Effects of Smoking of Conventional Cigarettes and of Hemoglobin Filter Cigarettes on Autonomic Cardiac Control

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Introduction: The short-term effects of smoking on Heart Rate Variability (HRV) in healthy young subjects are not well defined yet, especially concerning differences between habitual smokers and non-smokers, gender-related differences and the possible protective role of low yield cigarettes.

Methods: Thirty-two healthy volunteers (18 males, 14 females, 21 habitual smokers and 11 non-smokers, mean age 30 years), were studied sited, at the same place, the same hour of the day. Smokers were asked not to smoke for 12 hours prior the examination. Mean RR, SDNN, rMSSD, pNN50, Total Power, LF, HF, LF/HF, CCVLF and CCVHF, were measured for half an hour, divided in 6 periods of 5 minutes each. Smoking of a single cigarette took place during the second period.

Results: Contrary to previous reports no significant increase of the Mayer wave component (LF) was noticed. As expected, there was a statistically significant increase of the LF/HF ratio ($P<0.001$). Time domain indices and CCVHF were also significantly decreased. We also noticed that although the changes appear during smoking (2nd period) the maximal changes occur five to ten minutes after smoking cessation (3rd and 4th periods). Finally, no differences were noticed when the whole of the examinations was repeated using a new type of cigarette filter, based on hemoglobin (Biofilter).

Conclusions: Our data suggest that smoking causes an acute and constant decrease in vagal cardiac control. Analysis of smokers, non-smokers, male and female subgroups, revealed no significant differences when the changes, as a percentage of the initial values, were taken into account. Although the initial values differed significantly in some cases, the effects of smoking on HRV were almost identical. Our data, also note the lack of protective effects of the new filter as far as the autonomic cardiac control is concerned.

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The adverse effects of smoking on the cardiovascular system have been studied both in extensive epidemiological studies and on a basic research level. The conclusions of these studies are in such close agreement that smoking has been identified by the American Heart Association (AHA) as the most significant modifiable risk factor of coronary artery disease in the USA[1].

One of the mechanisms by which smoking impairs the cardiovascular function is its effect on Autonomic Nervous System (ANS) control[2-4]. Out of all the biologically active substances detected in cigarettes and tobacco products, nicotine, tar and carbon monoxide have been most widely studied. Nicotine acts as an agonist of nicotine receptors in the central and peripheral nervous system exerting a ganglioplegic effect and causing suppression of vagal (predominantly) and sympathetic control. However in the latter there seems to be a direct enhancing effect at the central nuclei level resulting in an overall increase of sympathetic tone. In addition, nicotine affects the chemoreceptors of the aorta and the carotid thus

causing reflex vasospasm, blood pressure elevation and respiratory rate acceleration.

The establishment of Heart Rate Variability (HRV) indices created a reliable and useful tool for studying the role of the autonomic nervous system in cardiac function[7]. Low values of heart rate variability (HRV) correspond to an increased sympathetic tone or a suppressed vagal tone. Today HRV clinical applications include the assessment of relative risk of death in post-myocardial infarction patients[8-9] as well as the assessment of diabetic neuropathy progression[10]. Moreover, recent studies enhance the value of the method, since it seems that decreased HRV rates in the general population are linked to increased cardiac morbidity, an observation derived from the prospective follow-up of 2513 healthy male subjects during the Framingham study[11].

The present study attempts to record the short-term changes in autonomic cardiac control that are directly caused by smoking in young healthy volunteers and to compare these changes to the changes caused by smoking hemoglobin filter (Biofilter) cigarettes. This comparison aims to show whether or not the latter provide some degree of protection against the adverse effects of smoking with respect to autonomic cardiac control. This last part of the study was triggered by the lack of clinical studies that associate the use of the aforementioned filters with beneficial or, to be more precise, less harmful effects on the cardiovascular system.

