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Publisher: Taylor & Francis

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International Journal of Environmental Health Research

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/cije20>

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Published online: 26 Jun 2013.

To cite this article: International Journal of Environmental Health Research (2013): Exposure to secondhand smoke promotes sympathetic activity and cardiac muscle cachexia, International Journal of Environmental Health Research, DOI: 10.1080/09603123.2013.800966

To link to this article: <http://dx.doi.org/10.1080/09603123.2013.800966>

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Exposure to secondhand smoke promotes sympathetic activity and cardiac muscle cachexia

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(Received 24 January 2013; final version received 1 April 2013)

Recent trials demonstrated that a single brief exposure to secondhand smoke (SHS) generates acute adverse health effects. We evaluated the acute (immediately after exposure) and short-term (0.5, 1, 2, 3 and 4 h after exposure) effects of SHS on cardiac autonomic control and myocardial integrity. Nineteen adult healthy never-smokers underwent a 1 h exposure to SHS at bar/restaurant levels and a 1 h control exposure. Heart rate variability (HRV), serum cotinine, and six cardiac protein markers were assessed before, during, and up to four hours following each exposure. SHS reduced the standard deviation of normal-to-normal intervals and increased cotinine levels, creatine kinase (CK)-MB, and myoglobin ($p < 0.05$). We conclude that acute exposure to SHS suppresses HRV and augments CK-MB and myoglobin. The SHS-induced elevations in CK-MB and myoglobin may reflect a generalized lytic state, especially of the cardiac muscle, which is apparent for at least 2 h following the SHS exposure.

Keywords: autonomic nervous system; creatine kinase-MB; HRV; myoglobin; passive smoking

Introduction

During the last decade, a number of studies in healthy never-smokers showed that brief exposures to secondhand smoke (SHS) can significantly impact the respiratory, immune, and endocrine systems (Metsios et al. 2007; Flouris et al. 2008, 2009, 2010). Similar findings have been reported for the cardiovascular system, suggesting that SHS generates acute haemodynamic alterations involved in the development of ischemic heart disease including platelet aggregation and endothelial dysfunction (Otsuka et al. 2001; Mahmud & Feely 2004). Moreover, some preliminary evidence in humans (Pope et al. 2001) and mice (Chen et al. 2008) showed that brief SHS exposures increase sympathetic drive and arrhythmia susceptibility within a few hours. However, the precise immediate impact of SHS on the autonomic nervous system function and myocardial markers of healthy never-smokers via a standardized experimental design remains

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to be elucidated (Dinas et al. 2013). Therefore, the aim of this experimental study was to investigate, for the first time, the immediate impact of SHS on cardiac autonomic control and myocardial integrity. Based on a recent analysis, (Dinas et al. 2013) we hypothesized that SHS would increase sympathetic drive and markers of myocardial injury.

Methods

The study conformed the standards set by the Declaration of Helsinki and was approved by the University of Thessaly ethics review board. *A priori* power calculations based on previous data (Metsios et al. 2007) demonstrated that a sample of 11 participants provided >95% statistical power. Nineteen healthy never-smokers (10 men; nine women; age 32.8 ± 5.9 years; body mass index: 23.5 ± 3.1 kg/m²) participated after providing written informed consent. Exclusion criteria included: smoking, pregnancy, evidence of cardiac disease, abnormal spirometry, recent (<8 weeks) respiratory tract infection, and previous disease and medications known to affect cardiovascular function.

Participants were evaluated individually during a SHS and a control condition administered in a random order and separated by ≥ 7 days (Figure 1(A)) to eliminate the effect of cotinine's comparatively long half-life (i.e. 24 h). Furthermore, in order to eliminate the effect of diurnal variation, all data were collected at the same time of the day (i.e. all trials started at 9:00 am). During the SHS condition, volunteers were submitted to a 1 h SHS exposure inside a 120 m³ environmental chamber (24 °C; humidity: 45%) with stable carbon monoxide (CO) concentration at bar/restaurant levels (23 ± 1 ppm) measured at 1.5 m from the floor (CO90 analyzer, Martindale Electric Ltd, Watford, UK). The desired CO concentration of the gas mixture was achieved by burning cigarettes placed on ashtrays at different areas in the chamber, as previously described (Flouris et al. 2008, 2009). The cigarettes were left to burn until reaching the filter. During the control condition, volunteers were exposed for 1 h to normal room air inside the same chamber.

