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A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects

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Abstract

Objectives: An increased sympathetic drive, in view of its proarrhythmic, proatherosclerotic, and prothrombotic actions, could contribute to the elevated cardiovascular risk of habitual smokers. However, the underlying mechanisms are still debated. In this study we address the hypothesis that spectral analysis of RR interval and systolic arterial pressure short-term variabilities may be used to assess the complex autonomic changes produced by habitual cigarette smoking. **Methods**: A cross-sectional design compared heavy (> 20 cigarettes/day) habitual smokers ($n = 20$; 40 ± 3 years), with similar age controls. Spectral analysis of RR interval variability provided markers of the sympatho-vagal balance modulating the SA node, by way of the normalised low frequency (LF ≅ 0.10 Hz) and high frequency (HF ≅ 0.25 Hz) components. The LF component of systolic arterial pressure (SAP) variability assessed the sympathetic vasomotor modulation. The frequency domain index (α) measured the baroreflex gain of the SA node. Subjects were studied at rest, and during the sympathetic excitation produced by active standing. **Results**: In smokers LF_{RR} was, at rest, greater than in controls (70.6 ± 3.8 vs 46.0 ± 2.5 normalised units, nu); concurrently HF_{RR} was reduced (22.1 ± 3.2 vs 42.0 ± 2.8 nu). Baroreflex gain and RR variance were also smaller in smokers. LF_{SAP} was, instead, similar in the smokers and control groups. The standing induced increase in LF_{RR} was blunted ($P < 0.001$) in smokers. **Conclusions**: Spectral analysis of RR interval and systolic arterial pressure variability indicates that habitual cigarette smoking induces selective alterations in neural control of the SA node. An increase at rest in markers of sympathetic modulation is accompanied by signs of reduced vagal drive and depressed baroreflex gain; while sympathetic vasomotor modulation appears similar in controls and smokers. Data are consistent with the hypothesis that autonomic alterations may contribute to the increased cardiovascular risk present in smokers.

Keywords: Autonomic nervous system; HRV; Blood pressure variability; Spectral analysis; Baroreflex; Cigarette smoking

1. Introduction

Heavy cigarette smoking poses costly hazards to human health, being responsible for about one-fifth of deaths attributable to cardiovascular disease [1].

Cigarette smoke might favour the development of atherosclerosis [2] by damaging the vascular endothelium [3] and producing an unfavourable lipid profile [4]. The adverse effects of smoking might depend, as well, on an increase in platelet aggregation [5] and vasomotor reactivity [1], leading to a prothrombotic state [5,6], favouring coronary spasm, particularly in women [7], and occlusion [1]. Acute events, such as ventricular fibrillation and sudden death, are increased by smoking [8], particularly in the presence of pre-existing coronary artery disease. However, the underlying trigger mechanisms are poorly understood.

In view of the proarrhythmic effect of sympathetic activity [9], the finding that cigarette smoking acutely increases plasma catecholamines [10,11] and cardiac norepinephrine spill-over [12] would suggest sympathetic overactivity as a possible component of the elevated coronary risk of smokers, as is the case in hypertension [13] or coronary artery disease [14]. This concept would also be in line with the concomitant reduction of vagal drive, recently

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observed in habitual smokers [15]. Surprisingly, short-term longitudinal studies employing direct electroneurographic techniques in man, documented a reduction in muscle efferent sympathetic activity during smoking a cigarette [16,17]. This finding, however, obtained from a peripheral sympathetic outflow in view of the highly differentiated nature of autonomic efferent activity [18], may not be applicable to cardiac innervation. Furthermore, a control, non-smoking population, was not examined.

The present cross-sectional, observational, study was therefore planned to address directly whether long-term exposure to cigarette smoking, as occurs in heavy habitual smokers, alters the baseline sympathetic and vagal modulation of the SA node and peripheral sympathetic vascular control, as compared to age-matched non-smokers.

To this end we employed spectral analysis of heart period and systolic arterial pressure variability [19,20] and non-invasive measurements, to minimise the possibility of disturbing resting autonomic activity, as it might occur with invasive or painful recording procedures [21].

As a secondary goal we addressed the autonomic changes induced by standing up, i.e. a manoeuvre which increases sympathetic modulation, as smoking has been reported to induce alterations in cardiovascular excitatory reflexes [16].