The heart rate increase and the increase of LF, LF/HF and CCVLF indices, as well as the decrease of all the remaining HRV indices are considered as changes compatible with the shifting of the ANS balance towards the sympathetic side. This shift, when it occurs, expresses the dual effect, sympathomimetic and parasympatholytic, of smoking on ANS cardiac tone. As far as the specific study of HRV indices is concerned, it is generally accepted that the HF (High Frequency component) index expresses vagal cardiac control, while the LF (Low Frequency component) index, although it mostly reflects sympathetic changes, does not seem to be influenced solely by these. The LF/HF ratio represents the composite of all effects on ANS tone as well as its dynamic change within small time intervals. From the time domain indices, r-MSSD and pNN50 show a greater correlation with parasympathetic system tone and are decreased when the ANS cardiac control balance shifts towards the sympathetic side.

Materials and method

Study population

Thirty-two healthy young volunteers participated in the study; table 1 shows the mean age of the subjects, gender-related distribution and their relation to smoking. The smokers group includes people who smoke more than 10 cigarettes per day. Young volunteers were chosen as it is a well-known fact that there is a progressive decrease of HRV after the fourth decade of life[12-13]. The non-smokers group, following a suggestion by the Ethics Committee of the Hospital where the study was presented, included volunteers who had been addicted to smoking in the past but had ceased to smoke for a period of two years at least.

All the participants in the study had a normal resting ECG and a history free of diabetes mellitus and chronic respiratory and cardiovascular diseases. All individuals with an acute health problem were excluded, including those with a plain respiratory infection, as well as individuals with diseases known to affect HRV, such as an old myocardial infarction or documented coronary artery disease, heart valve disease, congestive heart failure (NYHA II-IV), renal or hepatic failure, permanent pacemaker, pre-excitation syndrome, bundle branch block, sinus node disease, atrial fibrillation, frequent (>10/h) ventricular extrasystoles, as well as non-compliance with the study protocol.

Method

All the volunteers were subjected to two examinations which were carried out with a 15-day interval, at the same place and on the same hour of the day. The first examination studied the effect of conventional cigarettes. Three different types of "conventional" cigarettes were used which had the same content in tar and nicotine as the biofilter cigarettes,

Table 1. The population of the study.

	Men (30,3±3,1 έτη)	**Women** (29,6±2,5 έτη)	**N**
Smokers (29,9±3,3 έτη)	10	11	21
Non-Smokers (30,5±2,1 έτη)	8	3	11
Total	18	14	32

while these "conventional" cigarettes do not claim to having any specific protective property. In the second examination hemoglobin biofilter cigarettes were used.

The examinations were carried out with the subjects at a seated position, a position most frequently used by smokers while smoking. All the subjects received no medication and abstained from meals, alcohol or caffeine-containing beverages for 4 hours prior to the examination, while smokers were asked not to smoke for 8 hours at least prior to the examination. At the start of the examination, all subjects had blood pressure within normal ranges (<140/90 mmHg) and regular respiratory rate (10-16 respirations / minute).

Recording tool place for six successive 5-minute periods, using an Oxford Medilog ECG recorder with modified precordial leads. During the second 5-minute period the subjects were asked to smoke a specified cigarette length (up to 4 cm) within the aforementioned time interval. Five-minute periods were chosen as the study's time intervals because this way the minimum time period required for a reliable study of HRV indices is achieved, both in the scale of time (time domain measurements) as well as in the spectral scale (frequency domain measurements). Half an hour was chosen as the total observation time because it is the time interval that usually intervenes between the smoking of two successive cigarettes for the average smoker. Cigarette smoking took place in the second 5-minute period in order to obtain a five-minute recording of HRV indices baseline values before initiation of smoking. After the examination using a common filter cigarette was completed, all the volunteers were re-examined within a fortnight, under the same conditions, but this time biofilter cigarettes were used. We should note that all cigarettes used had the same tar and nicotine yields (14 and 1 mg respectively).

