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(ACUTE EFFECTS OF PASSIVE TOBACCO SMOKING ON HEART RATE VARIABILITY)

ΥΠΕΥΘΥΝΟΣ ΚΑΘΗΓΗΤΗΣ: Δρ. ΤΖΙΑΜΟΥΡΤΑΣ ΑΘΑΝΑΣΙΟΣ

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ABSTRACT

A vast number of studies have demonstrated that passive smoking (PS) generates a number of unfavourable health effects, including an increased risk for stroke, multiple cancers, lung cancer, emphysema, and heart disease. Heart rate variability (HRV) is a straightforward and cost effective technique to foresee health problems of cardiovascular nature and may be used to predict in advance smoking-induced health effects that may arise in the future. The aim of this study was to examine the PS effects and their duration on HRV. METHODS: Nineteen healthy adults (10 male, 9 female: age 32.8±5.9 years, BMI 23.5±3.1 kg/m^2) volunteered. On two separate days, participants arrived at the laboratory to complete, in a random order, an experimental and a control trial. HRV measurements were taken using a Polar PS800CX (Polar Electro Oy, Kempele, Finland) throughout the control and exposure trials. Data collected during each exposure were split into six 10-min intervals. Moreover, data collected from 0-10, 30-40, 60-70, 120-130, 180-190, and 240-250 minutes following the exposure each trial were used to derive HRV for the different time. The HRV indices studied were the root mean square of differences of successive RR intervals (RMSSD) and the percentage value of pairs of RR intervals that differ more than 50 ms (pNN50) for the time domain as well as the low (LF) and high (HF) frequency and their ratio (LF/HF) for the frequency domain. RESULTS: A factorial [sex (male, female), trial (control, PS), time (12 time points)] multivariate analysis of variance demonstrated main effects of sex on LF/HF and RMSSD ($P<0.05$), of trial on pNN50 ($P<0.05$), and of time on LF/HF ($P<0.05$). Interaction effects of trial*time on LF/HF and RMSSD ($P<0.05$) and of sex*trial*time on RMSSD ($P<0.05$) were also detected. CONCLUSIONS: PS influences the normal autonomic system functioning, as

measured by HRV by increasing the activation of the sympathetic nervous system which may indicate abnormalities in cardiovascular function.

ΠΕΡΙΛΗΨΗ

Μεγάλος αριθμός ερευνών έχει δείξει ότι το παθητικό κάπνισμα (ΠΚ) προκαλεί πολλές ανεπιθύμητες παρενέργειες στην υγεία του ανθρώπου, όπως προδιάθεση για εγκεφαλικό επεισόδιο, διαφόρους τύπους καρκίνου, εμφύσημα και καρδιαγγειακή νόσο. Η μεταβλητότητα του καρδιακού παλμού (ΜΚΠ) είναι ένας δείκτης που μας βοηθάει να διαγνώσουμε καρδιολογικά προβλήματα υγείας και μπορεί να χρησιμοποιηθεί για να προβλεφθούν προβλήματα που προκύπτουν από το κάπνισμα. Οι στόχοι της παρούσας εργασίας ήταν να εξετάσει τις επιδράσεις του ΠΚ και τη διάρκεια αυτών στη ΜΚΠ. ΜΕΘΟΔΟΣ: Δέκα εννέα υγιείς εθελοντές (10 άνδρες, 9 γυναίκες : ηλικίας 32.8±5.9 ετών, BMI 23.5±3.1 kg/m^2). Σε δύο ξεχωριστές ημέρες οι εθελοντές προσήλθαν στο εργαστήριο για να πραγματοποιήσουν μετρήσεις τη μία φορά με έκθεση σε παθητικό κάπνισμα (πειραματικό πρωτόκολλο) και την άλλη φορά για πρωτόκολλο ελέγχου. Οι μετρήσεις της ΜΚΠ πραγματοποιήθηκαν με καρδιοσυχνόμετρο Polar PS800CX (Polar Electro Oy, Kempele, Finland). Οι μετρήσεις κατά τη διάρκεια της έκθεσης στο ΠΚ διαχωρίστηκαν σε έξι τμήματα των 10 λεπτών και στο πειραματικό πρωτόκολλο και στο πρωτόκολλο ελέγχου. Μετρήσεις επίσης πραγματοποιήθηκαν μετά την έκθεση στο ΠΚ σε τμήματα των 10 λεπτών στο 0-10, 30-40, 60-70, 120-130, 180-190, και 240-250 λεπτό και στο πειραματικό και στο πρωτόκολλο ελέγχου. Οι δείκτες ΜΚΠ που μελετήθηκαν ήταν η μέση τετραγωνική ρίζα των διαφορών μεταξύ διαδοχικών διαστημάτων RR (RMSSD), το ποσοστό των ζευγαριών RR διαστημάτων που διαφέρουν περισσότερο από 50 ms (pNN50), η χαμηλή (LF) και η υψηλή συχνότητα (HF) και ο λόγος τους (LF/HF). ΑΠΟΤΕΛΕΣΜΑΤΑ: Μια παραγοντική ανάλυση διακύμανσης [φύλο (άνδρας, γυναίκα), δοκιμασία (ελέγχου, ΠΚ), χρόνος (12 χρονικά σημεία)] εντόπισε κύριες επιδράσεις του φύλου στους δείκτες LF/HF και RMSSD ($P < 0,05$), κύριες

επιδράσεις της δοκιμασίας στο pNN50 (P <0,05), και κύριες επιδράσεις του χρόνου στο LF/HF (P <0,05). Επίσης παρατηρήθηκε αλληλεπίδραση της δοκιμασίας και του χρόνου για το LF/HF και RMSSD (P <0,05) καθώς επίσης και του φύλου με το χρόνο για το RMSSD (P <0,05). ΣΥΜΠΕΡΑΣΜΑΤΑ: Το ΠΚ επηρεάζει τη λειτουργία του αυτόνομου νευρικού συστήματος όπως αυτή μετρήθηκε με την ΜΚΠ, αυξάνοντας την ενεργοποίηση του συμπαθητικού νευρικού συστήματος που υποδεικνύει ανωμαλίες στην καρδιακή λειτουργία.

