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Abstract—Elevated psychosocial stress might favor the occurrence of cardiovascular disease; however, mechanisms are incompletely understood. We hypothesized that patients (n=126; 44±1 years of age) referred to an internal medicine clinic because of symptoms related to chronic psychosocial stress would demonstrate signs of autonomic dysregulation compared with controls (n=132; 42±1 years of age). We used autoregressive spectral analysis of RR interval variability to obtain indirect markers of sympathetic and of vagal (respectively, low-frequency and high-frequency components, both expressed in normalized units) oscillatory modulation of sinoatrial node, as well as of sympathetic vasomotor regulation (low-frequency component of systolic arterial pressure variability) and of cardiac baroreflex sensitivity (α-index). Higher values of systolic and diastolic arterial pressure (respectively, 124±1 versus 117±1 mm Hg and 80±1 versus 75±1 mm Hg; both $P<0.001$), altered markers of autonomic regulation (increased normalized low-frequency and reduced high-frequency component of RR variability, $P<0.005$; increased-low frequency component of systolic arterial pressure variability, $P<0.002$), and reduced baroreflex sensitivity (19.3±1.4 versus 23.0±2.0 ms/mm Hg; $P<0.05$) were observed in patients compared with controls. Autonomic responses to active standing were also blunted in stressed patients. Autonomic markers were significantly correlated to stress perception score and were capable of discriminating between controls and patients with a high degree of accuracy. Chronic real-life stress in humans appears associated to increased arterial pressure and to impaired autonomic regulation of cardiovascular functions. The combination of sympathetic predominance, vagal withdrawal, and blunted baroreflex sensitivity might represent a treatable mechanistic link between psychosocial factors and future incidence of hypertension. **(*Hypertension*. 2005;46:1201-1206.)**

Key Words: autonomic nervous system ■ stress ■ risk factors ■ baroflex ■ behavior ■ hypertension, arterial

Mechanisms by which psychosocial factors increase the risk of cardiovascular diseases are various and complex, and among them, sympathetic overactivity seems to play a pivotal role.[1,2] Acute stress might induce short-lasting rises in arterial pressure and heart rate, impair endothelial function, and reduce the threshold for arrhythmia and sudden death.[3–6]

Chronic stress may also facilitate the occurrence of atherosclerosis[7] by way of the attendant elevated sympathetic activity, acting directly or in combination with inflammation,[8] elevated cortisol levels,[9] and unhealthy behaviors,[10] or other disturbances such as the metabolic syndrome.[11] Stress may also reduce baroreflex performance,[2,12] thus impairing one of the major cardioprotective autonomic reflex mechanisms,[13] ultimately favoring the occurrence of hypertension.

The mechanistic role of autonomic dysregulation in the context of stress has been explored in a variety of animal or laboratory models[11,14,15]; however, so far, relatively few studies have addressed the association between autonomic dysfunction and real-life stress in humans,[2,12] probably because stress may elude accurate quantification[16] because it consists of several (inter-related) elements, and its effects are characterized by pronounced between subjects' variability. In fact, response to stress may be difficult to assess even in the controlled laboratory environment.

Computer analysis of spontaneous blood pressure and heart rate fluctuations has been suggested to offer an insight into autonomic cardiovascular regulation,[17,18] with no need of external stimulation on the cardiac and vascular targets. This approach thus appears well suited to explore the impact of stress on autonomic cardiovascular control and the possible effects of suggested countermeasures.[19,20]

The aim of our study was to test whether patients with symptoms of chronic psychosocial stress, in absence of clinically manifested illness, show signs of autonomic dysregulation, as assessed through a noninvasive approach based on spectral analysis of cardiovascular variabilities.

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TABLE 1. Study Population*

Variables	Controls	Patients
No.	132	126
Age (years)	42±1	44±1
Gender		
% male	38.2	44.4
% female	61.8	55.6
BMI (kg/m^2)	23.0±0.3	23.3±0.3
Smoke (% smokers)	0	0
Drugs (% subjects on medication)	0	0
Physical activity		
% Sedentary life	46.66	46.34
% Occasional	30.66	20.73
% Regular light	22.66	32.90
% Regular heavy	0	0

*No statistical differences between controls and patients were present in any of these variables.