Heart rate variability (HRV) was measured (Polar RS800CX, Polar Electro Oy, Finland) for 10 min at baseline and throughout each condition. The measured HRV indices were: standard deviation of the average NN intervals, the standard deviation of the average NN intervals calculated over short-period recordings (SDNN), the root mean square of differences of successive NN intervals, the count number of NN intervals that differ more than 50 ms, the percentage value of pairs of NN intervals that differ more than 50 ms, low and high frequency, the ration of low to high frequency, and the total variance of all NN intervals (known as total power). Following data collection, each HRV data series was split into 10 min blocks using the HRV Analysis Kubios software (Version 1.1, Biomedical Signal Analysis Group, University of Kuopio, Finland). (Flouris & Cheung 2009; Flouris & Scott 2009) The blocks at baseline, during each exposure, as well as at 0, 0.5, 1, 2, 3, and 4 h following each exposure (Figure 1(A)) were used for analysis, while the remaining HRV data were not used. Time and frequency domain measures of HRV were derived using the aforementioned Kubios software.

Blood samples were drawn by a certified phlebotomist from an antecubital vein during both conditions at baseline, at 0.5 h during each exposure, and at 0, 0.5, 1, 2, 3, and 4 h following each exposure (Figure 1(A)). In both the conditions, the phlebotomist entered the chamber 0.5 h during each exposure to collect the blood samples for this time point. Serum cotinine levels were evaluated using a liquid chromatograph mass