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INTRODUCTION

A vast number of studies have demonstrated that active smoking generates a number of unfavourable health effects, including an increased risk for stroke, multiple cancers, lung cancer, emphysema, heart disease (Anon 1996; World Health Organisation 2002; Flouris, Vardavas et al. 2010). Based on recent evidence, there will be more than 8 million smoking-related deaths every year by 2030, while the total smoking-induced deaths during the 21st century will reach one billion (World Health Organization 2008). This is because, despite the adoption of stricter antismoking campaigns in many countries, more people smoke today than during any other time in human history [estimated to >1.25 billion adults]. (World Health Organisation 2002; WHO and Disease 2009) Indeed, the prevalence rates of smoking are steadily increasing (World Health Organisation 2002; Substance Abuse and Mental Health Services Administration 2007) primarily among young girls (Flouris, Faught et al. 2008; Warren, Jones et al. 2008) and a further global expansion of the tobacco epidemic is projected in the near future (World Health Organisation 2002). Given the widespread incidence of smoking as well as its deleterious health effects, it is crucial to examine practical and cost effective prognostic markers assessing the impact of smoking on health. This is particularly relevant for passive smoking (PS), the inhalation of smoke, called second hand smoke or environmental tobacco smoke, from tobacco products used by others. It occurs when tobacco smoke permeates any environment, causing its inhalation by people within that environment.

Heart rate variability (HRV) is a straightforward and cost effective technique to foresee health issues of cardiovascular nature and can be used to predict in advance smoking-induced health effects that may arise in the future (Hayano, Yamada et al. 1990). HRV describes the variations of the RR (NN) intervals (i.e., the time elapsing

between two consecutive R waves in the electrocardiogram) and can be used as a trustworthy expression of the many physiological factors modulating the normal heart rhythm (Anon 1996; Niskanen, Tarvainen et al. 2004). However, to our knowledge the evidence linking smoking with changes in HRV and, in turn, cardiovascular abnormalities have not been critically investigated. Moreover, it is also crucial to examine the existing biological evidence regarding the effects of passive smoking on HRV and their associated cardiovascular problems and to summarize fundamental information on the various HRV indicators and their diagnostic significance. This information will be valuable not only to physicians and scientists, but also to those interested in personal or public health, politics and economics.

Purpose

The aim of this study was to examine the PS effects and their duration on a variety of HRV indices.

Null Hypothesis

PS will not affect the HRV indices studied.

Alternative Hypothesis

PS will affect the HRV indices studied. Based on the limited published evidence in humans (Pope, Eatough et al. 2001) and mice (Chen, Chow et al. 2008), it is anticipated that PS will reduce HRV.

LITERATURE REVIEW

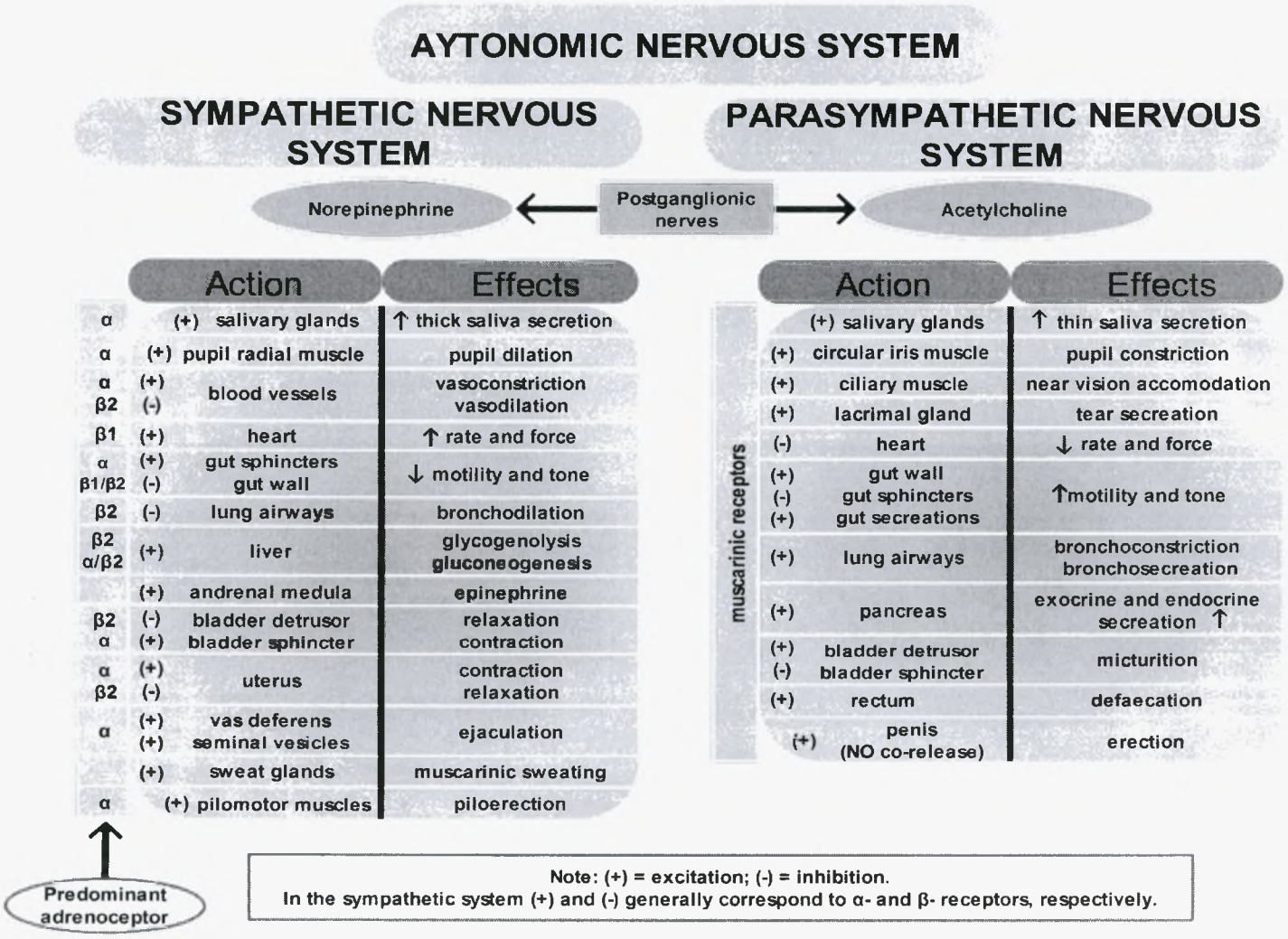
The aim of this literature review is to examine the existing biological evidence regarding the effects of passive smoking on HRV and their associated cardiovascular problems. In addition, I summarize fundamental information on the various HRV indicators and their diagnostic significance. I envisage that the information provided will be valuable not only to physicians and scientists, but also to those interested in personal or public health, politics and economics. In order to achieve the above, a comprehensive search in Pub Med was conducted using MeSH terms that are germane to active and passive smoking, HRV, autonomic function, and health effects (particularly of cardiovascular nature). The search also included the articles cited in the identified papers.

