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ORIGINAL ARTICLE

Sex Differences in the Prediction of Metabolic Burden from Physiological Responses to Stress

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Abstract
Background Heightened or prolonged physiological responses to stress may contribute to the development or progression of metabolic abnormalities.
Purpose This study aims to examine the prospective relationships between stress responses and metabolic burden, and to determine whether age and/or sex moderate these relationships.
Methods One hundred ninety-nine healthy men and women (M_{age}=41±11.5) were exposed to four stressors while blood pressure, heart rate, and heart rate variability were obtained. Residual change scores for reactivity (stress−baseline) and recovery (post-stress−baseline) scores were computed. Metabolic burden refers to the number of metabolic parameters for which participants were in the highest quartile (lowest for high-density lipoprotein cholesterol) for their sex. Metabolic burden was reassessed in 136 participants 3 years later.
Results Greater parasympathetic withdrawal in response to stress was associated with increased metabolic burden, though this was evident mostly in men. In women, dampened autonomic responses to stress were associated with higher metabolic burden.
Conclusions Cardiac autonomic responses to stress predict future metabolic abnormalities, though the direction of effect differs according to sex.

Keywords Stress · Physiological reactivity/recovery · Metabolic burden · Individual differences · Prospective

One of every five Canadians is diagnosed with metabolic syndrome, with prevalence rates reaching 40 % at 60 years of age [1, 2]. Metabolic syndrome refers to a cluster of interconnected factors that incurs significant risk for atherosclerosis, type II diabetes, and increased mortality from cardiovascular diseases [3–6]. It typically refers to the presence of at least three of the following: central adiposity, high blood pressure, elevated triglycerides and/or fasting blood glucose, and low levels of high-density lipoprotein (HDL) cholesterol [3, 7]. Psychological variables, such as hostility, appear to contribute to individual and combined parameters of metabolic syndrome [8–11]. This may occur, in part, as a result of heightened physiological responses to stress associated with these psychological variables [12].

The relationship between physiological responses to psychological stress and a global representation of metabolic syndrome has yet to be investigated. Some research exists, however, examining stress responses and the individual components of metabolic syndrome. For instance, a recent meta-analysis found hypertension to be consistently predicted by increased cardiovascular reactivity (CVR) to stress and poorer recovery from stress [13]. To our knowledge, the relationship between fasting blood glucose level and CVR and/or recovery from stress has not been examined, and the sparse research on other parameters of metabolic syndrome has yielded mixed findings. While some studies have reported heightened

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reactivity to laboratory stress in individuals with elevated total cholesterol and/or triglyceride levels [2, 14–17], others have reported no or opposite associations depending on the age and sex of the participants [18, 19]. Confusing the situation further, in these studies, stress responses have often been examined as outcomes of metabolic abnormalities rather than their predictors. The relationship between cardiovascular responses to stress and central adiposity has also yielded mixed results [20–23]. For example, in a cross-sectional study of middle-aged men and women, body mass index (BMI) and waist-to-hip ratio were both associated with delayed systolic (SBP) and diastolic (DBP) blood pressure recovery from stress but not with heightened CVR [24]. Delayed SBP recovery also predicted increases in waist–hip ratio after a 3-year follow-up in men but not in women [24]. In contrast, Carroll et al. [25] found that greater heart rate (HR) reactivity to a cognitive task was associated with less central adiposity in a large community sample of men and women. These associations were maintained prospectively, with high HR reactivity predicting a reduced likelihood of becoming obese over a 5-year follow-up [25]. Similar results were obtained in another independent sample [26].

Methodological differences relating to sample characteristics may explain some of the inconsistencies in the literature. While few investigations have specifically examined the moderating effects of sex and/or age on results obtained, there is some limited data to suggest that these may be of import [19, 24]. Given that older adults and men suffer disproportionately from metabolic syndrome in Canada [27] and exhibit heightened CVR to stress as compared to younger individuals or women (especially to cognitive- and performance-oriented tasks) [25, 28–30], it is possible that age and/or sex may similarly moderate the relationship between stress responses and metabolic syndrome.