Methods

Study Population

This study considers 288 consecutive subjects who, in the period 2001 to 2003, asked our advice because of symptoms related to stress lasting >3 months. A total of 162 subjects were excluded because of the presence of concomitant diseases, pharmacological treatment, or cigarette smoking, alcohol, or food abuse; the remaining 126 subjects (from now on defined as "patients"; 44±1 years of age) were enrolled. A total of 132 healthy volunteers of similar age (42±1 years) who did not report any stress-related symptoms were enrolled as "controls" (Table 1). The absence of clinically manifested disease was determined by history, physical examination, and laboratory and routine tests. Body mass index was 23.3±0.3 kg/m^2 in patients and 23.0±0.3 kg/m^2 in controls. Patients and controls were not on medications of any kind.

On the day of the study, subjects were instructed to avoid alcohol and caffeinated beverages for the 12 preceding hours, to abstain from heavy physical activity since the day before and, after a light breakfast, to come to the laboratory between 8:30 AM and 12:30 PM. All subjects were instructed about the study procedure and gave their informed consent. Our institution ethics committee approved the protocol of the study.

Symptom Evaluation

All subjects were assessed by a clinical psychologist through semistructured interview to establish the presence (patients) or absence (controls) of chronic psychosocial stress, the presence of stress-related symptoms, and to exclude patients with psychiatric diseases (with particular attention to depression and somatoform disorders) following *Diagnostic and Statistical Manual of Mental Disorders IV* criteria.[21]

As in a previous study on the autonomic effects of acute stress,[2] all subjects completed a self-administered questionnaire providing nominal self-rated scales that focused on overall stress perception and stress-related symptoms.

Autonomic Evaluation

After a preliminary 10-minute rest period in supine position, allowed for stabilization, blood pressure, ECG, and respiratory activity were recorded over a 10-minute supine baseline and over a subsequent 7-minute period of active standing.

To minimize possible emotional bias of the recording procedure, the ECG (CM5) and the respiratory signal were recorded in all subjects with a 2-way wireless radiotelemetry system while arterial pressure waveform (obtained in all patients and 83 controls) was continuously assessed noninvasively by Finapres device (Ohmeda). The accuracy of this device in tracking beat-by-beat blood pressure changes has been documented previously.[22] Data were acquired with a personal computer using an acquisition rate of 300 samples/channel per second.

From the simultaneous autoregressive spectral analysis of RR interval and systolic arterial pressure (SAP) variability, a series of indexes indirectly reflecting autonomic cardiovascular modulation were derived.[18] RR interval spectral powers were quantified in the low-frequency (LF; 0.03 to 014 Hz) and the high-frequency (HF; 0.15 to 0.35 Hz) regions. LF spectral powers were normalized according to the formula $P_{LF[nu]}=[(P_{LF[ms]}{}^2)/(VAR_{RR[ms]}{}^2-VLF_{[ms]}{}^2)]\times 100$, where $P_{LF[nu]}$=LF powers in normalized unit; VAR=tot variance; and VLF=very low frequency component <0.03 Hz; similar normalization was performed for HF powers. LF/HF of RR interval variability power ratio was also computed. SAP spectral powers were quantified in the LF region (0.33 to 0.14 Hz) and reported in absolute units.[23]

The sensitivity of arterial baroreflex control of RR interval was assessed by the α-index (average of the square root of the ratio between RR interval and systolic blood pressure spectral powers in the LF and HF regions).[24]

Monovariate and bivariate spectral analysis of RR interval variability and respiration were used to ensure that in all subjects included in the study, respiratory rate coincided with the HF component of RR variability, and no respiratory entrainment was present.

Statistics

Data in the text, figures, and tables are presented as means±SEM. Nonparametric tests (Mann–Whitney, Kruskal–Wallis, and Jonkheere–Terpstra) with Monte Carlo procedure were used to determine the significance of the observed differences as appropriate. Simple nonparametric correlation was used to assess the statistical link between stress scores and indices of autonomic cardiovascular regulation. Discriminant analysis was used to assess the integrated capacity of several psychometric and autonomic variables to correctly classify subjects as controls or patients. A P value <0.05 was considered significant. All computations were performed with a commercial statistical package (SPSS 13).

Results

Stress Evaluation

None of the control subjects reported any particular source of stress in their lives, as per enrollment criteria, whereas the chronic psychosocial stress reported by patients was related to personal problems (relationships with partners, friends, relatives, etc) in 24.3%, social work problems (possibility to lose job, mobbing, personal relationships with managers or other employees, dissatisfaction with their role or salary, lack of social support, etc) in 27.2%, and relatives' health problems (caregiving, worry for relative's health status, etc) in 48.5%. The presence of attendant somatic symptoms was assessed[25] by spontaneous reporting during consultation and by self-administered questionnaire. The majority of patients (76%) reported the same symptom/symptoms in both evaluations. Symptoms reported more frequently were related to heart, respiratory system, gastrointestinal tract, or muscle disturbances.