AUTONOMIC NERVOUS SYSTEM

HRV is calculated based on the time difference between repeated heart beats (Anon 1996; Niskanen, Tarvainen et al. 2004). As such, it characterizes changes in the activation of the autonomic nervous system which comprises of a neural network that automatically controls a number of bodily actions (e.g., circulation, digestion) through a series of positive and negative feedback loops (Kristal-Boneh, Raifel et al. 1995; Neal 2002; Niskanen, Tarvainen et al. 2004). The autonomic nervous system is divided anatomically into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). A number of organs, including the heart, are innervated by both SNS and PNS, which generally have opposing actions. The transmitter substance at SNS nerve endings is norepinephrine, while some preganglionic sympathetic fibres pass directly to the adrenal medulla that can release epinephrine. Both norepinephrine and epinephrine activate effector organs by acting

on α-, β_1-, or β_2-adrenoceptors (Neal 2002). On the other hand, the nerve endings of the PNS fibres release acetylcholine that acts on the different effector organs by activating muscarinic receptors (Neal 2002). The actions of SNS and PNS stimulation on a variety of organs are summarized in Figure 1.

Fig 1. Autonomic nervous system and the actions of the sympathetic (SNS) and parasympathetic (PNS) subsystems stimulation on a variety of organs.



HEART RATE VARIABILITY (HRV)

HRV describes the variations of the RR (NN) intervals (i.e., the time elapsing between two consecutive R waves in the electrocardiogram) and can be used as a trustworthy expression of the many physiological factors modulating the normal heart rhythm (Anon 1996; Niskanen, Tarvainen et al. 2004). As shown in Figure 1, the SNS acts to increase heart rate and plays a essential role in cardiovascular regulation in both health and disease (Sinski, Lewandowski et al. 2006). On the other hand, the PNS acts to lower heart rate (Neal 2002). Based on this mechanism, the heart rate rhythm and the contraction strength of the cardiac muscle are a consequence of the opposing influences exerted by the SNS and the PNS (Acharya, Joseph et al. 2006). At rest, the activation of the two systems must be comparable (Kristal-Boneh, Raifel et al. 1995; Acharya, Joseph et al. 2006). Increased SNS activation or decreased parasympathetic tone during periods of rest predispose to ventricular fibrillation and may indicate abnormalities such as hypertension, diabetes, cardiovascular diseases, or psychological problems (Kristal-Boneh, Raifel et al. 1995; Tsuji, Larson et al. 1996; Acharya, Joseph et al. 2006) and have been proposed as mechanisms explaining the associations of reduced HRV with increased mortality (Kleiger, Miller et al. 1987; Bigger, Fleiss et al. 1992; Tsuji, Larson et al. 1996). On the other hand, increased PNS activation at rest is an indicator of physical prowess, overall health and young biological age. It is evident, therefore, that HRV provides us with evidence that may predict heart abnormalities and it is linked with cardiovascular mortality (Kleiger, Miller et al. 1987; Bigger, Fleiss et al. 1992; Anon 1996; Tsuji, Larson et al. 1996).

Previous research has shown that active and passive smoking affect cardiovascular function by disrupting normal autonomic nervous system functioning (Acharya, Joseph et al. 2006). Indeed, HRV measurements have shown that active

(Hayano, Yamada et al. 1990) and passive (Zeskind and Gingras 2006) smoking generate both acute and chronic changes in autonomic cardiac control. Generally, active and passive smoking appear to decrease HRV and increase cardiac vulnerability (Hayano, Yamada et al. 1990; Niedermaier, Smith et al. 1993; Acharya, Joseph et al. 2006) and arrhythmia susceptibility (Chen, Chow et al. 2008).

INDICATORS OF HRV ANALYSIS

Wolf et al. (Wolf MM, Varigos GA et al. 1978) first recognized that reduced HRV is linked with an increased risk for post infarction mortality. Consequently, power spectral analysis of heart rate fluctuations was introduced to quantitatively evaluate beat-to-beat cardiovascular control (Akselrod, Gordon et al. 1981). These analyses provided with indicators that enhanced knowledge regarding the autonomic background of RR interval fluctuations in the heart rate record (Pomeranz, Macaulay et al. 1985; Pagani, Lombardi et al. 1986). At present, measurements of HRV are very common and are obtained usually through electrocardiography or specialized heart rate monitors (Anon 1996; Gamelin, Berthoin et al. 2006; Gamelin, Baquet et al. 2008; Nunan, Jakovljevic et al. 2008; Vanderlei, Silva et al. 2008). HRV recording is performed by an algorithm counting system which provides indicators connected with the autonomic nervous system activity with regards to the activation of the SNS and PNS. The various indicators of HRV are based either on the calculation of time difference between successive RR intervals (i.e., time-domain methods) or on the distribution of power (variance) as a function of frequency of the time difference between successive RR intervals (i.e., frequency-domain methods).

Time-Domain Methods

Time-domain methods are the simplest form of HRV analysis and are based on determining either the heart rate at any point in time or on the intervals between successive normal complexes. Using a continuous ECG record, each QRS complex is detected and the normal-to-normal (NN) intervals (i.e., intervals between adjacent QRS complexes resulting from sinus node depolarizations) or the instantaneous heart rate is determined. The time-domain methods provide with a number of HRV indices that derive from statistical or geometric analyses.

Statistical Indices

The statistical indices are based either on direct measurements of the NN intervals/instantaneous heart rate or on the differences between NN intervals. The most commonly used statistical indices include:

1. Standard deviation of the average NN intervals [SDNN; in milliseconds (ms)] calculated over successive short-period recordings with a normative SDNN value of 141±39 ms (Anon 1996; Niskanen, Tarvainen et al. 2004). Since SDNN is largely dependent on the duration of monitoring period, the common methodology used to derive SDNN values is to separate 24-h recordings into short term 5-min monitoring periods (Anon 1996). SDNN values reflect all the cyclic components responsible for variability in the period of recording and, if calculated based on 5-min recordings, it characterizes the PNS component of autonomic function. (Anon 1996; Karakaya, Barutcu et al. 2007).
2. SDANN, the standard deviation of the average NN intervals calculated over short-period recordings (usually 5 minutes) and it is driven by PNS activity (Anon 1996; Karakaya, Barutcu et al. 2007).

3. The root mean square of differences of successive NN intervals (RMSSD; in ms) with a normative value of 27±12 ms is driven primarily by PNS activation (Anon 1996; Niskanen, Tarvainen et al. 2004; Karakaya, Barutcu et al. 2007).
4. Count number of pairs of NN intervals that differ more than 50 ms (NN50) indicating PNS activity (Anon 1996; Niskanen, Tarvainen et al. 2004).
5. The percentage value of pairs of NN intervals (pNN50%) that differ more than 50 ms characterizing the PNS component of autonomic function (Anon 1996; Niskanen, Tarvainen et al. 2004).