Recording analysis was performed using the Oxford Medilog system (Excel - 2 version). All supraventricular and ventricular arrhythmias as well as conduction disorders that took place during the half-hour recording were identified. The presence of more than three per 5-minute interval or of more than 10 in the half-hour duration ectopic supraventricular contractions was considered a criterion for exclusion from the study and was encountered in two cases. No recording had more than 10 per hour (5 per half-hour) ventricular extrasystoles. In case extrasystoles were encountered within the aforementioned limits, the intervals immediately preceding and following the systole were excluded from the RR interval processing. Logarithmic processing of the data to determine the indices in the spectral scale was performed using Auto Regressive Transformation, a method usually preferred for studying small time intervals.

For the study of the ANS cardiac control changes, we used the HRV indices as specified by the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, both for the time domain (Mean, SDNN, rMSSD and pnn50) and for the spectral domain (Total Power, LF, HF and LF/HF)[14]. In addition, we studied the square root of LF/HF ratio divided by the mean RR (CCVLF [coefficient of component variance for LF] and CCVHF [coefficient of component variance for HF]) in order to determine the changes in the ANS state irrespectively of the heart rate changes. These indices are described in detail in table 2.

Table 2. Selected time and frequency domain measurements of heart rate variability according to the guidelines by the ESC and NASPE (reference No 14).

Variable	Unit	Description
Mean RR	ms	Mean of all filtered RR intervals over the length of the analysis
SDNN	ms	Standard deviation of all RR intervals
r-MSSD	ms	The square root of the mean value of the sum of the squares of differences between neighboring RR intervals over the length of the analysis
PNN50	ms	The percentage of the differences between neighboring filtered RR intervals that are greater than 50 ms
Total Power	ms^2	Total variation of all RR intervals
LF	ms^2	Variation in the low frequency range (0.04-0.15 Hz)
HF	ms^2	Variation in the high frequency range (0.15-0.4 Hz)
LF/HF		Ratio LF/HF
CCVLF	%	Square root of LF divided by mean RR
CCVHF	%	Square root of HF divided by mean RR

Statistical analysis

For a total of ten indices for both examinations of each volunteer, we calculated the mean values and their changes, expressed as a percentage of the initial values, with respect to the initial 5-minute period when the reference values were calculated. Results are presented as mean value ± standard deviation. For the comparisons performed between 5-minute intervals as well as between the two examinations, a paired t-test method was used. For comparisons between different subgroups (e.g. males-females or smokers-non-smokers), anova analysis was used. In both cases, numeric data were logarithmically converted before the statistical methods were applied in order to preserve at all times the criteria of their regular distribution. In all cases the statistical significance limit was set at the level of 5%.

Results

A. Time domain indices

All four time domain indices that were studied showed a statistically significant value decrease during the second 5-minute period (Table 3). The only exception was the SDNN index whose alteration during the second 5-minute period did not reach statistically significant levels (from 67±30msec to 65±23msec, P=0.926). Table 3 shows that the most significant changes took place in the third and fourth 5-minute period of the study, i.e. five to ten minutes after smoking cessation. The maximal quantitative changes that occurred are the following: Mean=11.8% in the 3rd period, SDNN=22.7% in the 5th period, r-MSSD =32.6% in the 3rd period and pNN50=49.1% in the 3rd period. The last two indices, which are primarily

Table 3. Measurements, changes of HRV variables and statistical significance of the differences compared with the first periods' values. Each period lasts five minutes.

