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Increased Sympathetic and Decreased Parasympathetic Activity Rather Than Changes in Hypothalamic-Pituitary-Adrenal Axis Activity Is Associated with Metabolic Abnormalities

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Journal of Clinical Endocrinology & Metabolism, 2010, 95(5):2458-2466

ABSTRACT

Context: Stress is suggested to lead to metabolic dysregulations as clustered in the metabolic syndrome, but the underlying biological mechanisms are not yet well understood.

Objective: We examined the relationship between two main stress systems, the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, with the metabolic syndrome and its components.

Design and Setting: The design was baseline data (years 2004–2007) of a prospective cohort: The Netherlands Study of Depression and Anxiety (NESDA). The study comprised general community, primary care, and specialized mental health care.

Participants: This study included 1883 participants aged 18–65 years.

Main Outcome Measures: Autonomic nervous system measures included heart rate, respiratory sinus arrhythmia (RSA; high RSA reflecting high parasympathetic activity), and pre-ejection period (PEP; long PEP reflecting low sympathetic activity). HPA axis measures included the cortisol awakening response, evening cortisol, and a 0.5 mg dexamethasone suppression test as measured in saliva. Metabolic syndrome was based on the updated Adult Treatment Panel III criteria and included high waist circumference, serum triglycerides, blood pressure, serum glucose, and low high-density lipoprotein cholesterol.

Results: RSA and PEP were both independently negatively associated with the presence of the metabolic syndrome, the number of metabolic dysregulations as well as all individual components except high-density lipoprotein cholesterol (all $p<.02$). Heart rate was positively related to the metabolic syndrome, the number of metabolic dysregulations, and all individual components (all $p<.001$). HPA axis measures were not related to metabolic syndrome or its components.

Conclusion: Our findings suggest that increased sympathetic and decreased parasympathetic nervous system activity is associated with metabolic syndrome, whereas HPA axis activity is not.

INTRODUCTION

It has often been hypothesized that stress leads to metabolic dysregulations[1–3]. In response to stress, two main stress systems, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, are both centrally activated[2,3]. Persistent (over)activation of these stress systems could lead to metabolic alterations, such as high blood pressure, serum triglycerides, serum glucose, waist circumference, and low high- density lipoprotein (HDL) cholesterol[3–5]. The metabolic syndrome consists of a cluster of these metabolic abnormalities and predisposes to cardiovascular disease[6,7] and diabetes[8]. Whether both stress systems are associated with the metabolic syndrome has only partially been examined[5,9,10].

Some studies have shown evidence for a role of ANS dysfunction in the metabolic syndrome. For sympathetic nervous system (SNS) activity, measured by, for example, muscle sympathetic nerve activity, elevated levels were found in subjects with metabolic syndrome[11,12]. However, Grassi *et al.*[12] showed that different measures of SNS activity show divergent associations with the metabolic syndrome; therefore, evidence for the relationship between purely sympathetic activity and the metabolic syndrome remains ambiguous and cannot be considered conclusive. More evidence is present for a negative relationship between parasympathetic nervous system (PNS) activity and the metabolic syndrome[13–15], although inconsistencies have been found. For example, PNS activity (as reflected by the high frequency spectra of heart rate variability) was unassociated[14,16] as well as negatively associated with having the metabolic syndrome[13, 15]. Studies have also shown inconsistent results for the association of PNS activity with various metabolic dysregulations[13–15]. In addition, some studies were limited by rather short periods of physiological recordings or no consideration of cardiovascular disease and cardiac medication[13–15].

Cortisol measured in saliva is considered a reliable and non-invasive indicator of HPA axis activity[17]. Although there are several studies that examined the association between salivary cortisol and the metabolic syndrome or its components, the relationship is still not elucidated. Results are inconsistent concerning the direction of the relationship as well as the aspect of the cortisol diurnal rhythm that might be involved. For instance, no[18,19], negative[20,21], and positive[22,23] associations have been reported between salivary morning cortisol or cortisol awakening response and components of the metabolic syndrome. Studies

specifically examining evening cortisol and metabolic syndrome components are scarce, mostly reporting no association[18,21]. Results regarding cortisol suppression after dexamethasone ingestion showed less suppression after dexamethasone to be associated with hypertension[21] and all other metabolic syndrome components[20], whereas Putignano *et al.*[19] reported no association with obesity. However, previous studies were rather small, measured morning cortisol by only one salivary sample, or did not adjust for important covariates such as sleep duration and awakening time.

