggests that the effect of SHS on WBC is dose-dependent. We previously demonstrated an acute dose–response effect of SHS on metabolism (Metsios et al. 2007), an effect also observed for acute active smoking (Perkins & Sexton 1995; Collins et al. 1996).

The WBC is a secondary assessment of inflammatory load. As such, it may be argued that it does not represent the most suitable indicator of chronic systemic inflammation. However, acute inflammatory markers such as WBC and C-reactive protein have been shown to be both prognostic and predictive of future cardiovascular events in several populations (Bakhru & Erlinger 2005). Moreover, the notion of a SHS-induced chronic low-grade systemic inflammation has been previously suggested based on the results from the main proteins for acute inflammatory load. Specifically, interleukins 4, 5, and 6, as well as interferon gamma show a prolonged increase following SHS (Flouris et al. 2009), while levels of C-reactive protein are higher in individuals exposed to SHS on a daily basis (Panagiotakos et al. 2004). The bonding of leucocytes to endothelial cells is also initiated within the first few minutes of smoke inhalation (Lehr et al. 1991), while lung inflammation (as measured by exhaled nitric oxide) and platelet activation are increased within the first 15–20 min of smoke inhalation (Davis et al. 1989; Yates et al. 2001). The latter is not confirmed in our study as we observed no changes on platelet activation. Following 1 h of smoke inhalation, nearly all systems are affected (Metsios et al. 2007; Flouris et al. 2008, 2009; Flouris, Vardavas et al. 2010). All these mechanisms are linked with low-grade systemic inflammation and the development and/or exacerbation of cardiovascular and chronic lung disease.

It is concluded that a 1-h exposure to SHS at bar/restaurant levels significantly increased the WBC assessed via CBC for at least 4 h following exposure in adult never-smokers. This effect of SHS on WBC has dose–response characteristics and should be taken into account when administering CBC, a test with major impact on clinical decision-making. WBC is expected to be relatively higher in individuals exposed to SHS on a daily basis and/or following an acute exposure to high SHS concentrations.

Acknowledgments

The salary of ADF is paid by the Centre for Research and Technology Thessaly. He has served as an expert consultant for the World Health Organization regarding electronic nicotine delivery systems. All authors, report no financial or personal relationships with other people or organizations that could influence (bias) their actions.
References


gaseous and particulate phase components of tobacco smoke in active and passive smokers.
Sochaczewska D, Czeszynska MB, Konefal H, Garanty-Bogacka B. 2010. Maternal active or pas-
sive smoking in relation to some neonatal morphological parameters and complications. Gine-
kol Pol. 81:687–692.
Venn A, Britton J. 2007. Exposure to secondhand smoke and biomarkers of cardiovascular dis-
White JR, Froeb HF. 1980. Small-airways dysfunction in nonsmokers chronically exposed to