	1st period	2nd period	3rd period	4th period	5th period	6th period
Mean RR	802±122 ms	719±102 ms –10,3% P<0,001	708±102 ms –11,8% P<0.001	736±111 ms –8,3% P<0,001	749±124 ms –6,6% P<0,001	756±123 ms –5,7% P<0,001
SDNN	67±30 ms	65±23 ms –2,7% P=0,926	52±20 ms –22,1% P<0,001	53±21 ms –20,6% P<0,001	52±20 ms –22,7% P<0,001	58±21 ms –15,6% P=0,003
r-MSSD	45±24 ms	37±21 ms –16,9% P=0,001	30±18 ms –32,6% P<0,001	32±17 ms –28,1% P<0,001	34±18 ms –24,5% P<0,001	36±18 ms –20,1% P<0,001
pNN50	19±18 ms	14±15 ms –27,5% P=0,002	10±11 ms –49,1% P<0,001	11±14 ms –41,1% P<0,001	13±15 ms –35,0% P<0,001	13±15 ms –30,7% P=0,001
Total Power	5218±5044 ms^2	4689±3195 ms^2 –10,1% P=0,435	2996±2180 ms^2 –42,6% P=0,002	3170±2410 ms^2 –39,2% P<0,001	2953±2157ms^2 –43,4% P<0,001	3570±2738 ms^2 –31,6% P=0,004
LF	2329±2061 ms^2	1946±1685 ms^2 –16,3% P=0,226	1368±983 ms^2 –41,1% P=0,002	1573±1334 ms^2 –32,3% P=0,001	1462±1213 ms^2 –37,1% P<0,001	1582±1207 ms^2 –31,9% P=0,014
HF	848±737 ms^2	721±853 ms^2 –15,0% P=0,015	390±400 ms^2 –54,% P<0,001	400±355 ms^2 –52,9% P<0,001	478±508 ms^2 –43,6% P<0,001	477±411 ms^2 –43,8% P<0,001
LF/HF	4,38±3,47	3,39±2,51 18,9% P=0,069	5,47±3,82 61,4% P<0,001	4,84±2,28 42,8% P=0,004	3,94±3,05 15,9% P=0,103	4.64±4.15 36,9% P=0,008
CCVLF	1,55±0,59	1,50±0,57 –3,2% P=0,577	1,27±0,49 –18,1% P=0,01	1,31±0,57 –15,5% P=0,012	1,27±0,47 –18,1% P=0,002	1,35±0,44 –12,9% P=0,032
CCVHF	0,92±0,37	0,85±0,45 –8,3% P=0,17	0,64±0,33 –31,4% P<0,001	0,66±0,26 –28,5% P<0,001	0,71±0,29 –23,7% P<0,001	0,72±0,27 –21,9% P<0,001

linked to changes in vagal control, showed the greatest percentage change in comparison to the others.

In the sixth five-minute period, all indices showed a trend to return to the initial values, however none had reached the initial levels at the end of the half-hour observation period.

B. Spectral domain indices

Total Power, LF and HF indices showed a statistically significant decrease at the start of smoking, a decrease that, as for the time domain indices, was maximized in the 3rd and 4th periods and lasted throughout the observation half-hour (table 3). An interesting observation which will be discussed below is the LF component decrease (16.3%, 41.1%, 32.3%, 37.1% and 31.9% for the five periods respectively in relation to the initial period). Despite the LF decrease, there was a statistically significant increase of the LF/HF ratio which maximized in the 3rd period (61%, $P<0.001$). This LF/HF ratio increase is caused by a significant decrease of the HF component which is a quantitative expression of vagal control, as will be analyzed below.

Despite the fact that both LF and HF indices showed a statistically significant decrease, the ratio of their square root divided by the mean RR interval (CCVLF and CCVHF) which expresses the change in their values irrespectively of heart rate showed a different quantitative change and different levels of statistic significance (Table 3).

C. Results regarding cigarettes with biological filter

In tables 5 which show the changes with both type of cigarettes in diagrammatic form, we can see that all

Table 4. Comparison of the changes produced by the two types of cigarettes, control cigarettes (con) and biofilter (bf), in relation to baseline values and statistical significance of percentile changes between the two types of cigarettes.