Overall Stress Perception Scale

Patients showed a significantly higher perception of stress compared with controls (respectively, 6.3±0.2 versus 3.2±0.2; P<0.001).

TABLE 2. Descriptive Statistics of RR Interval Variability in Controls and Patients Under Resting Conditions

Subjects	RR ms	VAR_{RR} ms^2	LF_{RR} mHz	LF_{RR} ms^2	LF_{RR} nu	HF_{RR} mHz	HF_{RR} ms^2	HF_{RR} nu	LF/HF	SAP mm Hg	DAP mm Hg	α-Index ms/mm Hg
Controls	933±14	2249±195	101±2	683±101	52±1	262±5	466±59	37±1	2.6±0.3	117±1	75±1	23.0±2.0
Patients	1046±21*	2575±204	87±2*	791±89	59±2*	237±6*	539±78	31±2*	4.3±0.5*	124±1*	80±1*	19.3±1.4†

VAR_{RR} indicates RR interval variance; LF/HF, ratio between spectral powers of LF and HF components of RR interval variability; DAP, diastolic arterial pressure; α-index, frequency domain measure of baroreflex gain; nu, normalized units.

Significant difference controls vs patients *$P \leq 0.005$; †$P < 0.05$.

Subjective Stress-Related Somatic Symptoms Questionnaire

The total subjective stress-related somatic symptoms questionnaire (4S-Q) score was significantly higher in patients compared with controls (respectively, 57.2±2.7 versus 19.7±2.2; $P<0.001$). A significant correlation was found between stress perception scale and 4S-Q scores ($r=0.58$; $P<0.001$).

Autonomic Evaluation

SAP and diastolic arterial pressure (Table 2), although still in the normal range, were significantly higher in patients compared with controls, whereas heart rate appeared significantly reduced in patients (60.3±1.2 versus 66.1±0.9 bpm; $P<0.005$). No significant difference between patients and controls was found when considering RR interval variance and absolute values of RR interval LF and HF spectral components. Conversely, the LF component of RR interval variability (LF_{RR}) expressed in normalized units (Table 2; Figure 1) was significantly higher in patients, whereas the HF component of RR interval variability (HF_{RR}), also expressed in normalized units,[18] was significantly smaller in patients than in controls. LF/HF ratio (Table 2)[18] was significantly more elevated in patients. No significant differences between the 2 groups were observed regarding total variance of RR interval variability. Similar differences were also observed considering males and females separately (Table 3).

During active standing, a stimulus that physiologically enhances sympathetic drive to sinoatrial (SA) node, we observed a significantly smaller increase of normalized LF_{RR} (Figure 1) and a significantly smaller reduction of normalized HF_{RR} in patients compared with controls, whereas, again, no difference was observed when considering absolute values.

Patients were also characterized by greater ($P<0.02$) LF component of SAP variability (LF_{SAP}; respectively, 4.9±0.6 versus 2.8±0.3 mm Hg^2)[23] and by a reduced α-index, expression of baroreflex sensitivity in the frequency domain[24] (respectively, 19.3±1.4 versus 23.0±2.0 ms/mm Hg; $P<0.05$). Respiratory rate was slightly lower in patients than in control subjects (14.2±0.3 versus 15.7±0.3 cycles/min; $P<0.005$).

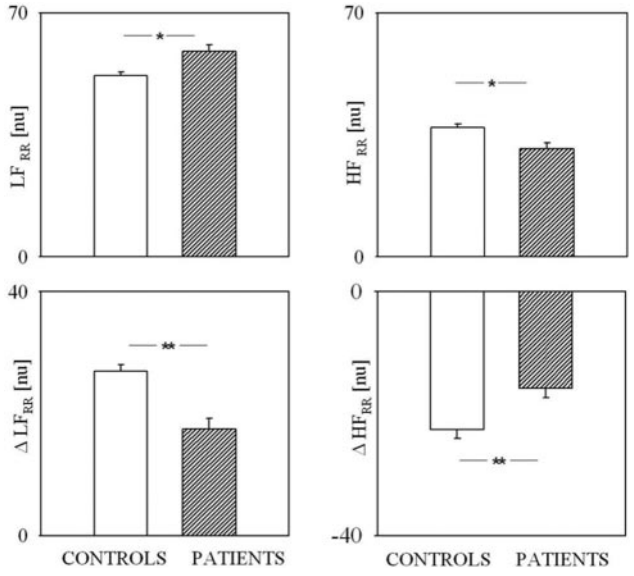

Figure 1. Average value (and SEM) of LF_{RR} (left top panel) and HF_{RR} (right top panel) at rest and of standing-induced changes of LF (ΔLF_{RR}; left bottom panel) and high (ΔHF_{RR}; right bottom panel) frequency in controls (open bars) compared with patients (lined bars). nu indicates normalized units. *$P<0.05$; **$P \leq 0.001$.