Therefore, we examined the association between metabolic syndrome and its components with multiple extensive measures of both ANS and HPA axis activity in a large cohort study considering important covariates to explore to what extent both stress systems are involved in metabolic abnormalities.

SUBJECTS AND METHODS

Study sample

Data are from the Netherlands Study of Depression and Anxiety (NESDA), a large longitudinal cohort study among 2981 adults (18 – 65 years old), 95.2% of North European ancestry (see[24]). Respondents were recruited from the community, in primary care through a screening procedure conducted among 65 general practitioners, and in specialized mental health care when newly enrolled at one of the 17 participating mental health organization locations. The research protocol was approved by the ethical committee of participating universities and all respondents provided written informed consent. Of the total sample, we excluded 80 persons using tricyclic antidepressants because of their effect on the ANS[25], HPA axis[26], and metabolic syndrome[27]. Of the 2901 remaining participants, we excluded 27 pregnant or breast-feeding women and 158 participants on corticosteroids because of their effects on the HPA axis, leaving a sample of 2716 respondents. Of 109 participants, no ANS data were available, another 695 did not return (sufficient) saliva samples for HPA axis activity assessment, and of 29 persons data on metabolic abnormalities were missing. Therefore, the present study sample consisted of 1883 participants. Participants in the present study sample (n=1883) did not differ from the excluded participants (n=1098) in presence of metabolic syndrome (21.1 vs. 23.4%, p=.17) or cardiovascular disease (5.8 vs. 7.1%, p=.18) but were less often female (64.9 vs. 68.9%, p=.02), older (43.0 vs. 39.9

years, $p<.001$), and more educated (12.4 vs. 11.7 years, $p<.001$).

Outcome measures

Metabolic syndrome

The metabolic syndrome was defined according to the American Heart Association and National Heart, Lung, and Blood Institute's update of the U.S. National Cholesterol Education Program-Adult Treatment Panel III criteria[28]. The National Cholesterol Education Program-Adult Treatment Panel III guidelines define metabolic syndrome as a presence of three or more of the following criteria: 1) waist circumference 102 cm or greater in men and 88 cm or greater in women, 2) triglycerides 1.7 mmol/liter or greater (150 mg/dl) or medication for hyper-triglyceridemia, 3) HDL cholesterol less than 1.03 mmol/liter (40 mg/dl) in men and less than 1.30 mmol/liter (50 mg/dl) in women or medication for reduced HDL cholesterol, 4) systolic blood pressure (SBP) 130 mm Hg or greater and/or diastolic blood pressure 85 mm Hg or greater or antihypertensive medication, and 5) fasting plasma glucose ≥ 5.6 mmol/liter (100 mg/dl) or antidiabetic medication. The number of metabolic syndrome components was used as an indicator of severity of metabolic abnormalities[27].

Metabolic syndrome components

In addition to metabolic syndrome, associations with continuous levels of individual metabolic components were examined to investigate consistency across components. Waist circumference was measured with a measuring tape at the central point between the lowest front rib and the highest front point of the pelvis on light clothing. Triglycerides, HDL cholesterol, and glucose were determined using routine standardized laboratorial methods. To incorporate medication use into the continuous variable, for persons using antidiabetic medication when glucose level was less than 7.0 mmol/liter (126 mg/dl), a value of 7.0 mmol/liter (126 mg/dl) was assigned[28]. Similarly, for persons using fibrates, 0.10 mmol/liter (3.8 mg/dl) was subtracted from HDL cholesterol and 0.67 mmol/liter (60 mg/dl) was added to triglycerides[28]. For persons using nicotinic acid, 0.15 mmol/liter (5.8 mg/dl) was subtracted from HDL cholesterol and 0.19 mmol/liter (17 mg/dl) added to triglycerides, based on mean changes after medication treatment[28]. SBP and diastolic blood pressure were measured twice during supine rest on the right arm with the Omron M4-I, HEM 752A, and were averaged over the two measurements. For persons using antihypertensive medication, 10 mm Hg was

added to the SBP[29].