	2nd period	3rd period	4th period	5th period	6th period
Mean RR (con)	–10,3%	–11,8%	–8,3%	–6,6%	–5.7%
(bf)	–7,9%	–9,6%	–6,8%	–5,2%	–4,0%
	P=0,103	P=0,199	P=0,241	P=0,237	P=0,240
SDNN (con)	–2,7%	–22,1%	–20,6%	–22,7%	–15,6%
(bf)	–3,2%	–15,5%	–19,0%	–18,2%	–10,3%
	P=0,842	P=0,209	P=0,752	P=0,388	P=0,467
rMSSD (con)	–16,9%	–32,6%	–28,1%	–24,5%	–20,1%
(bf)	–11,0%	–25,8%	–25,3%	–19,7%	–13,4%
	P=0,142	P=0,252	P=0,566	P=0,258	P=0,285
pNN50 (con)	–27,5%	–49,1%	–41,1%	–35,0%	–30,7%
(bf)	–19,1%	–35,6%	–36,9%	–28,1%	–12,2%
	P=0,06	P=0,602	P=0,630	P=0,660	P=0,156
Total (con)	–10,1%	–42,6%	–39,2%	–43,4%	–31,6%
Power (bf)	–9,1%	–27,8%	–35,1%	–33,6%	–18,5%
	P=0,479	P=0,094	P=0,557	P=0,203	P=0,126
LF (con)	–16,3%	–41,1%	–32,3%	–37,1%	–31,9%
(bf)	–2,3%	–16,6%	–25,9%	–20,1%	–5,1%
	P=0,162	P=0,054	P=0,399	P=0,094	P=0,137
HF (con)	–15,0%	–54,1%	–52,9%	–43,6%	–43,8%
(bf)	–22,8%	–37,5%	–41,9%	–36,3%	–21,0%
	P=0,631	P=0,344	P=0,675	P=0,704	P=0,162
LF/HF (con)	18,9%	61,4%	42,8%	15,9%	36,9%
(bf)	50,0%	55,6%	61,7%	68,1%	39,2%
	P=0,194	P=0,461	P=0,572	P=0,087	P=0,786
CCVLF (con)	–3,2%	–18,1%	–15,5%	–18,1%	–12,9%
(bf)	4,9%	–4,9%	–10,4	–6,9%	–1,4%
	P=0,182	P=0,054	P=0,375	P=0,098	P=0,201
CCVHF (con)	–8,3%	–31,4%	–28,5%	–23,7%	–21,9%
(bf)	–10,2%	–20,1%	–23,2%	–21,2%	–12,3%
	P=0,732	P=0,135	P=0,571	P=0,826	P=0,252

the indices which were studied follow exactly the same pattern of change with small differences in their values. In order to compare the mean percentage change per 5-minute period for both examinations (with conventional cigarettes and with biofilter cigarettes), a paired t-test method was applied aiming to identify the periods in which the difference in the mean percentage change between the two examinations represents a value different from zero at a statistically significant degree. As shown in table 4 the difference in the mean percentage change of HRV rates does reach statistically significant levels for any of the indices and in any 5-minute period.

D. Differences between smokers and non-smokers

As shown in table 5 the index values for the initial periods, i.e. before the effect of smoking, between smokers (n=21) and non-smokers (n=11) did not differ at a statistically significant level. However the initial heart rate of smokers appears increased compared to non-smokers (77±8 vs. 71±11, anova F=2.16, P=0.15, NS) at the start of the examination. For the total of time domain indices, smokers showed lower, i.e. worse, values compared to non-smokers. In the spectral domain, non-smokers showed a higher LF/HF ratio compared to smokers.

E. Gender-related differences

In the same table (Table 5) we can see that there are no statistically significant differences in the initial values between men (n=18) and women (n=14). An exception to the previous rule is the LF/HF index value which was found to be significantly higher in men (4.16±3,03 vs. 2.39±1.04, anova F=4.35, P=0.045). Nevertheless it seems that men had lower heart rate values at the start of the examination compared to women (73±9 vs. 76±9, anova F=0.438, P=0.51 NS), despite the fact that this difference does not reach statistically significant levels.

Discussion

It is well-known that smoking is responsible to a considerable extent for cancer in the upper and lower respiratory system. However, the greatest impact on morbidity and mortality in the general population comes from the unfavorable change in the epidemiological characteristics of coronary artery disease that is caused by smoking.