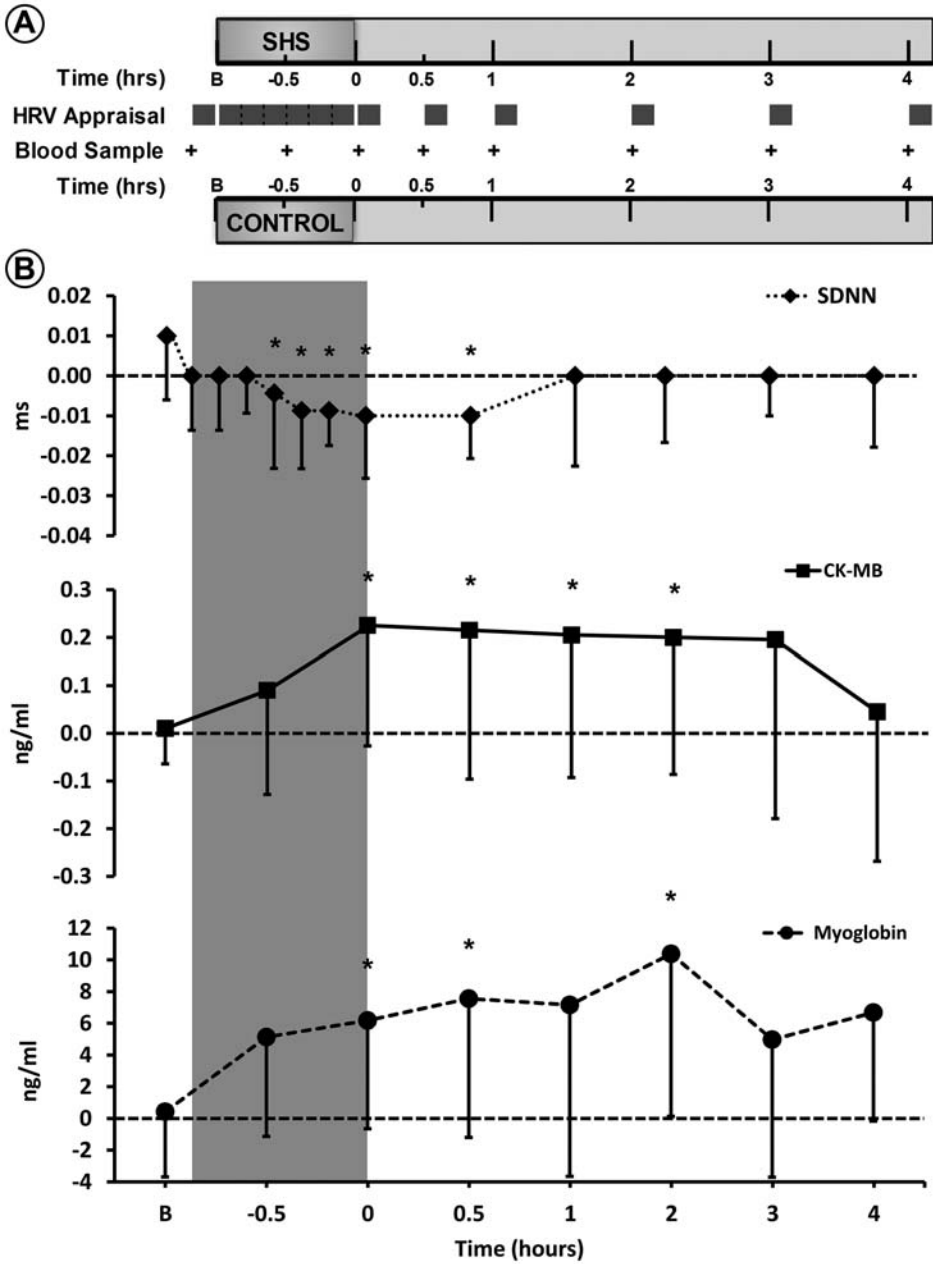


Figure 1. (A) Outline of the experimental design during the SHS and the control conditions. Crosses indicate the time points of blood sampling. Grey squares indicate the 10 min blocks of HRV assessment. “B” indicates baseline assessment, while “0” indicates the end of exposure. (B) Differences (i.e. SHS trial minus control trial) in SDNN, creatine kinase MB (CK-MB), and myoglobin levels measured prior to, during, and following the exposure. Asterisks indicate significant ($p < 0.05$) differences compared to baseline. The shaded region represents the exposure period. “B” indicates baseline assessment, while “0” indicates the end of exposure.

spectrometer system as previously described (Flouris et al. 2010, 2012). The Evidence_{investigator}TM (Randox Laboratories Ltd, UK) cardiac array, a sandwich chemiluminescent immunoassay, was used for the *in vitro* simultaneous quantitative detection of six cardiac biomarkers: troponin I, myoglobin, creatine kinase-MB (CK-MB), carbonic anhydrase III, fatty acid binding protein, and glycogen phosphorylase BB in serum samples.

Differential data analysis (Flouris 2012) was used to isolate the effect of SHS and remove the influence of diurnal variation in the examined parameters. Specifically, the difference between the SHS and the control trial was computed for each parameter (e.g. baseline value of CK-MB in the SHS trial minus baseline value of CK-MB in the control trial). Due to lack of normal distribution in our data, we used the non-parametric statistics Kruskal–Wallis one-way ANOVA followed by *post hoc* Mann–Whitney *U* tests to assess the effect of time on all variables. The level of significance was set at $p \leq 0.05$.

Results

Serum cotinine increased from 0.4 ± 0.7 at baseline (consisted with nonsmokers) (Flouris et al. 2009) to 14.0 ± 4.2 ng/mL immediately following SHS and remained increased for four hours (20.93 ± 4.4 ng/mL; $p < 0.05$). When analyzing the all time points simultaneously, no significant changes in HRV, myocardial markers, and respiration were detected ($p > 0.05$). However, subsequent *post hoc* tests detected a significant SHS-induced reduction in the standard deviation of normal-to-normal intervals ([SDNN] an HRV index measured in milliseconds characterizing the parasympathetic nervous system component of autonomic function), as early as 0.5 h during the exposure ($p = 0.034$; Figure 1(B)). The suppression of SDNN was sustained until 0.5 h after the exposure ($p = 0.047$). Regarding myocardial markers, the SHS lead to increased concentration of CK-MB and myoglobin which, compared with baseline values, remained significantly elevated for two hours after the exposure ($p < 0.05$; Figure 1(B)). A similar trend was observed in carbonic anhydrase III, however the comparisons against baseline values did not reach statistical significance ($p = 0.08$).