It has been proposed that prolonged physiological arousal following exposure to stress may more greatly contribute to allostatic load and resulting disease outcomes than CVR [31, 32]. In support of this, Steptoe and Marmot [33] reported that delayed SBP and DBP recovery from stress predicted increases in blood pressure after a 3-year follow-up more consistently than heightened CVR during stress. However, few studies have examined the relationships between recovery responses and metabolic syndrome parameters [13, 24, 33].

Finally, no research has specifically examined the relationships between autonomic responses to psychological stress and metabolic syndrome parameters. Yet, the autonomic nervous system (ANS) plays a key role in the regulation of metabolism, with major organs involved in metabolism (e.g., heart, brain, liver, pancreas) innervated by the sympathetic and parasympathetic nervous systems [34]. Importantly, disruptions of the ANS have been associated with the development of individual or combined parameters of metabolic syndrome [35–38].

In sum, physiological responses to stress may contribute to metabolic abnormalities, although conflicting findings have been reported, depending on sample characteristics and metabolic measure. To date, no investigations have examined the relationships between stress responses and metabolic syndrome. Yet, the importance of metabolic syndrome to cardiovascular and related outcomes, and its independence of its individual components have repeatedly been shown [39–43]. The objective of the current study was to examine the prospective relationships between stress responses to a laboratory protocol and a global index of metabolic dysfunction, herein referred to as metabolic burden, in a healthy adult sample of men and women. Healthy participants were sought to minimize the impact of disease processes or medications on stress responses and their relation with metabolic dysfunction. However, this rendered assessing metabolic syndrome unfeasible, as its prevalence in the sample was low. Metabolic burden was therefore defined as the number of metabolic syndrome parameters for which participants were in the highest quartile (lowest for HDL) for their sex. Such count-based summary measures have been shown to be predictive of a larger spectrum of health outcomes than individual parameters [44]. Reactivity and recovery were examined for HR, BP, and for indices of ANS responses to stress obtained via the analysis of heart rate variability (HRV) [45]. Finally, the moderating effect of age and sex on the associations between stress responses and metabolic burden was evaluated. It was hypothesized that heightened reactivity to and delayed recovery from stress would contribute to increased metabolic burden prospectively, particularly in men and older adults.

Methods

This prospective study was part of a larger project that sought to examine the association of psychological and psychophysiological variables with intermediary coronary artery disease risk factors (for examples of data published from this project, see [11, 46–50]).

Time 1

Participants

One hundred and ninety-nine healthy adult men (n=81) and women (n=118) were recruited through advertisements in newspapers and community centers within the greater Montreal area. Individuals were excluded from the study if they (a) used any mental health services within the past year; (b) had any current/diagnosed health problems (for example, asthma, hypertension, diabetes, hypercholesterolemia, heart disease, cancer, autoimmune disorders, disorders of the adrenal gland) or were taking medication (for example, statins, beta-blockers,

anti-inflammatory) capable of affecting cardiovascular, immune, or neuroendocrine functions; (c) had any cognitive disabilities rendering them unable to complete questionnaires or understand instructions; and (d) were undergoing any form of hormone replacement therapy. Individuals were interviewed by phone to ensure that they met the criteria before they were invited to participate. Similar numbers of participants were selected from three age groups (18–34, 35–44, and 45–65 years) to ensure a broad age range. Women were over-sampled to include a substantial number of post-menopausal women (n= 34) for a separate component of the study not discussed here. Complete data for these analyses were obtained for 193 participants (78 men, 115 women) at time 1.