Moreover it is a fact that during the past three decades significant efforts have been made to limit the adverse effects of smoking through alternative ways or means of smoking. Despite the fact that experimental data were often encouraging, a reduction in mortality from smoking these alternative products has never been proven from an epidemiological standpoint. This last observation also underlines the current lack of knowledge both in relation to the pathophysiology of the results of smoking as well as for the behavior of smokers.

This study aims to supplement the research efforts in this field, especially after the emergence of new data that showed that HRV low rates in the general population are linked to an increased likelihood of occurrence of new cardiovascular events. The direct effects of smoking on cardiac function with respect to ANS control changes have been investigated in the past by Hayano and his team[2] and they showed a significant change in ANS control immediately after the start of smoking and lasting

Table 5. Initial values of the subgroups of the study population in relation to smoking status and gender.

	Non-Smokers (N=11)	Smokers (N=21)	ANOVA	Men (N=18)	Women (N=14)	ANOVA
Mean RR	845±169	780±85	P=NS	815±136	786±104	P=NS
SDNN	72±31	65±31	P=NS	66±32	68±26	P=NS
r-MSSD	46±25	44±23	P=NS	43±24	46±23	P=NS
PNN50	20±19	18±18	P=NS	18±17	20±19	P=NS
Total Power	5531±3477	5054±5771	P=NS	5239±1401	3795±1014	P=NS
LF	2320±1270	2333±1270	P=NS	2439±2435	2187±1529	P=NS
HF	780±674	883±781	P=NS	802±786	906±693	P=NS
LF/HF	4,33±3,38	2,89±1,81	P=NS	4,16±3,03	2,39±1,04	P=0,045

for twenty minutes at least after its cessation. In this study smoking was performed after a twenty-minute period in a supine position. It is known however that staying in this position causes by itself a significant change in ANS control, shifting its balance towards the parasympathetic side. It is therefore reasonable to assume that the sympathetic response is expected to be increased when triggered by an external intervention in a person who has remained in a supine position for twenty minutes.

Thus the primary goal in our study was to investigate the effects of smoking on ANS control in a seated position, on the one hand because this position is the most frequent smoking position and on the other hand because this limits the supine position's vagal control which was the starting point in previous studies.

Our results, as presented above, show that in young healthy individuals (habitual smokers and non-smokers, male and female subgroups) smoking causes a direct change in HRV, indicative of the shift of ANS control towards the sympathetic side. Although this change appears during smoking, the maximal changes occur five to ten minutes after smoking cessation. This last observation is in agreement with experimental data which showed that smoking causes an immediate (within three minutes) suppression of vagal cardiac control in the sinus node but also a long-term suppression through a potent ganglioplegic effect and a modification of baroreceptor control in the large vessels[15]. The above conclusions are in complete agreement with our own observations.

Furthermore, it has been shown that smoking causes an immediate release of catecholamines in the peripheral circulation. Hayano and his team[2] attributed the observed increase of the LF component in the spectral domain directly after smoking to this experimentally documented response. In the present study, this component appeared decreased instead of increased. There are two possible explanations for this. According to one hypothesis, the smaller change in the level of catecholamines after smoking observed in our study is explained by the different postural effect. According to another scenario, the possible sympathomimetic activity of catecholamines did not ultimately become apparent due to the potent parasympatholytic effect that was mentioned before. Moreover, the population of this study had a higher mean age by approximately 5 years and age is known to significantly affect ANS behavior.

Given that at the end of the half hour period the values of HRV indices remained significantly decreased compared to the initial values, we can reasonably conclude that an average smoker that smokes one cigarette per half hour (i.e. 20-30 cigarettes per day), is constantly under the harmful influence of smoking, at least with respect to ANS cardiac control. In the light of recent studies that associate HRV values with the likelihood of cardiovascular events occurring in healthy individuals, it is obvious that the study of HRV constitutes another, directly measurable, mechanism for the evaluation of the harmful effects of smoking on the cardiovascular system. This evaluation of the effects of smoking becomes even more valuable when we consider that it is practically impossible to directly monitor the clinical results of other smoking effects (e.g. carcinogenesis, atheromatosis). Safe conclusions with respect to these can only be drawn after painstaking, long-term prospective studies.