2. Methods

This study involved 40 volunteers. Twenty subjects (Table 1) were heavy habitual cigarette smokers, as judged by high Fagestrom scale score [22] (mean 8.2 ± 0.3, range 6 to 11). This scoring system considers the number of cigarettes/day, the duration of the habit, and the intensity of the craving for cigarettes. Subjects had smoked for an average of 18 ± 2 years (range 5 to 40). Twenty non-smoking volunteers of similar age (Table 1) served as controls.

Subjects were judged to be healthy on the basis of medical history, physical examination, electrocardiogram and for a sample of smokers ($n = 5$) spirometry. None was on any medication.

Table 1
Characteristic of the study population

	Smokers	Non-smokers
Age (years)	40 ± 3	40 ± 3
Weight (kg)	71 ± 3	73 ± 2
Height (cm)	172 ± 2	172 ± 2
Systolic arterial pressure (mmHg)	112 ± 2	115 ± 2
Diastolic arterial pressure (mmHg)	72 ± 2	73 ± 2
Heart rate (beats/min)	79 ± 3	66 ± 2 *

Data are presented as means ± s.e.m. * $P < 0.001$ vs smokers.

Since the hemodynamic and autonomic effects of cigarette smoking vary in time [23], and show habituation [17], we considered it important to perform our studies after smokers had smoked several ($n = 7$) cigarettes (i.e., 1 cigarette with 1.3 mg nicotine, per hour, since waking up) to ensure a sufficiently stable baseline. Accordingly, recordings were performed in a quiet room, with a comfortable temperature (22–24°C), always in the same time window (between 1.00 and 3.00 h p.m.). On the morning of the study, every subject had a light breakfast, with no caffeinated beverages (coffee or tea) and postponed lunch after recording was completed. In the hours preceding the study all subjects attended their usual daily activity, and avoided any bout of heavy physical exercise, which might produce long-lasting autonomic effects [24].

All subjects had been carefully instructed about the study procedure, and all had given their written informed consent. This study was approved by the local Ethics Committee. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Each participant was connected to a two-channel telemetry system (Marazza, Italy) which provided continuous electrocardiographic and respiratory signals (obtained with a piezoelectric transducer). Arterial pressure was continuously estimated with a non-invasive device (Finapres, Ohmeda, USA), which had been already validated both for time and autoregressive frequency domain measures, against invasive pressure recording [21].

After a 10-min period, allowed for stabilisation, a control recording of 10 min was obtained, to be followed by a further period of 7 min recording, which was obtained while the subjects maintained the upright posture (active standing). This is a condition which enhances sympathetic drive. The time elapsed since the last-smoked cigarette and initiation of the recording procedure was always at least 30 min, which allowed acute hemodynamic effects to wane [16,17].

Analog signals were channelled after appropriate amplification and filtering, to an analogue to digital board (Data Translation USA), inserted into a PC (Compaq, USA). During experimental sessions continuous acquisition at 300 samples/s per channel was performed. Data stored on the hard disk were subsequently processed off-line, and saved on back-up digital tape.

2.1. Data analysis

From the ECG signal [19,20] a continuous RR interval series (i.e. tachogram) was initially obtained, with the peak of the R wave as fiducial point. Tachogram sections, of adequate length and stationarity, were used to calculate simple statistics and the best autoregressive estimate of the power spectral density. The power and frequency of every spectral component are presented both in absolute (i.e.