Correlation Between Psychological and Autonomic Indices

Overall stress perception scale score showed significant correlations with arterial pressure and several autonomic indices; notably, the strongest link was with SAP ($r=0.263$; $P<0.001$). Regarding the correlations with spectral indices of RR interval variability, only normalized but not absolute spectral components were significantly linked to stress perception (Table 4). Similar significant, albeit weaker, correlations were found considering 4S-Q scores; again, correlations with absolute spectral components of RR interval variability were not significant.

To assess the integrated capacity of used indices to correctly categorize the study subjects into either controls or patients, discriminant analysis was also performed. Figure 2 shows that, whereas the combination of psychological and autonomic variables provided a correct classification in ≈90%, the separate use of all psychometric or all autonomic variables reduced correct classification to ≈80%. Notably, progressively restricting the number of variables to the top-ranking 10 and subsequently 5, determined a further trivial loss of classification capacity. When only the 3 top-ranking variables (resting SAP, rest–stand difference of LF_{RR} in normalized units, and α-index at rest) were used, the correct classification was still near 80% (79.5% for the original cases and 77.6% for the cross-validated cases).

Discussion

In this study on patients with symptoms of chronic psychosocial stress, we observed signs of cardiovascular dysregula-

TABLE 3. Descriptive Statistics of RR Interval Variability in Male and Female Controls and Patients Under Resting Conditions

		RR	VAR_{RR}	LF_{RR}			HF_{RR}			LF/HF	SAP	DAP	α-Index
		ms	ms^2	mHz	ms^2	nu	mHz	ms^2	nu	—	mm g	mm g	ms/mm Hg
Males	Controls	916±20	2338±376	102±3	796±177	60±2	267±8	378±71	31±2	3.6±0.6	121±1	76±1	22.3±3.8
	Patients	1051±31	2569±295	84±2	923±157	65±2	228±7	380±84	25±2	5.6±0.8	128±1	82±1	18.3±2.0
Females	Controls	943±19	2191±211	99±3	610±120	47±1	258±7	523±85	40±1	1.8±0.2	114±1	73±1	23.3±1.9
	Patients	1041±27	2579±283	88±3	685±198	53±2	245±9	666±122	35±2	3.2±0.5	121±1	77±1	20.2±1.9
		*		*		*	*		*	*	*	*	†

VAR_{RR} indicates RR interval variance; LF/HF, ratio between spectral powers of LF and HF components of RR interval variability; DAP, diastolic arterial pressure; α-index, frequency domain measure of baroreflex gain; nu, normalized units.

Significant difference among groups by Kruskal–Wallis *$P \leq 0.05$; by Jonkheere–Terpstra test †$P \leq 0.05$.

tion, as shown by higher values of arterial pressure and altered markers of autonomic control.

The potentially confounding influences of chronic or psychiatric conditions,[26] or of drugs and behaviors affecting symptom profile or cardiovascular regulation, were carefully avoided by the selection procedure.

Although still in the normal range, patients of this study were characterized by slight but significantly higher values of SAP and diastolic arterial pressure levels compared with controls. As with real-life stressors of shorter duration,[2] the level of perceived stress was correlated to arterial pressure values. Whether stress may contribute by its association with higher arterial pressure levels to the elevated cardiovascular risk observed in patients with arterial pressure in the high-normal range[27] remains to be assessed.