Measurements

ANS

During the visit to the research centres, The Netherlands Study of Depression and Anxiety subjects were wearing the VU University Ambulatory Monitoring System. The VU-AMS is a light-weight, unobtrusive device that records the electrocardiogram (ECG) and changes in thorax impedance (dZ) from six surface electrodes placed at the chest and on the back of the subjects[30,31]. The interbeat interval time series was extracted from the ECG signal to obtain heart rate, an indicator of combined SNS and PNS activity. To separately index the cardiac effects of both ANS branches, pre-ejection period (PEP; long PEP reflects low SNS activity) and respiratory sinus arrhythmia (RSA; high RSA reflects high PNS activity) were extracted from the combined dZ and ECG signals. The PEP reflects noradrenergic inotropic drive to the left ventricle and was obtained from the dZ/dt signal, ensemble averaged across 1-minute periods time locked to the R-wave of the ECG. The PEP was defined as the interval from the B point (upstroke) to the X point (incisura) of the dZ/dt signal, as described in detail elsewhere[31]. The RSA reflects cardiac parasympathetic activity and was obtained by combining the interbeat interval time series with the filtered (0.1-0.4 Hz) dZ signal, which corresponds to the respiration signal. RSA was obtained by subtracting the shortest interbeat interval (IBI) during heart rate acceleration in the inspirational phase from the longest IBI during deceleration in the expirational phase for all breaths, as described in detail elsewhere[30]. Automated scoring of IBI, RSA, and PEP was checked by visual inspection, and valid data were averaged over 90.2 ± 23 minutes to create a single PEP, RSA, and heart rate value. To additionally investigate whether patterns of sympathetic and parasympathetic co-activation or parallel activation/inhibition were related to the metabolic syndrome, two measures of autonomic balance were acquired following the approach of Berntson *et al.*[32]. Cardiac autonomic balance (CAB) was calculated as the difference between normalized values of RSA and PEP [formula= zRSA - (-zPEP) (because increased sympathetic control is associated with shortened PEP values, PEP was multiplied by -1)] such that low values reflect parallel high sympathetic and low vagal cardiac control (unfavourable cardiac pattern) and high values reflect low sympathetic and high vagal cardiac control (favourable cardiac pattern). Cardiac autonomic regulation (CoAR) was calculated as the sum of the normalized values of RSA and

PEP [formula = zRSA + (-ZPEP)] and low values represent co-inhibition (low SNS and low PNS activity) and high values co-activation (high SNS and high PNS activity) of the two cardiac autonomic branches.

HPA axis

As described in more detail elsewhere[33], respondents were instructed to collect saliva samples at home on a regular (working) day, which has shown a reliable and minimally intrusive method to assess the active, unbound form of cortisol[17]. The median time between the interview and saliva sampling was 9.0 d (25th to 75th percentile: 4-22). Saliva samples were obtained using Salivettes (Sarstedt, Germany) at seven time points. The cortisol awakening response includes four sampling points; at awakening (T1) and 30 (T2), 45 (T3), and 60 (T4) min later. Two evening values were collected at 2200 h (T5) and 2300 h (T6). Dexamethasone suppression was measured by cortisol sampling the next morning at awakening (T7) after ingestion of 0.5 mg dexamethasone directly after the saliva sample at 2300 h (T6). Samples were stored in refrigerators and returned by mail. After receipt, Salivettes were centrifuged at 2000 g for 10 minutes, aliquoted, and stored at −80°C. Cortisol analysis was performed by competitive electrochemiluminescence immunoassay (E170; Roche, Basel, Switzerland), as described in Van Aken *et al.*[34]. The functional detection limit was 2.0 nmol/liter and the intra- and interassay variability coefficients in the measuring range were less than 10%. Data cleaning excluded values greater than 2 SD above the mean (i.e. above 59.6 –123.6 nmol/liter for T1– T4, 40.9 nmol/liter for T5, 59.8 nmol/liter for T6, and 35.6 nmol/liter for T7).

One-hour awakening cortisol: The area under the curve with respect to the increase (AUCi) and ground (AUCg) were calculated using the formulas described by Pruessner *et al.*[35]. The AUCg is an estimate of the total cortisol secretion over the first hour after awakening, and the AUCi is a measure of the dynamic of the cortisol awakening response, more related to the sensitivity of the system, emphasizing changes over time[36]. For area under the curve calculations, all four morning samples were required (n=1584).

Evening cortisol: Because the correlation between the two evening values was high (r = 0.52, p<.001), the mean of the two values was used for analyses to reflect evening cortisol (n=1871).