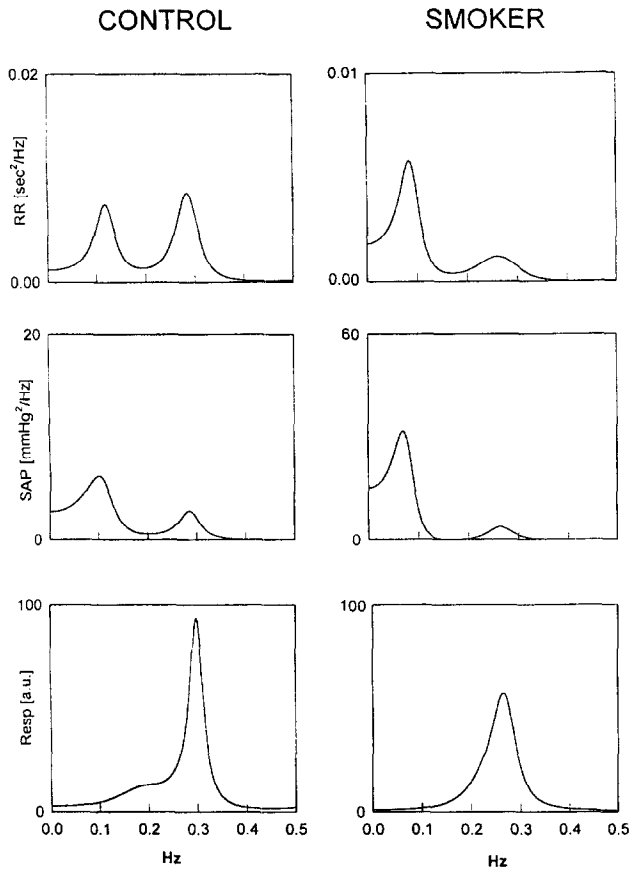

Fig. 1. Autospectra of RR interval, and systolic arterial pressure variabilities and of respiration in a control subject (left panels) and in a smoker (right panels). In this representative example the total power of RR variability (corresponding to the area under the curve) in the smoker appears reduced as compared to the control subject (notice the change in scale; total power is respectively 2715 and 4020 ms^2). The absolute values of both LF and HF spectral components are also smaller in the smoker (respectively, LF, 772 and 1185 ms^2; HF, 307 and 1569 ms^2). Vice versa the normalized power of the LF component is greater in the smoker as compared to the control subject (LFnu: 70 and 41), while HF is smaller (HFnu: 27 and 51). It is also apparent that the main respiratory frequency coincides in both subjects with the center frequency of the respiratory components of RR interval and systolic arterial pressure.

[s^2]) and normalised units (i.e. [nu]) [20]. Normalised units are computed by dividing the absolute power of a given high-frequency (HF) or low-frequency (LF) component by total power (i.e. variance) after having subtracted from it the power of the component with a center frequency near 0 Hz (less than 0.03 Hz) and multiplying this ratio by 100. The analysis of these very low-frequency components, which nevertheless may contain important information, requires a different methodology [25] and is not within the scope of the present study. Unless otherwise specified, spectral power will be presented in normalised units. Spectral analysis was also performed on the systolic arterial pressure and the respiratory signals using a similar procedure (Fig. 1).

From the simultaneous analysis of arterial pressure and RR interval variabilities, a frequency domain index (α) can be derived [26,27], which is a measure of the overall gain of the heart period–arterial pressure relationship and provides results similar to those obtained with the phenylephrine slope approach [27]. The index α is computed both in correspondence of LF and HF oscillatory components; an average index is obtained with the formula:

$$\alpha = \left[(P_{RR}/P_{SAP})^{1/2}_{LF} + (P_{RR}/P_{SAP})^{1/2}_{HF}\right]/2$$

where P_{RR} and P_{SAP} represent the spectral power of the RR interval and of the systolic arterial pressure components, respectively. The validity of this calculation requires that the value of the coherence function between the two variability signals is, at the relevant frequencies, greater than 0.5 [26–28], and the direct influence of respiration on RR period variability is minimal, as has been found in the case of the human subjects [29]. Elevated short-term [26] and long-term (2 month) [30] reproducibility of this measure has been reported.

2.2. Statistics

Data are presented as means ± s.e.m. The following statistical procedures were employed, as appropriate: Student's t test, and two-way repeated measures ANOVA with Bonferroni correction using a commercial statistical package (Sigmastat, Jandel). A α level of 0.05 was considered significant.

Table 2
Descriptive statistics of RR interval and of its variability, in controls and in smokers, at rest and during active standing

	RR (ms)	σ^2 (ms^2)	DC (%)	LF			HF			LF/HF
				Hz	ms^2	nu	Hz	ms^2	nu	
Rest										
Controls	921 ± 30	3742 ± 830	41 ± 5	0.10 ± 0.01	1007 ± 271	46.0 ± 2.5	0.26 ± 0.01	907 ± 207	42.0 ± 2.8	1.3 ± 0.2
Smokers	774 ± 28 ^	1320 ± 260 ^	52 ± 5	0.10 ± 0.01	495 ± 142 ^	70.6 ± 3.8 ^	0.32 ± 0.01 ^	152 ± 42 ^	22.1 ± 3.2 ^	6.7 ± 1.6 ^
Standing										
Controls	806 ± 27 *	3491 ± 783	54 ± 5	0.09 ± 0.01	1330 ± 504	76.8 ± 3.0 *	0.26 ± 0.02	265 ± 80	17.0 ± 3.0 *	8.4 ± 1.7 *
Smokers	690 ± 26 * ^	999 ± 150 ^	49 ± 6	0.08 ± 0.01	441 ± 107 ^	84.5 ± 2.0 * ^	0.28 ± 0.02	43 ± 8 ^	12.0 ± 2.0 * ^	12.0 ± 2.0 * ^