Discussion

This study shows for the first time that an average SHS exposure – such as that used in the present study – suppresses HRV within 0.5 h and the augments CK-MB and myoglobin within one hour. The observed HRV suppression confirms our first hypothesis and is associated with the impaired cardiac electrical conduction leading to confirmed detrimental long-term effects (Dinas et al. 2013). We recently proposed that this acute SHS-induced HRV suppression is caused either by a nicotine-induced up-regulation of catecholamine release or by inhaled suspended particles in smoke (Dinas et al. 2013). Indeed, low HRV is prognostic of myocardial infarction, heart failure conduction disturbances, and ventricular dyssynchrony (La Rovere et al. 2003; Dinas et al. 2013). Moreover, increased SNS activity has been related to the development of hypertension, diabetes, and cardiovascular diseases (Hayano et al. 1990; Flouris et al. 2009).

Our second hypothesis that SHS would increase markers of myocardial injury was partly confirmed. Indeed, as SHS did not influence troponin I, it is unlikely that the rise in CK-MB was of cardiac origin. This is also true for myoglobin, given the upward (yet not statistically significant) trend observed in carbonic anhydrase III. The

augmented myoglobin and carbonic anhydrase III strongly indicate that the source of serum myoglobin is skeletal in origin. Tobacco smoke contains various components that may promote skeletal muscle protein breakdown (Rom et al. 2012a) and catabolic processes (Metsios et al. 2007). Among smokers, several epidemiological and experimental studies (reviewed elsewhere) (Rom et al. 2012a) have confirmed that smoking increases the risk of sarcopenia by impairing muscle protein synthesis and augmenting the expression of genes associated with impaired muscle integrity (Petersen et al. 2007).

A cellular model proposed recently (Rom et al. 2012b) to explain the smoke-induced skeletal muscle catabolism, suggests that volatile and soluble components (including aldehydes and reactive oxygen and nitrogen species) inherent in tobacco smoke lead to increased oxidative stress which, in turn, activates intracellular signaling pathways that up-regulate muscle-specific E3 ubiquitin ligases leading to muscle protein degradation. Increased inflammatory activity has been also identified as one of the main systemic effects of tobacco smoke inhalation that may promote skeletal muscle catabolism (Rom et al. 2012a). While most of these mechanisms were studied during active smoking, there is no reason to suggest that they do not apply also to SHS. Indeed, previous research demonstrated that even brief exposures to SHS can generate acute disruptions in normal catabolic processes indicating protein breakdown (Metsios et al. 2007). Furthermore, several lines of evidence have confirmed a rapidly developing systemic inflammatory reaction occurring during SHS exposure. Leucocytes start bonding to endothelial cells within five minutes, (Lehr et al. 1991) while within 60 min of SHS a pronounced inflammatory response has been documented (Flouris et al. 2008, 2009, 2012). According to the above lines of evidence, it can be inferred that the lytic state, especially of the skeletal muscle, observed immediately following SHS in the present study was manifested through oxidative stress and systemic inflammation.

In conclusion, this is the first study to demonstrate that a 1 h SHS exposure at bar/restaurant levels suppresses HRV within 0.5 h and augments CK-MB and myoglobin within one hour. While the effect on cardiac autonomic control recedes within 1 h after the exposure, the generalized lytic state indicated by the SHS-induced elevations in CK-MB and myoglobin is apparent for at least 2 h following SHS.

Acknowledgments

This study received no funding from external sources and the authors report no financial or personal relationships with other people or organizations that could inappropriately influence (bias) their actions. Andreas Flouris' salary is paid by the Centre for Research and Technology Thessaly. He has served as an expert consultant for the World Health Organization regarding nicotine delivery systems.

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