Procedure

Participants were scheduled for a laboratory appointment at 8:00 a.m. on a weekday to control for circadian rhythms. They were asked to abstain from drinking (with the exception of water), smoking, and strenuous exercise for 12 h prior to testing. They were also asked to refrain from the use of drugs or alcohol for the 24 h preceding their appointment. Participants were sent home and their appointment rescheduled if they did not adhere to these instructions or if they exhibited physical symptoms such as a cough, cold, or headache. Research assistants were trained to maintain a neutral tone and expression during testing and were paired with participants of the same sex. Once participants provided written consent, anthropomorphic data (weight, height, and waist circumference) were obtained. The electrodes for electrocardiographic (ECG) monitoring were then attached in a bipolar configuration to the lower side of the rib cage and a ground electrode was placed on the left hip. Participants completed sociodemographic, medical, and psychological questionnaires, after which they rested quietly during a 10-min baseline period. Blood samples were taken following the baseline period as well as after the final recovery session. Participants engaged in four psychological challenges of 5 min each (a neutral reading task, two role-plays, and a nonscripted debate). Each stressor was preceded by a 5-min taped autogenic relaxation procedure and a 2-min preparation phase, and followed by a 5-min recovery period. The ECG was obtained continuously during laboratory testing. SBP and DBP were measured every 2 min in the laboratory through a standard inflatable cuff placed on the participant's non-dominant arm. After completing the stress protocol, participants were equipped with ambulatory BP and ECG equipment, and measures were obtained continuously over a 24-h period. Participants were compensated $200 for their time and travel. This study was approved by the Research and Ethics Board of the Montreal Heart Institute.

Laboratory Tasks

In order to augment task stressfulness, participants were informed that their performance would be rated and that they would be videotaped during each task. The tasks led to significant affective and physiological reactivity in pilot testing or prior studies (e.g., [51–53]).

Public Reading of a Neutral Text Participants read an affectively neutral text on Antarctica's geography aloud in the presence of a same-sex confederate.

Role-Plays As with a prior study [51], participants engaged in two scripted role-play scenarios manipulating quarrelsome behavior. Participants were asked to play the role of a supervisor providing feedback to an employee whose performance had been mediocre. The script of the first role-play scenario contained agreeable assertions while the script of the second role-play contained an equal number of quarrelsome assertions. The participant was asked to enact the script as authentically as possible with a confederate acting as the employee. These role-plays were counterbalanced across participants.

Debate In the final task, the participants engaged in a nonscripted debate regarding abortion. They argued from a partisan position and alternated speaking and listening for 1-min periods with a confederate who was debating the opposite position. The participant began the debate, which resulted in a total of 3 min of active debate for the participant, and 2 min of listening while the confederate spoke.

Measures

Sociodemographic Variables Data on sex, age in years, ethnicity, weight, height, marital status, income, and years of schooling were obtained. Behavioral risk factors, such as daily tobacco and alcohol consumption and hours of physical activity, were reported by the participant.

Metabolic burden was defined as the number of metabolic syndrome parameters for which participants were in the highest quartile (lowest for HDL) for their sex, for a total range of 0 to 5. For the sex-specific cutoff values for each of the metabolic parameters used to assess metabolic burden, please refer to Table 1. Serum samples were analyzed for lipids and glucose at the Montreal Heart Institute. These determinations were made using respective reagent Flex on the multianalyzer Dimension RxL Max (Dade Behring Diagnostics, Marburg, Germany) with heparinized plasma, as simultaneously as possible following the blood draw. Waist circumference was measured using a standard measuring tape. Twenty-four-hour ambulatory BP measures were obtained at 20-min intervals during the daytime and at 1-h intervals from 2200 to 0600 hours, using

Table 1 Metabolic profile of participants and sex-specific cutoffs used to create the metabolic burden construct