$\sigma 2$ = variance; DC = very low-frequency component; LF = low-frequency component; HF = high-frequency component; nu = normalised units. Data are means ± s.e.m. * $P < 0.05$ rest vs standing; ^ $P < 0.05$ controls vs smokers.

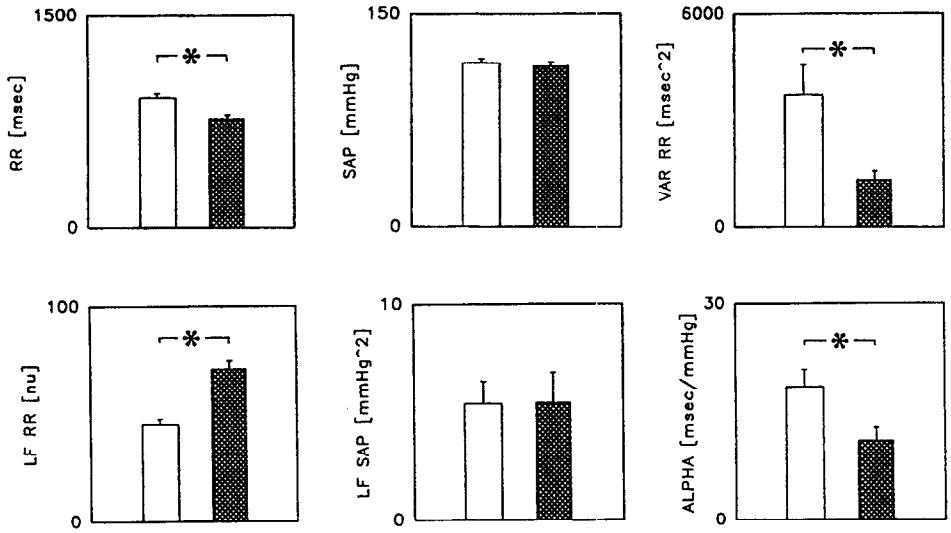

Fig. 2. Hemodynamic values, and time and frequency domain markers of autonomic circulatory modulation in controls (open bars) and in smokers (lined bars). * $P < 0.05$.

3. Results

3.1. Rest condition

At rest RR interval was significantly reduced in habitual smokers as compared to controls (Table 2 and Fig. 2), while systolic and diastolic arterial pressure values were similar in the two groups (Table 3). RR interval variance was reduced in smokers as compared to controls (Table 2), while no significant differences were observed in SAP variance.

Frequency domain indices of neural control of the cardiovascular system showed significant differences between the two groups (Table 2). Low-frequency component (LF) of RR interval variability, expressed in normalised units was significantly greater in smokers (Fig. 2) and, conversely, high-frequency component (HF) of RR interval variability, expressed in nu, was significantly lower (Table 2). No significant differences were observed in LF_{SAP} when comparing the two groups.

The resting value of the index α, a frequency domain measure of the overall gain of baroreflex control of the heart period, was significantly lower in smokers as compared to controls (Fig. 2 and Table 3).

3.2. Active standing

In this study active standing was used to enhance sympathetic drive to the heart and blood vessels. This stimulus produced a significant reduction in RR period in the control group. A similar reduction was present in the group of smokers (Table 2), although in this group RR interval reached a lower value.

Systolic arterial pressure was slightly increased in both groups, as compared to resting values (Table 3). RR and SAP variance were not significantly modified by active standing.

LF_{RR} was significantly increased in both groups, but in smokers this increase was less than in controls ($\Delta = 13 \pm 4$ nu and $\Delta = 31.2 \pm 3$ nu respectively, $P < 0.001$). A parallel, opposite change was observed in HF_{RR}. LF_{SAP} was similarly increased in both groups by standing up, the index α was significantly reduced in both groups and reached a significantly smaller value in smokers (Table 3).