Geometric Indices

The geometric indices derive from converting the time-domain series of NN intervals into a geometric pattern such as the sample density distribution of NN interval durations, sample density distribution of differences between adjacent NN intervals. Thereafter, the variability of the resulting pattern is assessed based on the geometric and/or graphic properties. The most commonly used geometric indices include:

1. The integral of the sample density distribution of NN intervals divided by the maximum of the density distribution (NN triangular index) with a normative value of 37±15 ms (Anon 1996; Niskanen, Tarvainen et al. 2004). This indicator characterizes overall HRV measured over 24 hours and it is driven primarily by SNS but it is also influenced to some degree by the PNS (Anon 1996).
2. Baseline width of the minimum square difference triangular interpolation of the maximum of the sample density distribution of NN intervals (TINN; in ms) that characterizes primarily the SNS but may be also influenced to some degree by the PNS (Anon 1996).

Frequency-domain methods

The frequency-domain indicators of HRV are based on the distribution of power (variance) as a function of frequency of the time difference between successive NN intervals, also known as power spectral density. The methodologies used to estimate the latter are classified into parametric and nonparametric which, in most cases, provide comparable results. A detailed discussion of the advantages and disadvantages of parametric and nonparametric methods is beyond the scope of the present review and can be found elsewhere (Anon 1996). The most commonly used frequency-domain indices distinguished in a spectrum calculated from short-term recordings of 2-5 minutes (Sayers 1973; Akselrod, Gordon et al. 1981; Hirsh 1981; Pagani, Lombardi et al. 1986; Malliani, Pagani et al. 1991) are:

1. Very Low Frequency (VLF) in the range of 0.0033 – 0.04 Hz. (Niskanen, Tarvainen et al. 2004; Haensel, Mills et al. 2008). The physiological interpretation of VLF in relation to autonomic function warrants further elucidation.
2. Low Frequency (LF) band in the range of 0.04—0.15 Hz is driven primarily by SNS but it is also influenced to some degree by the PNS (Anon 1996; Niskanen, Tarvainen et al. 2004; Felber Dietrich, Schwartz et al. 2007; Haensel, Mills et al. 2008).
3. High Frequency (HF) band in the range of 0.15—0.40 Hz is driven by respiration and appears to mainly indicate PNS activity (Anon 1996; Niskanen, Tarvainen et al. 2004; Haensel, Mills et al. 2008).
4. The ratio of LF and HF frequency band powers (LF/HF), with normative values of 1.5-2.0 indicating the balance between SNS and PNS (Anon 1996; Niskanen, Tarvainen et al. 2004).

5. The total variance of all NN intervals, called total power, corresponds to the sum of all spectral bands (i.e., 0.0—0.5 Hz) (Anon 1996; Niskanen, Tarvainen et al. 2004; Haensel, Mills et al. 2008).

It is important to note that frequency-domain indicators can be also used to analyze the sequence of NN intervals of the entire 24-hour period. In this case, however, the result also includes an ultra low frequency (ULF) band (in addition to VLF, LF, and HF components) between 0 and 0.0033 Hz (Anon 1996).

ACTIVE SMOKING AND HRV

Chronic Effects

The influence of chronic active smoking on HRV has been studied extensively. The first published evidence was provided by Penny and Mir (Penny and Mir 1986) demonstrating a decreased HRV in chronic cigarette smokers compared to non-smokers. In the following years, a number of epidemiological studies were conducted, the vast majority of which confirmed that HRV is decreased in chronic active smokers. Specifically, Hayano and colleagues found a decreased vagal activation in heavy smokers compared to non smokers or moderate smokers (Hayano, Yamada et al. 1990). Levin and colleagues found that the HRV of heavy smokers was significantly lower than that of non smokers (Levin, Levin et al. 1992). Kupari and colleagues observed that HRV was lower in individuals who smoke $\leq$10 cigarettes/day compared to a non-smokers or to smokers who smoke >10 cigarettes/day (Kupari, Virolainen et al. 1993). In line with these findings, Eryonucu et al. showed that the total HRV parameters were significantly lower in smokers than in non-smokers (Eryonucu, Bilge et al. 2000) which was confirmed by a recent study showing that regular healthy smokers demonstrate increased LF and LF/HF as well as

decreased HF compared to healthy non-smokers (Alyan, Kacmaz et al. 2008). Chronic active smoking has been also shown to reduce HRV during pregnancy of both the mother (Fifer, Fingers et al. 2009) and the offspring (Thiriez, Bouhaddi et al. 2009) with detrimental effects to the child's health (Fifer, Fingers et al. 2009) The decrease in HRV caused by chronic active smoking has been also supported by studies showing that HRV is dramatically decreased in smokers but not in non-smokers when exposed to air pollution (Xu and Wang 1998; Pope, Burnett et al. 2004; Min, Min et al. 2009). Further confirmation of the diminishing effect of chronic active smoking on HRV is provided by studies demonstrating that smoking cessation increases HRV within 7 days in chronic active habitual (Minami, Ishimitsu et al. 1999) as well as heavy (Yotsukura, Koide et al. 1998) smokers.

It is important to mention that, although the majority of published evidence suggests that chronic active smoking is associated with decreased HRV, some studies failed to find such a relationship. Murata and colleagues did not observe an association between tobacco consumption and HRV in healthy male and female smokers (Murata, Landrigan et al. 1992). Similarly, Kageyama did not find a link between current smoking status and HRV, although PNS modulation among heavy smokers tended to be lower than that among non-smokers (Kageyama, Nishikido et al. 1997).

Acute Effects

The literature presents with five studies investigating the acute effects of active smoking on HRV. The first experiment was conducted by Hayano and colleagues who assessed HRV in smokers before smoking one cigarette as well as 3, 10, 17 and 24 minutes after smoking (Hayano, Yamada et al. 1990). Results demonstrated a

reduced PNS modulation within three minutes after smoking (Hayano, Yamada et al. 1990). Similarly, Niedermaier and colleagues found that PNS activity to the cardiac muscle is reduced by active smoking (Niedermaier, Smith et al. 1993). Interestingly, this study also revealed that active smoking differentially affects SNS outflow to various target organs. Specifically, smoking was found to increase SNS traffic to the skin, heart, and adrenal glands but to reduce SNS traffic to the musculature (Niedermaier, Smith et al. 1993). A subsequent study also found that habitual smokers demonstrate a marked disturbance of the neural control of the heart as compared to non-smoking controls characterized by SNS predominance and reduced PNS modulation and overall HRV (Lucini, Bertocchi et al. 1996). In line with these results, Kobayashi and colleagues found that SNS activity increases and PNS activity decreases within five minutes from smoking one cigarette in smoker taxi drivers during work (Kobayashi, Watanabe et al. 2005). Finally, Karakaya and colleagues measured HRV in 15 smokers 5 minutes before as well as 5, 10, 15, 20, 25, and 30 minutes after smoking one cigarette (Karakaya, Barutcu et al. 2007). The results demonstrated that smoking reduced the LF/HF, the mean NN interval, the SDNN, and the RMSSD within the first 5 minutes (Karakaya, Barutcu et al. 2007).