Dexamethasone suppression test: A total of 1712 of the 1781 subjects with cortisol sample T1 and T7 (96.1%) had taken the 0.5 mg dexamethasone after 2300 h

on the first sampling day. We used a cortisol suppression ratio calculated by cortisol value at awakening on the first day (T1) divided by cortisol value at awakening the next day (T7) after ingestion of 0.5 mg dexamethasone the evening before.

Covariates

Sociodemographic factors included sex, age, and years of attained education. Use of oral contraceptives (yes/no) and menopause (yes/no) were identified by self-report. Smoking status was categorized into never smoked, former smoker, and current smoker. Daily alcohol use was categorized into no, mild to moderate (maximum 2 alcohol consumptions a day), and heavy (more than 2 alcohol consumptions a day). Physical activity was assessed by the International Physical Activity Questionnaire[37] and expressed in 1000 metabolic equivalent minutes in the past week. Cardiovascular disease (including coronary disease, cardiac arrhythmia, angina, heart failure, and myocardial infarction) was ascertained by self-report. Furthermore, it was determined whether subjects were using heart medication by copying the names of medicines from the containers brought in by the subjects. Using the World Health Organization's anatomical therapeutic chemical (ATC) classification, medication was classified. Use both of 1-blockers (ATC code C07, used daily or more than 50% of the time) and other heart medication [ATC codes C01 (cardiac therapy), C02 (antihypertensives), C03 (diuretics), C04 (peripheral vasodilators), C05 (vasoprotectives), C08 (calcium channel blockers), or C09 (renin and angiotensin agents)] was ascertained. Additionally, for analyses with cortisol measures, sampling factors that have been shown to influence cortisol measures by Vreeburg *et al.*[33] were included. Respondents reported time of awakening and working status on the sampling day. Season was categorized into dark months (October through February) and months with more daylight (March through September). Average sleep duration during the last week was assessed using the Insomnia Rating Scale[38] and dichotomized into sleeping more or less than six hours a night.

Statistical analyses

Baseline characteristics were compared across metabolic syndrome status using χ^2 and ANOVA statistics. Partial correlation coefficients (adjusting for age, sex, and education) between ANS and cortisol measures were calculated to examine the intercorrelations between both stress systems. Multiple logistic

Table 1. Sample Characteristics for Individuals With and Without the Metabolic Syndrome

	Participants, %		
	Metabolic Syndrome		
	No (n=1484)	Yes (n=399)	*p*[a]
Sociodemographics			
Age, mean (SD), y	41.1 (13.0)	50.4 (10.1)	<.001
Female sex	68.5	51.6	<.001
Education, mean (SD), years	12.7 (3.2)	11.3 (3.3)	<.001
Health factors			
Physical activity, mean (SD), 1000 MET min/week	3.7 (3.0)	3.6 (3.0)	.36
Smoking			
Non-smoker	32.0	22.6	
Former smoker	35.2	45.1	<.001
Current smoker	32.8	32.3	
Alcohol use			
Non drinker	14.4	19.5	
Mild/moderate drinker	69.9	61.2	.003
Heavy drinker	15.7	19.3	
Use beta-blockers	4.3	22.1	<.001
Use other heart medication	5.7	30.3	<.001
Use of oral contraceptives	19.5	6.8	<.001
Post-menopausal women	18.7	26.6	.001
Cardiovascular disease	5.7	11.5	<.001
Time of awakening, mean (SD), h:min	7:31 (1:16)	7:19 (1:06)	.006
Working on day of sampling	63.4	56.4	.01
Sampling in month with more day light	58.6	57.9	.79
≤ 6 hours of sleep	24.7	32.3	.002

Table 1. Continued

Autonomic measures			
Respiratory Sinus Arrhythmia, mean (SD), ms	46.1 (24.5)	32.2 (19.8)	<.001
Heart rate, mean (SD), beats/min	71.1 (9.3)	72.0 (10.5)	.06
Pre-ejection Period, mean (SD), ms	122.9 (16.2)	119.8 (21.9)	.005
Cardiac Autonomic Balance, mean (SD)	0.158 (1.33)	-0.593 (1.57)	<.001
Cardiac Autonomic Regulation, mean (SD)	0.053 (1.33)	-0.323 (1.42)	<.001
HPA-axis measures			
AUCg, mean (SD), nmol/l/h	19.0 (7.1)	19.6 (6.9)	.18
AUCi, mean (SD), nmol/l/h	2.4 (6.2)	2.1 (6.6)	.48
Mean evening level, mean (SD), nmol/l	5.4 (3.5)	5.6 (3.0)	.31
Cortisol suppression ratio[b], mean (SD)	2.9 (1.7)	2.7 (1.6)	.10
Continuous measures of the Metabolic Syndrome			
Waist circumference, mean (SD), cm	85.0 (11.2)	103.3 (11.8)	<.001
Systolic blood pressure, mean (SD), mmHg	133.0 (17.8)	151.8 (19.4)	<.001
Glucose, mean (SD), mmol/l[c]	4.9 (1.1)	5.9 (1.2)	<.001
HDL cholesterol, mean (SD), mmol/l	1.7 (0.4)	1.3 (0.4)	<.001
Triglycerides, mean (SD), mmol/l[c]	1.0 (1.5)	1.8 (1.6)	<.001