With respect to the absence of any protective effect of cigarettes with biological filter on ANS cardiac control, we ought to note that it was never claimed by manufacturers that this new type of filter (i.e. hemoglobin filter) provides special protection against the specific harmful influences. However the fact remains that the changes in ANS cardiac control play an important role in adversely affecting cardiovascular risk for smokers and, more importantly, they are directly measurable. Conversely, the other, potentially protective properties of this new type of cigarette cannot be clinically documented but rather they have to proven based on long-term epidemiological data. In conclusion, we ought to mention the American Heart Association's official position on smoking which underlines that "The use of alternative smoking products or low yield cigarettes has not reduced the risk of cardiovascular events. The absence of this reduction may be due both to the products themselves and the change in the behavior of the users of these products".

In any case, it is presently both socially and scientifically accepted that words such as "protection" should not accompany tobacco products, at least not until such time as independent prospective epidemiological studies will prove the possible protective role of such products at a population level.

Limitations

The lack of biochemical indices for better monitoring of the changes in ANS status has been one of

the limitations in our study. In addition, a larger number of individuals in the study population would allow the extrapolation of safe conclusions for the comparative changes between the different subgroups. Finally, it would be worthwhile to observe the participants for a longer time period in order to estimate the time until full recovery of HRV.

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References

1. Cigarette smoking and cardiovascular diseases: AHA Scientific/Medical Position 1996 (http://www.americanheart.org).
2. Hayano J, Yamada M, Sakakibara Y, et al: Short and Long-term effects of cigarette smoking on Heart Rate Variability, Am J Cardiol, 1990; 65: 84-88.
3. Luchini D, Bertocchi F, Maliani A, et al: A controlled study of the autonomic changes produced by habitual cigarette smoking in healthy subjects. Cardiovascular Research, 1996; 31: 633-639.
4. Niedermaier O, Smith M, Beightol L, et al: Influence of Cigarette Smoking on Human Autonomic Function. Circulation, 1993; 88: 562-571.
5. Aronof WS, Dendinger J, Rokaw SN: Heart rate and carbon monoxide level after smoking high-low and non-nicotine cigarettes. A study in male patients with angina pectoris. Ann Intern Med 1971; 74: 697-702.
6. Nicotine, Pharmacology/Pharmacokinetics: USP DI, Drug information for the health care professionals-United States Pharmacopeia 1995.
7. Malik M, Camm J: Heart Rate Variability. Clin Cardiol 1990; 13: 570-576.
8. Kleiger RE, Miller JT, Bigger JT, et al: The Multicenter Post-Infarction Research Group. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987; 59: 256-262.
9. Odemuyiwa O, Malik M, Farell T, et al: Comparison of the predictive characteristics of heart rate variabilty index and left ventricular ejection fraction for all cause mortality, arrhythmic events and sudden death after acute myocardial infarction. Am J Cardiol 1991; 68: 434-439.
10. Bellavere F., Balzani I., De Masi G: Power spectral analysis of heart rate variation improves assesment of diabetic cardiac autonomic neuropathy. Diabetes 1992; 41: 633-640.
11. Tsuji H, Larson MG, Venditti FJ, et al: Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation 1996; 94: 2850-2855.
12. Liao D, Barnes RW, Chambless LE, et al: Age, Race and Sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability-The ARIC study. Am J Cardiol 1995; 76: 906-912.
13. Schwartz JB, Gibb WJ, Tran T: Aging effects on Heart Rate Variability. J Gerontol 1991; 46: M99-M106.
14. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart Rate Variability. Standards of measurements, physiological interpretation and clinical use. Eur Heart J, 1996; 17: 354-381.
15. Nadeau RA, James TN: Effects of nicotine on heart rate studied by direct perfusion of sinus node. Am J Physiol 1967; 212: 911-916.
16. Cryer PE, Haymond MW, Santiago JV, Shah SD: Norepinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Eng J Med 1976; 296: 573-577.