TABLE 4. Simple Correlation Between Indices of Autonomic Cardiovascular Regulation and, Respectively, Stress Perception Scale Score and 4S-Q Score

Autonomic Indices		Correlation With Stress Perception Scale Score		Correlation With 4S-Q Score	
Variables	Units	*r*	*P*<	*r*	*P*<
RR	**ms**	**0.173**	**0.009**	0.114	0.086
VAR_{RR}	ms^2	−0.013	0.849	0.029	0.661
LF_{RR}	**mHz**	**−0.205**	**0.002**	**−0.219**	**0.001**
LF_{RR}	ms^2	0.007	0.921	0.044	0.505
LF_{RR}	**nu**	**0.153**	**0.021**	**0.158**	**0.016**
HF_{RR}	**mHz**	**−0.168**	**0.011**	**−0.148**	**0.025**
HF_{RR}	ms^2	−0.103	0.119	−0.082	0.215
HF_{RR}	**nu**	**−0.191**	**0.004**	**−0.170**	**0.010**
LF/HF		**0.175**	**0.008**	**0.160**	**0.015**
ΔLF_{RR}	**nu**	**−0.217**	**0.001**	**−0.149**	**0.023**
ΔHF_{RR}	**nu**	**0.203**	**0.002**	**0.171**	**0.009**
SAP	**mm Hg**	**0.263**	**0.000**	**0.160**	**0.035**
DAP	**mm Hg**	**0.176**	**0.008**	**0.233**	**0.000**
α-Index	**ms/mm Hg**	**−0.212**	**0.005**	**−0.167**	**0.028**

VAR_{RR} indicates RR interval variance; LF/HF, ratio between spectral powers of LF and HF components of RR interval variability; ΔLF_{RR}, changes in LF component of RR interval variability; ΔHF_{RR}, changes in HF component of RR interval variability; DAP, diastolic arterial pressure; α-index, frequency domain measure of baroreflex gain; nu, normalized units.

Significant correlations are printed in bold characters.

The intriguing finding of a slightly lower heart rate observed in patients compared with controls should be contrasted with the well-known tachycardia produced by acute psychological stress. Recent studies[28] on nonanesthetized instrumented mice subjected to prolonged stress show that after an initial increase in heart rate, a subsequent adaptation determines a clear bradycardia after about a week of continued exposure to stress. Chronic instrumentation avoided the hyper-responsiveness produced by handling and novelty, thus allowing the development over time of the unexpected bradycardia. Likewise in our patient population, the lack of any form of drug treatment, smoking, and use of a totally noninvasive wireless approach might have kept experimental bias to a minimum, thus allowing the emergence of a modest bradycardia. In line with this finding, Furlan et al[29] reported a modest bradycardia in humans exposed to chronic work-related stress. Patients also displayed a slower respiratory rate,[30] which, as shown previously with direct recordings of muscle sympathetic nerve activity,[31] might have induced a dissociation between indices of average autonomic tone and of oscillatory autonomic modulation.

Our study provides new information on the selective role of different autonomic oscillatory mechanisms using markers derived from autoregressive spectral analysis of cardiovascular variabilities.[18] A unique property of this technique derives from its ability to provide indices not only of the power of individual components but also, as with the Wiegner–Wille approach,[32] of their center frequency, thus describing more comprehensively the oscillatory behavior of cardiovascular autonomic centers. Increases in sympathetic drive are signaled by a relative increase in the normalized power of the LF component (whereas nonsignificant changes were observed in absolute spectral power) and in a leftwards shift of its center frequency because it occurs with upright posture[32] in normal individuals or with essential hypertension.[33] A similar leftward shift in the LF frequency with sympathetic stimulation was also shown by broadband spectral analysis in elderly subjects.[34]

Patients of this study, compared with controls, presented clear differences in spectral profile: the LF_{RR} (in normalized units) and LF/HF were all elevated at rest, whereas the increase of these measures with active orthostatism was blunted. Simultaneously, the center frequency of the LF component was shifted to the left. Overall, these changes