PASSIVE SMOKING AND HRV

Chronic Effects

To the best of my knowledge, only one study has assessed the chronic effects of passive smoking on HRV. In this experiment, Felber Dietrich and colleagues measured HRV through 24-h electrocardiogram recordings in 1218 non-smokers aged ≥50 years (Felber Dietrich, Schwartz et al. 2007). The results demonstrated that individuals who were passively exposed to smoke at home or at work for more than 2

h/day revealed decreased total power, LF and LF/HF as well as increased HF. These results suggest that chronic passive smoking at home and work is associated with lower HRV (Felber Dietrich, Schwartz et al. 2007).

Acute Effects

Until relatively recently, we were unaware of the acute influence of passive smoking on HRV. However, two germane experiments have been conducted during the past decade both showing that HRV is decreased by acute passive smoking. Specifically, Pope and colleagues evaluated the effects of acute passive smoke exposure in a commercial airport on HRV in 16 adult non-smokers via ambulatory electrocardiographic monitoring for 8-hr periods while participants alternated 2 hr in non-smoking and smoking areas (Pope, Eatough et al. 2001). Results demonstrated that acute exposure to passive smoke significantly reduced HRV as indicated by changes in VLF, LF, HF, triangular index, and SDNN indices (Pope, Eatough et al. 2001). These findings were recently confirmed by a study in mice using a 6-hour exposure to passive smoke for three consecutive days (Chen, Chow et al. 2008). The results demonstrated that the passive smoking exposure decreased HRV not only during but also beyond the exposure period. Moreover, acute passive smoking was associated with an increase susceptibility for arrhythmia (Chen, Chow et al. 2008).

MECHANISMS

As observed in the previous sections, the vast majority of evidence provided to date suggests that acute and chronic active and passive smoking generate marked disruptions in the normal autonomic nervous system functioning characterized by increased SNS drive and reduced PNS modulation and overall HRV. Two main

mechanistic pathways have been proposed to explain this smoking-induced effect on neurocardiovascular regulation. The principal biomarker in the first mechanism put forth is nicotine, the main constituent of tobacco smoke. Indeed, nicotine up-regulates catecholamine release generating potent acute and chronic effects on cardiovascular regulation mainly through SNS activation (Karakaya, Barutcu et al. 2007). This is confirmed by evidence showing that plasma catecholamine levels increase within one minute after smoking a cigarette (Hill and Wynder 1974; Cryer, Haymond et al. 1976; Trap-Jensen, Carlsen et al. 1979; Baer and Radichevich 1985; Grassi, Seravalle et al. 1994). Interestingly, some evidence suggests that the smoking-induced adrenergic activation does not originate centrally and it is independent of ganglionic sympathetic transmission stimulation (Grassi, Seravalle et al. 1994). Indeed, smoking has been suggested to act on peripheral sympathetic sites to augment catecholamine release and/or to reduce its clearance at the neuroeffector junctions, although the mechanisms involved in the central sympathoinhibition have not been elucidated (Grassi, Seravalle et al. 1994).

While much of the smoking-induced effects on autonomic function have been ascribed to the nicotinic pathway, research has shown that nicotine patches generate a much smaller reduction in HRV compared with smoking, suggesting the involvement of other factors (Lucini, Bertocchi et al. 1998). In this light, a second mechanistic hypothesis has been proposed according to which respirable particles affect neural control of the heart (Stone and Godleski 1999). Indeed, suspended particles, resulting from the incomplete cigarette combustion may play an important role in the smoke-induced reduction in HRV (Pope, Eatough et al. 2001). While this notion requires further exploration, it is supported by several animal (Godleski 2000) and human (Liao, Creason et al. 1999; Pope, Verrier et al. 1999; Gold, Litonjua et al. 2000; Pope

and Dockery 2006) studies demonstrating that exposure to particulate matter, especially in the fine and ultra fine range, is linked with a decreased HRV.

CLINICAL IMPLICATIONS

The mechanism(s) by which the smoking-induced reduction in autonomic function contributes to cardiovascular-related mortality is not well understood. A recent study in mice suggests that the smoking-induced reduction in autonomic function increases arrhythmia susceptibility (atrial fibrillation, ventricular fibrillation or tachycardia), abnormalities in cardiac electrical conduction, and AV block (Chen, Chow et al. 2008). This may account for the findings in the epidemiological data linking active and passive tobacco smoking to cardiac arrhythmias and sudden cardiac death (Kritz, Schmid et al. 1995; Barnoya and Glantz 2006; Bhatnagar 2006; Metsios, Flouris et al. 2007; Flouris, Metsios et al. 2008; Flouris, Metsios et al. 2009; Flouris, Vardavas et al. 2010). Lower HRV is also associated with an increased risk for coronary heart disease, (Dekker, Crow et al. 2000; Sinski, Lewandowski et al. 2006; Flouris, Faught et al. 2008; Flouris 2009; Flouris and Oikonomou 2010) cardiovascular morbidity and mortality (Anon 1996; Acharya, Joseph et al. 2006; Thayer and Sternberg 2006; Carrillo, Metsios et al. 2009; Faught, Flouris et al. 2009; Metsios, Flouris et al. 2009) as well as on-going subclinical inflammation (Lanza, Sgueglia et al. 2006; Haensel, Mills et al. 2008). As such, low HRV has been proposed as an indicator of disease (Anon 1996; Thayer and Sternberg 2006) given its prognostic attributes myocardial infarction (Bigger, Fleiss et al. 1993) and chronic heart failure (La Rovere, Pinna et al. 2003) patients. Moreover, increased SNS activity has been proposed as an indicator for hypertension, diabetes, and cardiovascular diseases (Hayano, Yamada et al. 1990; Flouris and Scott 2009). Therefore, the reductions in HRV induced by both acute and

chronic active and passive smoking supported in the majority of the published studies to date may have serious clinical implications characterized by marked disruptions in the normal autonomic nervous system functioning which, in turn, impair cardiac electrical conduction with confirmed detrimental long term effects.