AUCg/i, area under the curve with respect to the ground/increase; HDL, high density lipoproteins; MET, metabolic energy turnover
[a] Based on χ^2 and ANOVA-statistics for dichotomous or categorical and continuous measures respectively.
[b] Cortisol suppression ratio= cortisol T1/ cortisol T7 after 0.5 mg dexamethasone
[c] Glucose and triglyceride levels are back-transformed

regression analyses were conducted with ANS measures (i.e. heart rate, RSA, or PEP) and salivary cortisol measures (i.e. AUCg, AUCi, evening cortisol, or cortisol suppression ratio) as independent variables and metabolic syndrome as the dependent variable. Multiple linear regression, adjusted for all covariates, was used to analyze the association of ANS and salivary cortisol measures with either the number of metabolic syndrome components (0-5) or continuous individual metabolic syndrome components as dependent variables. All metabolic syndrome components were normally distributed, except for triglycerides and glucose levels, which were log transformed before analyses. If linear regression with the number of metabolic syndrome components yielded significant results; fully corrected analysis of covariance analyses were performed to compare the mean ANS and HPA axis values of persons with increasing number of metabolic syndrome components (i.e. 0, 1, 2, 3, 4, and 5) and investigate linearity. $p \leq .05$ was regarded as statistically significant. All analyses were conducted using SPSS version 15.0 (SPSS, Chicago, IL).

RESULTS

In our sample, 21.2% of the subjects met the criteria for the metabolic syndrome; 25.1% met none of the criteria, 31.3% one, 22.4% two, 12.9% three, 6.4% four, and 1.9% all five criteria. Sample characteristics are presented in **Table 1**. Persons with the metabolic syndrome were more likely to be male, older, and less educated, a non-drinker or heavy drinker, a former smoker, using heart medication, or having prevalent cardiovascular disease and were less likely to be using oral contraceptives than persons without the metabolic syndrome. Persons with the metabolic syndrome showed on average a lower RSA, CAB, and CoAR, higher heart rate, and shorter PEP, whereas no differences were seen in cortisol measures, except for a trend toward less suppression after dexamethasone.

Table 2 shows the results of the partial correlations between HPA axis measures and ANS measures adjusted for age, sex, and education. In contrast to an expected intercorrelatedness because of shared central activation of both stress systems, ANS measures did not significantly correlate with HPA axis measures (all $p > .11$).

After full adjustment, RSA, heart rate, and PEP were significantly related to the metabolic syndrome as well as the number of metabolic abnormalities (**Table 3 and Fig. 1**). The odds for the metabolic syndrome increased when RSA

and PEP decreased, indicating that decreased parasympathetic and increased sympathetic activity are associated with increased likelihood of metabolic syndrome. Lower RSA and PEP were also associated with the number of metabolic syndrome components present (Table 3 and Fig. 1).

Table 2. Correlations Coefficients of Partial Correlation between HPA-axis and ANS Measures[a]

	AUCg, nmol/l/h	AUCi, nmol/l/h	Evening Cortisol, nmol/l/h	Suppression ratio
RSA, ms	-.031	-.027	-.016	-.010
HR, beats/min	.040	.038	.020	.039
PEP, ms	.037	.006	.046	-.038
CAB	.020	-.002	.043	-.037
CoAR	-.039	-.013	-0.030	.041

[a]Adjusted for age, sex and education.

A higher heart rate was associated with increased odds for metabolic syndrome and an increase in number of metabolic syndrome components. None of the HPA axis measures was associated with the metabolic syndrome or with the number of its components (Table 3).