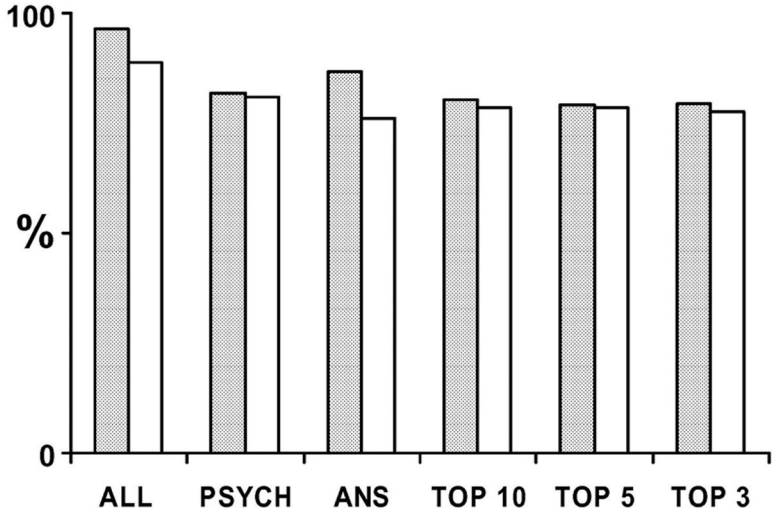

Figure 2. Discriminant analysis showing the modest loss of classification power produced by progressively restricting the number of variables used in the model. Notice that even using only the 3 top-ranking variables (resting SAP, rest–stand difference of LF_{RR} in normalized units, and α-index at rest) the correct classification is still near 80%, suggesting that these variables capture a large fraction of the information necessary to correctly allocate subjects to either controls or patients. Stippled bars represent original cases; open bars, cross-validated cases.

suggest a predominant oscillatory sympathetic modulation of the SA node[18,35,36] and a reduced responsiveness to excitatory stimuli. As in subjects with high normal arterial pressure,[33] a concomitant derangement of vagal oscillatory modulation of the SA node is suggested by smaller resting HF_{RR} and by reduced baroreflex sensitivity (assessed by the α-index). Given the major protective role of this vagally mediated reflex mechanism in the setting of coronary artery disease,[13] this finding might partly account for the well-known facilitating influence of stress on ischemia-linked arrhythmias and sudden death.[3,37] Furthermore, the increased LF_{SAP} suggests the presence in this patient population of enhanced sympathetic vasomotor modulation, as it occurs in acute mental stress,[2] with a strong potential to also induce endothelial dysfunction in this instance.[5] These data are in line with the results of broadband spectral analysis of arterial pressure fluctuations in elderly subjects exposed to postural sympathetic activation.[34]

No differences were observed in resting RR variance, suggesting that this simple time domain measure of heart rate variability, although reduced in patients with hypertension,[38] may be suboptimal to assess the influence of chronic real-life stress on autonomic cardiac regulation in absence of manifested cardiovascular disease.

Whether our reported changes contribute to the increased cardiovascular risk observed in chronic stress remains speculative, although our findings are in line with previous animal experiments showing a strong influence of sympathetic activation on stress-induced coronary artery disease.[3,7]

In keeping with previous observations in acute stress,[2] a strong association was also found between levels of perceived chronic stress and somatic symptoms, as assessed by 4S-Q questionnaire. Although the molecular mechanisms of this link were not examined, recent experimental data[39] suggest that stress-mediated sympathetic overstimulation might increase protein kinase C activity in central structures, such as the prefrontal lobe, leading to a dysregulation of thought, affect, or behavior. Such a frontal cortical dysfunction might interfere with individual autonomic response patterns[40] to potentially stressful situations, disturbing the interactions between cognitive appraisal and general state of health, which, in turn, reflects the interplay between genetic factors, behaviors, and lifestyle choices.[10,41] This mechanism might also be playing a role in other clinical conditions, such as orthostatic intolerance[42] or chronic fatigue,[43] in which subjective symptoms accompany alterations of autonomic regulation.

Study Limitations

In this observational study, we did not measure sympathetic nerve activity directly, which requires invasive techniques.[23] We only inferred[44] information on resting autonomic oscillatory properties and responses to an excitatory stimulus indirectly from spectral analysis of RR interval and SAP variability.[18,35]

Perspectives

Using a noninvasive, wireless approach, based on spectral analysis of short-term cardiovascular variability, we observed that chronic real-life stress in humans appears associated to an increase in arterial pressure and to impaired autonomic regulation of cardiovascular functions. Sympathetic predominance, vagal withdrawal, and baroreflex impairment might represent the autonomic counterpart of the complex psychophysiological changes underlying the increase in cardiovascular risk associated to chronic stress.[16] Optimizing the autonomic profile with behavioral or pharmacological means[45] might thus represent a testable strategy to reduce the link between psychosocial factors and future incidence of hypertension.[33,46,47]

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References

1. Julius S. Sympathetic hyperactivity and coronary risk in hypertension. *Hypertension*. 1993;21:886–893.
2. Lucini D, Norbiato G, Clerici M, Pagani M. Hemodynamic and autonomic adjustments to real life stress conditions in humans. *Hypertension*. 2002;39:184–188.