LITERATURE REVIEW CONCLUSIONS

Overall, the vast majority of published evidence suggests that acute and chronic active and passive smoking generate marked disruptions in the normal autonomic nervous system functioning characterized by increased SNS drive and reduced PNS modulation and overall HRV. This phenomenon is partly attributed to an up-regulation of catecholamine release by nicotine generating potent acute and chronic effects on cardiovascular regulation mainly through SNS activation. In addition, suspended particles resulting from the incomplete cigarette combustion have been also hypothesized to play an important role in the SHS-induced reduction in HRV. The reductions in HRV similar to those induced by acute and chronic active and passive smoking are associated with an impaired cardiac electrical conduction with confirmed detrimental long term effects. Thus, it seems logical to postulate that the smoke-induced HRV reductions may account, at least in part, for the findings in the epidemiological data linking active and passive tobacco smoking to cardiac arrhythmias and sudden cardiac death.

Since the first study that assessed the effects of tobacco smoke on HRV, research in this topic has spread into different areas and new scientific evidence continues to accumulate. However, many germane studies are inherently limited. For instance, a large number of epidemiological studies base tobacco smoke exposure on self-report without an objective measurement of exposure, they adopt a cross-

sectional design, and they provide little data on the duration of the exposure or tobacco use. On the other hand, many mechanistic studies rely on animal models which are inherently limited, particularly in relation to the level and duration of tobacco exposure and use, as well as their relevance to humans. Nevertheless, the literature also contains outstanding experiments that have provided valuable evidence effects of tobacco smoke on cardiac autonomic regulation.

Notwithstanding the increased attention on the effects of tobacco smoke on HRV and the excitement for the continuously emerging discoveries in this area, we remain largely naive to issues as critical as the mechanisms causing the smoke-induced decrease in HRV. Furthermore, a standardization of the HRV indicators used in future research should be conducted, as studies thus far tended to evaluate different HRV indicators making their results difficult to compare. Finally, future research should address the effects of acute and chronic passive tobacco smoke on HRV given the dearth of published data on this topic.

METHODS

Participants

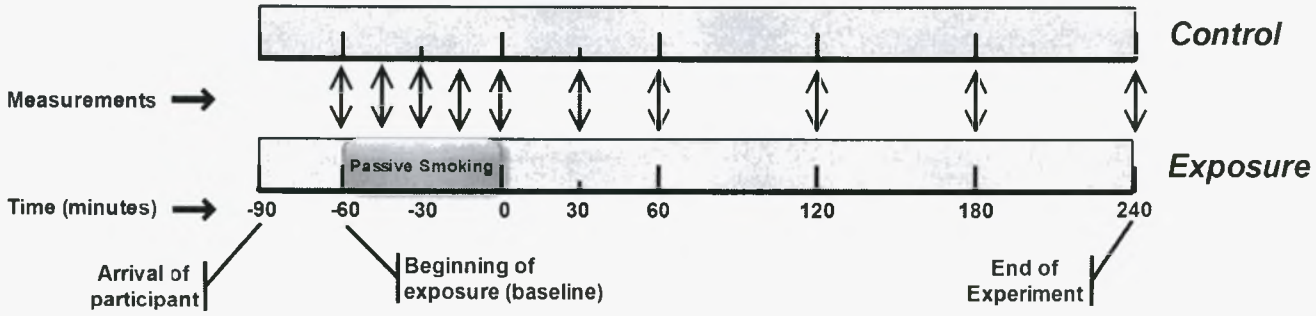
Nineteen healthy adults (10 male, 9 female: age 32.8±5.9 years, BMI 23.5±3.1 kg/m^2) volunteered. Exclusion criteria included: smoking, pregnancy, evidence of cardiac or pulmonary disease, and previous disease and medications. The experimental protocol was approved by the ethical review board at the University of Thessaly.

Experimental Design

On two separate days, participants arrived at the laboratory (9 a.m.) to complete, in a random order, an experimental and a control trial. They were welcomed by an

investigator and, during their first visit, they were subjected to height and weight measurements. Thereafter participants were asked to put on a Polar PS800CX that is capable of measuring HRV indices. The subjects were exposed for 60 min to PS during the exposure trial and to normal room air during the control trial (fig 2). Following the exposure, participants were monitored for 4 hours. Throughout their stay in the laboratory and to minimize influences on HRV values, participants were instructed to remain silent and as calm as possible throughout data collection. For both trials, subjects were given a small sandwich consisting of (30% fat, 55% carbohydrate, and 15% protein) (Michailidis, Jamurtas et al. 2007) exactly 1 hours following the exposure. All participants arrived at the laboratory following a 10-hour fast, and were instructed to refrain from strenuous physical activity and other excessive stressors for 72 hours prior to each trial.

Fig 2: Experimental protocol.



PS Exposure

The PS exposure was achieved by cigarette combustion from a variety of popular brands and was adjusted at known carbon monoxide concentrations for bars/restaurants (Flouris, Metsios et al. 2008). The concentration of carbon monoxide was measured to 23 ppm by a CO analyzer (Martindale Electric, Watford, England).

During the exposure to PS participants remained seated at rest for 1 hour inside a 6X5X4 m environmentally controlled chamber (air temperature: 24°C; air velocity: 0.05 m/s; humidity: 45%).

HRV Measurements

HRV measurements were taken using a Polar PS800CX (Polar Electro Oy, Kempele, Finland) throughout the control and exposure trials. Data collected during each exposure were split into six 10-min intervals. Moreover, data collected from 0-10, 30-40, 60-70, 120-130, 180-190, and 240-250 minutes following the exposure each trial (fig. 2) were used to derive HRV for the different time points.

The heart rate monitor signal was transferred to the Polar Precision Performance Software (release 3.00; Polar Electro Oy), and R-R intervals were exported under ASCII format. Both frequency domain and time domain measures were analyzed using HRV analysis software version 1.1 (Finland: Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland 2002). The root mean square of differences of successive RR intervals (RMSSD) and the percentage value of pairs of RR intervals that differ more than 50 ms (pNN50) were studied for the time domain and the low (LF) and high (HF) frequency and their ratio (LF/HF) for the frequency domain.