	Metabolic profile				Sex-specific cutoffs for metabolic burden			
	Time 1—mean (SD)		Time 2—mean (SD)		Time 1		Time 2	
	Men (n=78)	Women (n=115)	Men (n=55)	Women (n=81)	Men	Women	Men	Women
24-h SBP (mmHg)	116.4 (10.04)	106.9 (12.85)	116.9 (9.69)	108.0 (13.53)	123.0	115.0	120.0	117.0
24-h DBP (mmHg)	72.8 (8.22)	67.8 (9.26)	73.3 (8.50)	68.4 (9.68)	75.0	72.0	74.0	74.0
Glucose (mmol/L)	5.3 (0.50)	5.2 (0.51)	5.3 (0.47)	5.2 (0.53)	5.6	5.4	5.5	5.4
HDL (mmol/L)	1.2 (0.33)	1.5 (0.35)	1.2 (0.35)	1.5 (0.33)	1.0	1.2	1.0	1.2
Triglycerides (mmol/L)	1.4 (0.92)	0.9 (0.65)	1.4 (0.98)	0.9 (0.44)	1.7	1.2	1.7	1.2
WC (cm)	90.3 (11.64)	84.3 (13.01)	91.0 (11.50)	84.6 (12.75)	97.0	93.0	96.3	92.0
Metabolic burden (sum score)	1.39 (1.35)	1.44 (1.32)	1.42 (1.53)	1.37 (1.36)	–	–	–	–

Metabolic burden is the number of metabolic syndrome parameters for which participants were in the 75th percentile (25th percentile for HDL cholesterol) for their sex. Significant sex differences emerged for each metabolic burden parameter (except for glucose) at time 1 and time 2, all p values <0.001. There was no significant sex difference for metabolic burden sum scores.

WC waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL* high density lipoprotein cholesterol

Spacelab Ambulatory Blood Pressure Units, which use an oscillometric method. The BP measures were based on values averaged over the 24 h. Twenty-four-hour ambulatory BP measures were chosen as they are more predictive of cardiovascular endpoints as compared to laboratory or clinic readings [54, 55] and have been recommended as the gold standard measurement of BP in the diagnosis of hypertension and metabolic syndrome [56]. Usable ambulatory BP data were obtained for 98 % of the sample.

Physiological Responses During the Stress Protocol The stress protocol began following a 20-min adaptation period in the laboratory. BP during the stress protocol was assessed using an AccutorPlus automated BP monitor from Datascope. This model uses an oscillometric method and has been recommended by the European Society of Hypertension [57]. A mean of two readings per period (baseline, stressor, recovery) was used for analysis. Baseline BP was based on the average of two readings obtained during the last 5 min of the 10-min rest period preceding the blood draw and exposure to the stress tasks.

The ECG was obtained using disposable electrodes and the Biopac acquisition system (Biopac Systems Canada, Inc., Montreal Canada) using Acq-Knowledge 3.7.3 software (Goleta, CA, USA). Signals were first filtered with a digital band-pass filter and a 1,000-Hz sampling rate. Interbeat intervals were generated using a peak detection algorithm, after which the series was screened by hand and corrected for artifacts. Spectral analysis of HRV was performed offline using fast Fourier transformations of the interbeat intervals (RR) in MATLAB using published algorithms [58] and was characterized by the high frequency (HF; 0.15–0.40 Hz) and the low frequency components (LF; 0.04–0.15 Hz) as recommended by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [45]. HF-HRV reflects parasympathetic control of the heart. LF-HRV was once used as an index of sympathetic activity, though recent evidence suggests that, under some circumstances, it may actually reflect vagal influences [59]. Given debate on this issue, LF/HF ratio was used instead as a measure of sympathovagal balance in the main analyses. Traditionally, it is expected that as stress increases acutely (as per a stress protocol), sympathovagal balance increases, while parasympathetic control of the heart (as denoted by HF) decreases. HF-HRV was assessed in both absolute and normalized units (nu). The latter is a relative measure that accounts for changes in total spectral power [45], such as may occur during a stress protocol.