Table 3
Descriptive statistics of systolic arterial pressure, of its variability and of α index, in controls and in smokers, at rest and during active standing

	SAP	σ^2	DC	LF		HF		α
	(mmHg)	($mmHg^2$)	(%)	Hz	$mmHg^2$	Hz	$mmHg^2$	(ms/mmHg)
Rest								
Controls	115±3	30±5	66±3	0.09±0.01	5.3±1.0	0.25±0.01	3.2±1.3	18.3±2.4
Smokers	113±3	33±8	67±4	0.09±0.01	5.4±1.0	0.31±0.01 ^	1.9±0.6	10.9±1.9 ^
Standing								
Controls	122±3 *	40±8	64±3	0.10±0.01	10.3±2.0 *	0.25±0.01	3.0±1.2	10.7±1.4 *
Smokers	124±4 *	41±7	50±6	0.08±0.01	14.2±3.0 *	0.29±0.02	2.1±0.6	6.0±0.8 * ^

SAP = systolic arterial pressure; σ^2 = variance; DC = very low-frequency component; LF = low-frequency component; HF = high-frequency component. Data are means ± s.e.m. * $P < 0.05$ rest vs standing; ^ $P < 0.05$ controls vs smokers.

3.3. Respiration

The frequency of respiration, which corresponds to the center frequency of the HF component of both RR and SAP variabilities (Fig. 1), was higher in smokers, both at rest and during standing up (Tables 2 and 3).

4. Discussion

The main finding of this study is that habitual smokers reveal a marked disturbance of the neural control of the SA node as compared to non-smoking controls. At rest smokers were characterised by signs of sympathetic predominance, as well as by reduced vagal modulation and blunted baroreflex gain. These alterations were likely to be selective, as markers of sympathetic vasomotor control were, instead, similar in the two populations examined.

4.1. Spectral analysis of RR interval and systolic arterial pressure variability to assess neural control of the circulation

The instantaneous RR interval depends on the continuous interplay between vagal and sympathetic efferent activity to the SA node and the intrinsic heart rate [20]. Spectral analysis [19] extracts two major oscillatory components, at ~ 0.1 Hz, low frequency (LF), and ~ 0.25 Hz, high frequency (HF), that are present in the continuous series of RR intervals. Although both LF and HF components are likely to originate from a central and peripheral interaction of vagal and sympathetic mechanisms [20,31], their relative powers have been shown to provide, in multifarious experimental conditions, quantitative markers of the state of the sympatho-vagal balance [20].

In fact, in the presence of sufficient stationary conditions and variance, sympathetic excitations seem always to be accompanied by a relative increase in LF, while vagal excitations lead to a relative increase in HF. This reciprocal peripheral organisation might reflect the central reciprocal organisation of the two rhythms [31]. Hence, a normalisation procedure is necessary to assess the relative power of LF and HF, independently of total power or variance [19,20]: alternatively, the LF/HF ratio also provides similar information.

By using this approach it was possible to quantify with greater accuracy than using the RR interval, the variance, or absolute values of single spectral components, the various levels of graded tilt, likely to induce progressive changes in sympatho-vagal balance [32]. Moreover, during reductions in arterial pressure produced in man by progressive doses of nitroprusside i.v., the normalised power of the LF component appeared highly correlated with similar oscillations present in the efferent sympathetic muscle nerve activity [33].

By using a bivariate technique, which considers simultaneously RR interval and systolic arterial pressure values [27,28], it is also possible to obtain an estimate of the overall gain of baroreflex mechanism, by way of the frequency domain index α [26,27]. This latter provides measures that are comparable to those obtained with time domain approaches [27], such as the traditional slope technique [34] or the ramp method [35], without requiring artificial changes in arterial pressure levels, or preselection of the data.

The spectral methodology also permits the assessment of the sympathetic modulation of vasomotor control, by way of the LF power of sympathetic arterial pressure variability [20]. The totally non-invasive nature of the approach is important to minimise the influence of fear or emotions, as might happen with invasive or intrusive procedures [21].