Statistical Analysis

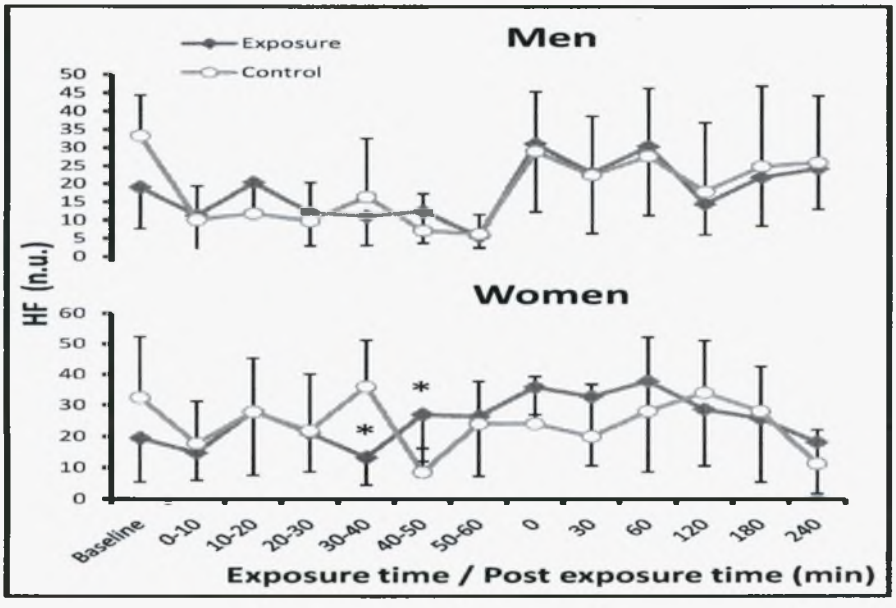
Given the sexual dimorphism, in which has been found difference between men and women (Flouris, Metsios et al. 2008), it was examined the effect of PS on HRV, a factorial [sex (male, female), trial (control, PS), time (six 10-min intervals)] multivariate analysis of variance (MANOVA) was conducted for RMSSD, pNN50,

LF, HF as well as LF/HF. All statistical analysis were completed using SPSS 15.0 and the level of significance was set at $P<0.05$.

RESULTS

The HRV indices studied prior to, during and following the exposure are shown in figures 3 to 7. An increase is observed in HF values during the PS exposure mostly in women (fig 3). A significant decrease is observed in LF/HF values, in both sexes during the PS exposure, especially in the last 20 minutes (fig 5). Furthermore, a reduction is apparent in pNN50 values in both sexes in post exposure time (fig 6), whereas no changes are observed in LF values (fig 4). An increase in RMSSD values was found during the PS exposure for the men and a reduction in post exposure for women (fig 7). In addition, the MANOVA detected main effects of sex on LF/HF (fig.5) and RMSSD (fig.7) ($P<0.05$), of trial on pNN50 (fig.6) ($P<0.05$), and of time on LF/HF ($P<0.05$). Interaction effects of trial*time on LF/HF and RMSSD ($P<0.05$) and of sex*trial*time on RMSSD ($P<0.05$) were also detected.

Fig 3. HF HRV Index



* : significant difference between trials for the same time point ($P<0.05$).

Fig 4. LF HRV Index

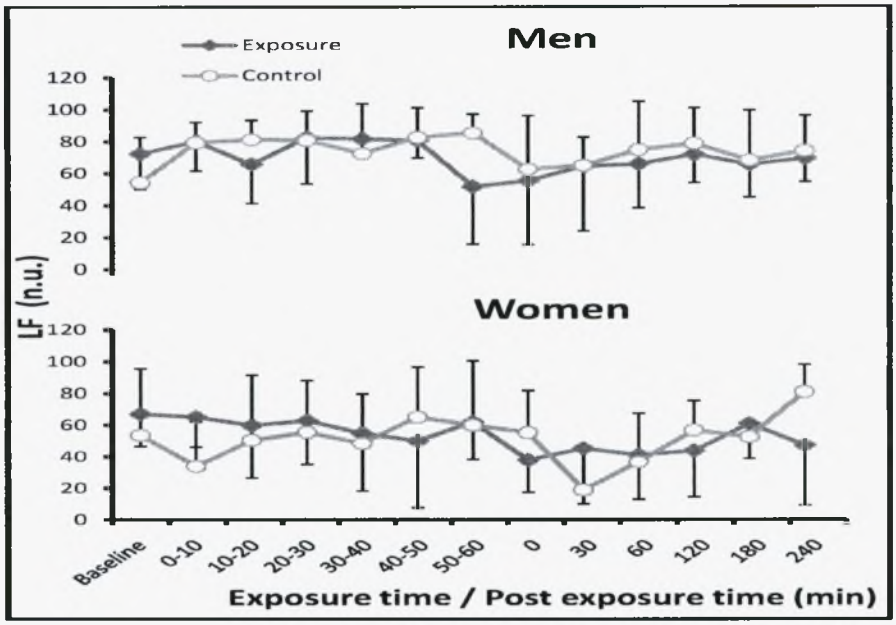
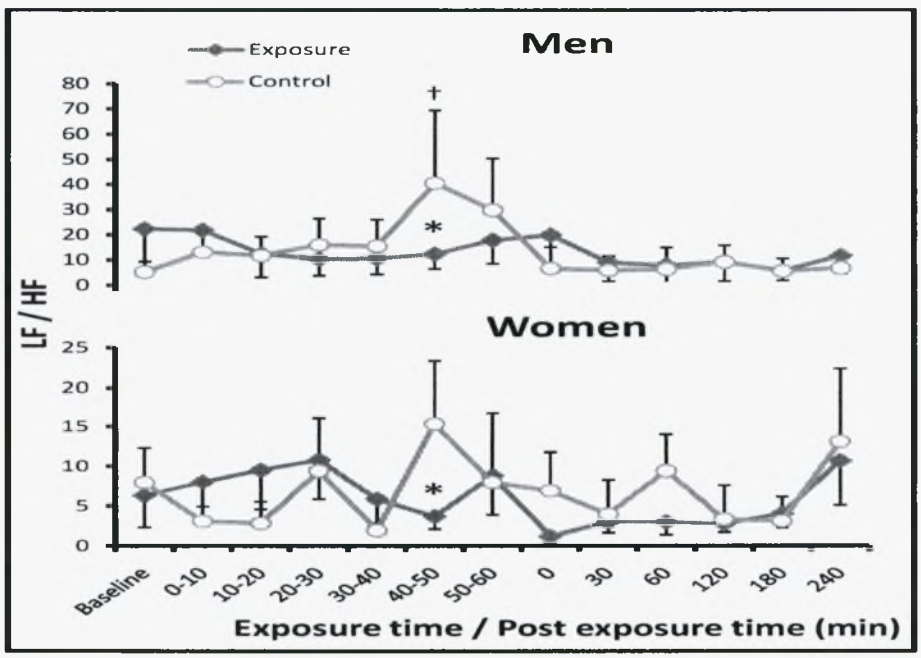


Fig 5.LF/HF HRV Index



* : significant difference between trials for the same time point (P<0.05).
† : significant difference from baseline within the same trial (P<0.05).