Table 3. Adjusted[a] Associations between the Stress Systems (per 10 Units Increase) and Metabolic Syndrome and Number of Metabolic Syndrome Components.

	Metabolic syndrome		Number of metabolic syndrome components	
	OR (95% CI)	*p*	β	*p*
Autonomic nervous system				
RSA, per 10 ms increase	0.81 (0.74-0.90)	<.001	-.110	<.001
HR, per 10 bpm increase	1.72 (1.46-2.02)	<.001	.220	<.001
PEP, per 10 ms increase	0.87 (0.80-0.94)	<.001	-.132	<.001
CAB, per 1 unit increase	0.75 (0.67-0.84)	<.001	-.163	<.001
CoAR, per 1 unit increase	1.06 (0.94-1.20)	.31	.045	.12
HPA axis				
AUCg, per 10 nmol/l/h increase	1.07 (0.88-1.29)	.50	.008	.72
AUCi, per 10 nmol/l/h increase	1.05 (0.85-1.30)	.65	-.003	.87
Evening cortisol, per 10 nmol/l increase	0.84 (0.57-1.23)	.36	-.012	.56

[a]Based on logistic and linear regression analyses adjusted for age, sex, education, oral contraceptive use, menopause, cardiovascular disease, physical activity, smoking, alcohol use, use of beta-blockers and other heart medication. For HPA axis, analyses are additionally adjusted for working, awakening time, season and sleep

Table 4 shows the associations of ANS and HPA axis measures with the different continuous metabolic syndrome components. Again, salivary cortisol measures