4.2. Altered neural control of the SA node in habitual smokers

The design of this study considered a cross-sectional comparison of habitual smokers and age-matched, non-smoking controls, in order to focus on the long-term effect of several years of heavy (> 20 cigarettes/day) smoking on autonomic activity, while avoiding the potentially confounding effects of the brief increase in arterial pressure that accompanies cigarette smoking [16,17]. Thus, smokers were examined, under relatively steady effect, always at the same time of the day and at least 30 min after smoking the last of several cigarettes (see Methods), when the attendant neural and hemodynamic short-term effects are no longer evident [16,17].

Given the highly selective nature of sympathetic efferent control [19] and the possibility that neural effects of smoking might be dishomogeneous in different autonomic outflows [16], we addressed simultaneously markers of sympathetic modulation of the SA node and of the peripheral vasculature. The observation that only the former ones differed in the two populations, while markers of sympathetic vasomotor control were similar, indicated the selectivity of the effect and confirmed the potential risk of extrapolating to cardiac regulation the results obtained from a peripheral sympathetic outflow. The simultaneous reduction in HF, a marker of vagal modulation [20], can be viewed as a shift of the autonomic balance towards sympathetic predominance. Concurrently, the elevated sympathetic drive might explain, as well, the reduced gain of baroreflex mechanism as it has been demonstrated that a reflex sympathetic excitation is, as a complex pattern, associated with a reduced baroreflex gain [36]. In keeping with this, we recently reported a strong negative correlation between sympathetic modulation and frequency domain measures of baroreflex gain in man [30].

The observation that standing induced increases in LFnu [20,21], a marker of sympathetic modulation of the SA node, and that the attendant reductions of HFnu were blunted in smokers while the increases in LF_{SAP}, a marker

of sympathetic vasomotor control [20], were similar in the two groups, suggests the presence of an alteration of sympatho-vagal responsiveness to excitatory stimuli which affects cardiac innervation rather selectively. This is in keeping with a prior study by Hayano et al. [15], who reported a reduction in postural responses of vagal modulation of heart rate, but did not examine sympathetic modulation. On the contrary, some excitatory peripheral sympathetic responses, such as the activation of muscular sympathetic efferent traffic occurring during Valsalva straining [16], may be enhanced transiently by smoking a cigarette.

We cannot offer a direct explanation of the mechanisms that underlie the differences of the resting profile of autonomic modulation, as well as the selective cardiac alteration of autonomic responses initiated by active standing observed in smokers. Possibilities include stimulation by nicotine [37] of receptors located at a central, ganglionic or peripheral level, including sensory endings of afferent fibres, such as those innervating the lungs and their circulation [38], which might be capable of initiating pressor sympathetic reflexes [39]. In this latter case other irritants and components of cigarette smoke might also take part in stimulation of respiratory sensory endings [37]. Finally the role of the higher respiratory rate observed in smokers (19.2 versus 15.6 breaths/min) should be discussed in view of the known inverse relationship between breathing frequency and respiratory related oscillations in RR interval. This point has been addressed in detail by Brown et al. [40], who reported a clear reduction in absolute power of both LF and HF components of RR interval variability with increasing breathing frequency, particularly evident in the approximate range 7.5 to 15 breaths/min. Although these authors did not calculate normalised units (nor the LF/HF ratio) from their data (Fig. 4 of their study) it is clear that changes in the breathing range of 15–20 breaths/min produced only minor, if any, influence on this ratio.

4.3. Clinical implications

Habitual smokers presented a reduced total heart rate variability and depressed baroreflex gain. These alterations are considered negative prognostic indicators in patients recovering from a prior myocardial infarction [41,42]. Smokers also showed an increase in sympatho-adrenal activity, which in patients with congestive heart failure, correlates with mortality [43]. An increase in sympathetic activity has also been recently proposed [13] as an important component of the elevated coronary risk of hypertension. In particular sympathetic activity would represent a common link among many of the "non-pressure-related" risk factors, such as the tendency for thrombosis and increased vascular reactivity which are also observed in smokers [1,5,6]. The presence of complex autonomic alterations could thus provide a novel hypothesis to delve into the increased propensity of habitual smokers to acute coronary death.

Obviously this concept, derived from an observational study on a small group of healthy smokers, needs confirmation from prospective, interventional, studies on larger populations. Such studies should explicitly address the issue of occult coronary artery disease [44], while from a practical point of view, they could benefit from the totally non-invasive nature of this approach.

Acknowledgements

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