3. Rozanski A, Blumenthal JA, Saab PG, Davidson KW, Kubzanski L. The epidemiology, pathophysiology, and management of psychosocial risk factors in cardiac practice: the emerging field of behavioral cardiology. *J Am Coll Cardiol*. 2005;45:637–651.
4. Schwartz AR, Gerin W, Davidson KW, Pickering TG, Brosschot JF, Thayer JF, Christenfeld N, Linden W. Toward a causal model of cardiovascular responses to stress and the development of cardiovascular disease. *Psychosom Med*. 2003;65:22–35.
5. Ghiadoni L, Donald DE, Cropley M, Mullen JM, Oakley G, Taylor M, O'Connor G, Betterridge J, Klein N, Steptoe A, Deanfield JE. Mental stress induces transient endothelial dysfunction in humans. *Circulation*. 2000;102:2473–2478.
6. Yeung AC, Vekshtein VI, Krantz DS, Vita JA, Ryan TJ, Ganz P, Selwyn AP. The effect of atherosclerosis on the vasomotor response of coronary arteries to mental stress. *N Engl J Med*. 1991;325:1551–1556.
7. Kaplan JR, Pettersson K, Manuck SB, Olsson G. Role of sympathoadrenal medullary activation in the initiation and progression of atherosclerosis. *Circulation.* 1991;84:VI-23–VI-32.
8. Lewthwaite J, Owen N, Coats AJS, Henderson B, Steptoe A. Circulating human heat shock protein 60 in the plasma of British civil servants: relationship to psychological and psychosocial stress. *Circulation.* 2002;106:196–201.
9. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med*. 1995;332:1351–1362.
10. Everly GS Jr, Lating JM. *A Clinical Guide to the Treatment of the Human Stress Response*. 2nd ed. New York, Boston, Dordrecht, London, Moscow: Plenum Publishers; 2002.
11. Brunner EJ, Hemingway H, Walker BR, Page M, Res M, Clarke P, Juneja M, Shipley MJ, Kumari M, Andrew R, Seckl JR, Papadopoulos A, Checkley S, Rumley A, Lowe GDO, Stansfeld SA, Marmot M. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome—nested case-control study. *Circulation*. 2002;106:2659–2665.
12. Mezzacappa Sibolboro E, Kelsey MR, Katkin ES, Sloan RP. Vagal rebound and recovery from psychological stress. *Psychosom Med*. 2001; 63:650–657.
13. La Rovere MT, Specchia G, Mortara A, Schwartz PJ. Baroreflex sensitivity, clinical correlates, and cardiovascular mortality among patients with a first myocardial infarction. A prospective study. *Circulation*. 1988;78:816–824.
14. Bristow JD, Honour AJ, Pickering GW, Sleight P, Smyth HS. Diminished baroreflex sensitivity in high blood pressure. *Circulation*. 1969;39:48–54.
15. Pagani M, Mazzuero G, Ferrari A, Liberati D, Cerutti S, Vaitl D, Tavazzi L, Malliani A. Sympathovagal interaction during mental stress. A study using spectral analysis of heart rate variability in healthy control subjects and patients with prior myocardial infarction. *Circulation.* 1991;83:II-43–II-51.
16. Rosengren A, Hawken S, Ôunpuu S, Sliwa K, Zubaid M, Almahmeed W. A, Blackett KN, Chitr Sitthi-amorn, Sato H, Yusuf S. Association of psychosocial risk factors with risk of acute myocardial infarction in 11 119 case and 13 648 controls from 52 countries (The INTERHEART study): case control study. *Lancet*. 2004;364:953–962.
17. Parati G, Saul JP, Di Rienzo M, Mancia G. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation. A critical appraisal. *Hypertension*. 1995;25:1276–1286.
18. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, Turiel M, Baselli G, Cerutti S, Malliani A. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympathovagal interaction in man and conscious dog. *Circ Res*. 1986;58:178–193.
19. Ornish D, Scherwitz LW, Billings JH, Gould KL, Merritt TA, Sparler S, Armstrong WT, Ports TA, Kirkeeide RL, Hogeboom C, Brand RJ. Intensive lifestyle changes for reversal of coronary heart disease. *J Am Med Assoc*. 1998;280:2001–2007.
20. Benson H, Feldman CL. Decreased premature ventricular contractions through use of the relaxation response in patients with stable ischaemic heart-disease. *Lancet*. 1975;2:380–382.
21. *Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV)*. Washington, DC: American Psychiatric Society; 1994.
22. Parati G, Casadei R, Groppelli A, Di Rienzo M, Mancia G. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension*. 1989;13:647–655.
23. Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett CL, Somers VK. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation*. 1997;95:1441–1448.
24. Pagani M, Somers VK, Furlan R, Dell'Orto S, Conway J, Baselli G, Cerutti S, Sleight P, Malliani A. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension*. 1988;12:600–610.
25. Kroenke K. Studying symptoms: sampling and measurement issues. *Ann Intern Med*. 2001;134:844–853.
26. Simon GE, VonKorff M, Piccinelli M, Fullerton C, Ormel J. An international study of the relation between somatic symptoms and depression. *N Engl J Med*. 1999;341:1329–1335.
27. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, Levy D. Impact of high normal blood pressure on the risk of cardiovascular disease. *N Engl J Med*. 2001;345:1291–1297.
28. Bernatova I, Key M. P, Lucot J. B, Morris M. Circadian differences in stress-induced pressor reactivity in mice. *Hypertension*. 2002;40: 768–773.
29. Furlan R, Barbic F, Piazza S, Tinelli M, Seghizzi P, Malliani A. Modifications of cardiac autonomic profile associated with a shift schedule of work. *Circulation*. 2000;102:1912–1916.
30. Anderson DE, Chesney M. Gender-specific association of perceived stress and inhibited breathing pattern. *Int J Behav Med*. 2002;9:216–227.
31. van de Borne P, Montano N, Narkiewicz K, Degaute JP, Malliani A, Pagani M, Somers VK. Importance of ventilation in modulating interaction between sympathetic drive and cardiovascular variability. *Am J Physiol.* 2001;280:H722–H729.
32. Jasson S, Medigue C, Maison-Blanche P, Montano N, Meyer L, Verneiren C, Mansier P, Coumel P, Malliani A. Instant power spectrum analysis of heart rate variability during orthostatic tilt using a time-/frequency-domain method. *Circulation*. 1997;96:3521–3526.
33. Lucini D, Mela GS, Malliani A, Pagani M. Impairment in cardiac autonomic regulation preceding arterial hypertension in humans. Insights from spectral analysis of beat-by-beat cardiovascular variability. *Circulation*. 2002;106:2673–2679.
34. Parati G, Frattola A, Di Rienzo M, Castiglioni P, Mancia G. Broadband spectral analysis and blood pressure and heart rate variability in very elderly subjects. *Hypertension*. 1997;30:803–808.
35. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation.* 1991;84:482–492.
36. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation.* 1996;93:1043–1065.
37. Lown B. Clinical studies of the relation between behavioral factors and sudden cardiac death. In: Lown B, Malliani A, Prosdocimi M, eds. *Neural Mechanisms and Cardiovascular Disease.* Berlin, Germany: Liviana Press/Springer-Verlag; 1986:495–512.
38. Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension*. 1998;32:293–297.
39. Birnbaum SG, Yan PX, Wang M, Vijayraghavan S, Bloom AK, Davis DJ, Gobeske KT, Sweatt JD, Manji HK, Arnsten AFT. Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science*. 2004;306:882–884.
40. Saper CB. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Ann Rev Neurosci.* 2002;25:433–469.
41. Jeanmonod P, von Känel R, Maly FE, Fischer JE. Elevated plasma C-reactive protein in chronically distressed subjects who carry the A allele of the TNF-a -308 G/A polymorphism. *Psychosom Med.* 2004;66:501–506.
42. Narkiewicz K, Somers VK. Chronic orthostatic intolerance—part of a spectrum of dysfunction in orthostatic cardiovascular homeostasis. *Circulation*. 1998;98:2105–2107.
43. Pagani M, Lucini D. Chronic fatigue syndrome: a hypothesis focusing on the autonomic nervous system. *Clin Sci*. 1999;96:117–125.
44. Malliani A, Pagani M, Montano N, Mela GS. Sympathovagal balance: a reappraisal. *Circulation*. 1998;98:2640–2643.
45. Broadley AJM, Korszun A, Abdelaal E, Moskvina V, Jones CJH, Nash GB, Ray C, Deanfield JE, Frenneaux P. Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. *J Am Coll Cardiol*. 2005;46:344–350.
46. Everson SA, Kaplan GA, Goldberg DE, Salonen JT. Hypertension incidence is predicted by high levels of hopelessness in Finnish men. *Hypertension*. 2000;35:561–567.
47. Matthews KA, Katholi CR, McCreath H, Whooley MA, Williams DR, Zhu S, Markowitz JH. Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA study. *Circulation*. 2004;110: 74–78.