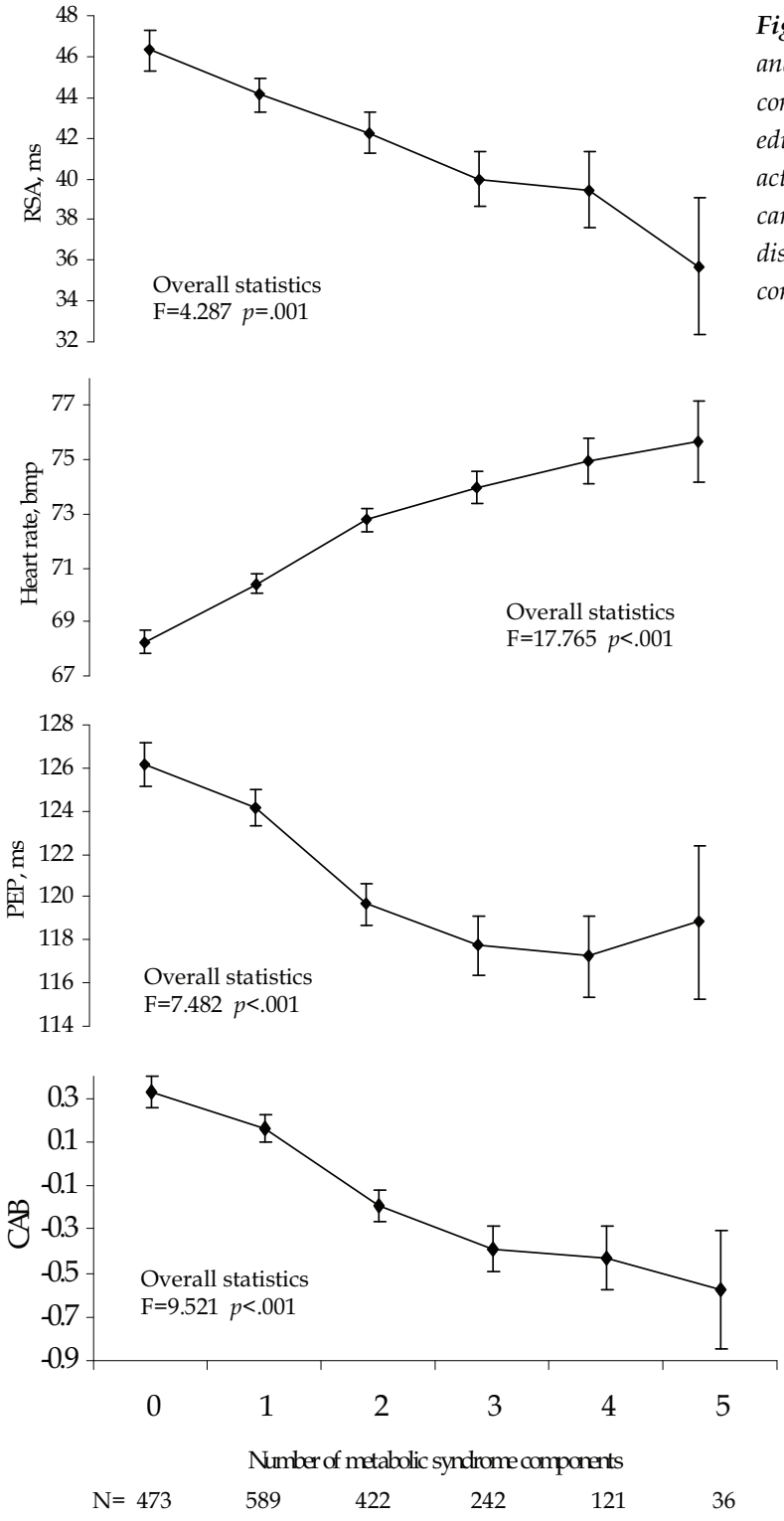

Figure 1. *Mean adjusted RSA, HR, PEP and CAB for the number of MetSyn components. Corrected for age, sex, education, alcohol use, smoking, physical activity, use of betablocking agents, other cardiac medication, cardiovascular disease, menopause and use of oral contraceptives*

were not significantly related to the continuous metabolic syndrome components. However, RSA (PNS activity) and PEP (sympathetic drive) were negatively associated with waist circumference (β=-0.078, p=.005 and β=-0.143, p<.001, respectively), triglycerides (β=-0.092, p=.002 and β=-0.081, p=.001, respectively),

and SBP (β=-0.111, *p*<.001 and β=-0.115, *p*<.001, respectively). RSA was also negatively associated with glucose (β=-0.066, *p*=.02). Heart rate was positively associated with waist circumference (β=0.111, *p*<.001), triglycerides (β=0.186, *p*<.001), SBP (β=0.150, *p*<.001), and glucose (β=0.140, *p*<.001) and negatively associated with HDL cholesterol (β=-0.062, *p*=.01). When we performed a multivariable analysis in which PEP and RSA were entered together, the odds ratios (ORs) and βs remained largely similar to the separate univariable analyses [e.g. for the metabolic syndrome, RSA OR=0.83 higher CAB was negatively associated with the number of metabolic dysregulations and all individual components of the metabolic syndrome (except for HDL cholesterol). The CoAR, reflecting SNS and PNS co-activation, did not associate with any of the metabolic measures.

Table 4. Adjusted[a] Associations between the Stress Systems and the Individual Components of the Metabolic Syndrome

	Waist circumference		Log Triglycerides		HDL cholesterol		SBP		Log Glucose	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
ANS										
RSA, ms	-.078	.005	-.092	.002	.003	.93	-.111	<.001	-.066	.02
HR, bpm	.111	<.001	.186	<.001	-.062	.01	.151	<.001	.140	<.001
PEP, ms	-.143	<.001	-.081	.001	.031	.20	-.115	<.001	-.031	.20
CAB	-.155	<.001	-.113	<.001	.023	.36	-.150	<.001	-.056	.02
CoAR	.083	.01	.019	.49	-.030	.26	.038	.12	-.011	.69
HPA axis										
AUCg, nmol/l	-.028	.21	.024	.31	.021	.36	.000	.99	-.015	.52
AUCi, nmol/l	-.039	.07	.013	.57	-.015	.50	-.014	.50	-.016	.47
Evening cortisol,	-.023	.25	.029	.18	.002	.92	.035	.08	-.014	.53
Suppression ratio	.023	.25	.003	.91	-.005	.83	-.014	.49	.005	.81

[a] Based on linear regression analyses adjusted for age, sex, education, oral contraceptive use, menopause, cardiovascular disease, physical activity, smoking, alcohol use, use of beta-blockers and other heart medication. For HPA axis, analyses are additionally adjusted for working, awakening time, season and sleep