Fig 6. pNN50 HRV Index

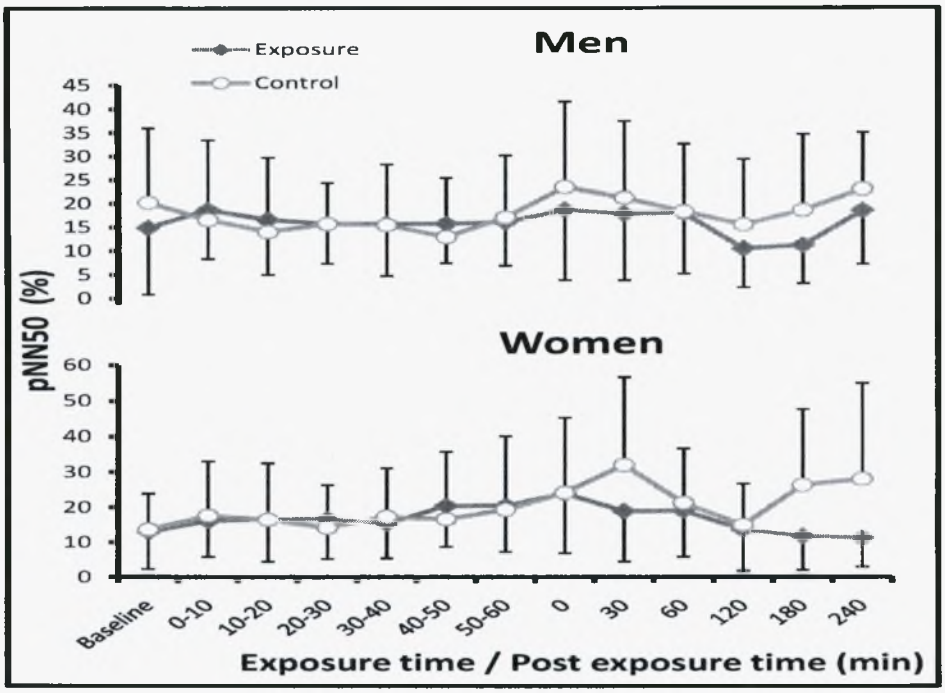
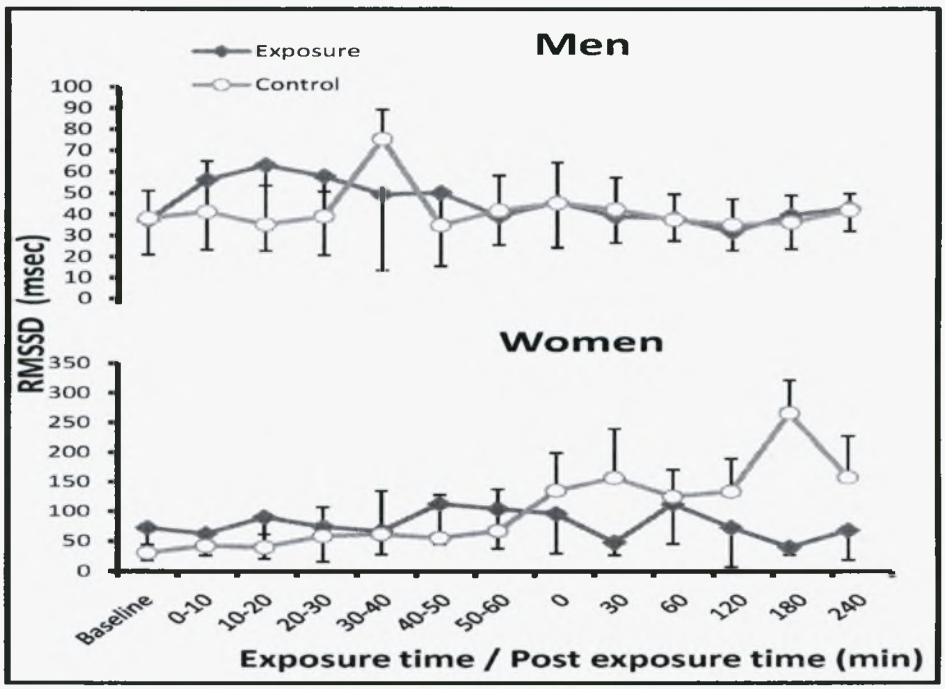


Fig 7. RMSSD HRV Index



DISCUSSION AND CONCLUSIONS

The purpose of this study was to examine the acute effects of moderate passive smoking on HRV measurements. To the best of my knowledge this is the first experiment to use a standardized experimental protocol in order to examine the effects of PS and their duration on the cardiovascular function in healthy non-smokers. I found that PS influences the function of the autonomic system, as measured by HRV, and more specific the activation of SNS which may indicate abnormalities in cardiovascular function (Kristal-Boneh, Raifel et al. 1995; Tsuji, Larson et al. 1996; Acharya, Joseph et al. 2006) and has been proposed as a mechanism explaining the associations of reduced HRV with increased mortality (Kleiger, Miller et al. 1987; Bigger, Fleiss et al. 1992; Tsuji, Larson et al. 1996). Specifically, in this study it was found that PS significantly alters LF/HF and HF as well as that the latter effect is subject to sex. This finding mirrors recent evidence (Flouris, Metsios et al. 2008) showing that PS is accompanied by increased IL-1b and thyroid hormone secretion as well as higher systolic blood pressure only in men. Further investigations are required in order to understand the reasons for the observed sex differences which relate to the cardiac and hormonal responses during and after exposure to PS.

LF/HF data supports the hypothesis that HRV was disrupted during and 4-hours after exposure to PS. This finding shows that acute exposure to PS significantly reduces HRV as indicated by changes in LF/HF ratio, an important HRV index, which is associated with an increased susceptibility for arrhythmia (Chen, Chow et al. 2008). However, reduced HRV is also connected with cardiovascular function by disrupting normal autonomic nervous system functioning (Acharya, Joseph et al. 2006; Zeskind and Gingras 2006).

In conclusion, the evidence supports that PS disrupts HRV and therefore increases cardiac vulnerability (Hayano, Yamada et al. 1990; Niedermaier, Smith et al. 1993; Acharya, Joseph et al. 2006) as well as arrhythmia susceptibility. Future studies should examine the chronic effects of PS on HRV.

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