DISCUSSION

In this large study, we found that decreased PNS and increased SNS activity were associated with metabolic syndrome and its components, whereas HPA axis measures were not. The activity of the ANS and HPA stress systems was

not correlated. These results suggest that in contrast to HPA axis dysregulation, ANS dysregulation is strongly associated with metabolic syndrome and might therefore partly be involved in its unfavourable consequences such as the incidence of cardiovascular disease. The association of low PNS activity with the metabolic syndrome corroborates the findings of some groups[13,15] and contrasts with other groups reporting no association. Previous studies had also reported on the associations between measures of SNS activity and metabolic syndrome, but the evidence was scarce and results were inconsistent[10,12]. The present study provides consistent evidence for an association of increased SNS activity (i.e. shorter PEP) with the metabolic syndrome and individual metabolic components. Although the effects of PEP and RSA on metabolic syndrome and its components were partly independent, a pattern of parallel high SNS and low PNS activity was most strongly associated with the metabolic syndrome. In contrast, a pattern of low SNS activity and low PNS activity or high SNS activity with high PNS activity did not show association with the metabolic syndrome. Our findings are strikingly congruent to results of Berntson *et al.*[32], who reported a similar relationship between ANS and diabetes. Taken together, these results suggest that especially the combination of increased SNS activity and decreased PNS activity is related to the metabolic syndrome, whereas high SNS activity in the presence of high PNS activity or low PNS activity in the presence of low SNS activity are not.

In line with several studies[10,18], we found no relationship between salivary HPA axis measures and the metabolic syndrome or its components. These results suggest that the HPA axis is not dysregulated in persons with metabolic syndrome. Most studies that did find associations between salivary cortisol measures and several metabolic syndrome components were not comparable with our study because they studied solely men, used small samples, included only obesity measures, or used just one or two morning samples[22,23]. Important work has been done by Rosmond *et al.*[39], who reported that in men, a reduced variation in the diurnal cortisol pattern was associated with metabolic dysregulations and predicted higher risk of cardiovascular events after five years. However, it is unclear how this abnormal cortisol pattern relates to our salivary cortisol measures. Other studies have found metabolic syndrome to be more frequently accompanied by increased urinary cortisol rather than plasma or saliva cortisol (e.g.[10]), which could be a result of increased cortisol excretion in combination with increased metabolism. Alternatively, HPA axis hyperresponsiveness after corticotrophin releasing hormone (CRH) stimulation[40] or acute stress might be

more strongly related to metabolic abnormalities, whereas basal activity remains intact.

It is a general belief that the autonomic nervous system and the HPA axis stress systems are highly intertwined[41] because both systems are centrally activated in response to stress, e.g. by the hypothalamus. In addition, both stress systems arouse each other: CRH, which drives HPA axis activity, also seems to stimulate sympathetic flow[42], and central catecholamines, an ANS marker, seem to stimulate the HPA axis[43]. Although many hypotheses linking the two systems have emerged, previous studies directly correlating ANS and HPA axis measures under resting conditions are scarce. Our results suggest that both systems do not correlate very strongly, and only ANS activity is associated with an unfavourable metabolic state. Both stress systems are responsive and dynamic systems with different temporal courses. Previous studies showed that ANS activity remained high after repeated stress, whereas the HPA axis was desensitized and did not respond with hyperactivity[44], which might explain why the ANS and HPA axis did not correlate in our study. In addition, results of a study on the metabolic syndrome in relation to HPA axis and ANS measures in a sample of 180 men[10] are in accordance with ours; strong associations between ANS measures and the metabolic syndrome were reported, whereas HPA axis measures were not associated. Finally, it is possible that correlations between the ANS and the HPA axis become more apparent in response to acute stress but are lower when subjects are not experiencing acute stress, such as in our study.

Our study had several strengths, including a large sample size and multiple measures of the HPA axis and sympathetic as well as parasympathetic control. In addition, it was presented that a specific pattern of parallel decrease in parasympathetic and increase in sympathetic control was most strongly associated with metabolic dysregulations and the metabolic syndrome. Furthermore, all components that constitute the metabolic syndrome were separately analyzed, and the intercorrelation of both stress systems was investigated. Finally, our sample size enabled us to consider important covariates. However, some limitations have to be acknowledged as well. First, because analyses were cross-sectional, our results do not indicate any causal direction of the associations found. Future longitudinal studies are warranted to further examine the relationship between the HPA axis, the ANS, and metabolic dysregulations. Second, non-compliance with cortisol sampling could have occurred because it was logistically and financially not feasible to electronically monitor compliance.

To conclude, although the ANS was strongly associated with metabolic syndrome and its individual components, the HPA axis was not. In particular, a parallel decrease in parasympathetic and increase in sympathetic control were associated with metabolic dysregulations and could therefore have an important role in its higher risk of cardiovascular disease.

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