Affect and Arousal were assessed by means of the affect grid, a one-item instrument measuring both valence and intensity of affect [60]. The participant is asked to indicate the extent to which he is feeling pleasure–displeasure on the horizontal axis and arousal–sleepiness on the vertical axis of a 9×9 grid of squares. Elevated scores on both axes suggest high arousal and pleasant affect. Intensity of specific affects (e.g., anger, fear, happiness) was also measured using a seven-point rating scale from 1 (not at all) to 7 (very much).

Psychological Variables Various psychological questionnaires were administered for a separate component of the overall investigation that was examined here in post hoc analyses only. These include the Anxiety Sensitivity Index [61, 62], the Beck Depression Inventory-II [63, 64], and the Beck Anxiety Inventory [65–67].

Data Reduction

SBP, DBP, HR, HF-HRV, HF_{nu}, LF-HRV, and LF/HF readings were averaged over each baseline, stress, and post-stress period. The four stress periods were averaged to create a stress composite score. A composite post-stress score was similarly created. Prior research has shown that such aggregate measures, when compiled from multiple stressors that induce similar physiological responses, are more reliable and reflective of a person's typical or trait-like reactivity and recovery compared to responses to individual tasks [68–70]. Stress reactivity (stress−baseline) and recovery (post-stress score−baseline) change scores were then created as per established methods [71]. Finally, in order to minimize the impact of individual differences in baseline values on the change scores, the latter were further regressed on baseline values. These residual change scores were utilized for the primary analyses.

Time 2

Method

Participants were contacted for a follow-up visit approximately 3 years later (M=2.87, SD=0.30 years). Of the 184 individuals who were successfully contacted, six participants were excluded for medical reasons including pregnancy (n=3), cancer (n=2), and sleep apnea (n=1), while 35 refused due to lack of interest (n=16), scheduling issues (n=15), or perception that the protocol was too demanding (n=4). Complete data for current analyses was obtained for 136 participants. Metabolic parameters were obtained as per time 1.

Participants who returned at follow-up were significantly older (M=42 vs. M=39 years), had spent more years on the job market (24 vs. 20 years), smoked fewer cigarettes a week (M=8 vs. M=20), and had higher HR post-stress compared to baseline (M=2.18 vs. M=0.88) (all p values <0.05) compared to those who did not return at follow-up. No other significant differences emerged.

Analyses

All distributions were verified for normality; BMI, number of hours of exercise per week, and HF-HRV were log-transformed to increase the normality of their distributions. Bivariate correlations were employed to investigate the relationship between metabolic burden and possible covariates, such as sociodemographic (sex, age, marital status, income, education) and behavioral variables (smoking, exercise, caffeine, alcohol consumption). Potential covariates were selected based on prior research suggesting an association with metabolic syndrome.

Various preliminary analyses were performed. To evaluate whether it was appropriate to create a composite score from the four stressors, we examined the internal consistency of each physiological measure across the four tasks and post-task periods. The extent to which metabolic burden serves as a proxy for metabolic syndrome was assessed using a one-way ANOVA. Sex differences in the reactivity and recovery change scores were also examined using one-way ANOVAs. Finally, partial correlations were performed between each residual reactivity or recovery change score obtained at time 1 and metabolic burden at time 2, while controlling for metabolic burden at time 1, to evaluate the extent to which stress responses predicted change in metabolic burden in the overall sample.

The main analyses consisted of hierarchical linear regressions on the outcome measure of metabolic burden at time 2. Covariates at time 1 were chosen based on their univariate associations of p<0.25 with metabolic burden at time 2. Block 1 included age, sex, marital status, and metabolic burden at time 1. BMI was not included given its statistical and theoretical overlap with waist circumference. A reactivity or recovery change score was entered in block 2. The interactions of the reactivity or recovery change score with age and/or sex were entered stepwise in block 3. Analyses were repeated for each physiological measure of reactivity and recovery. Significance was set at p<0.05. Significant interactions were evaluated using simple slopes analyses with lower and higher estimates for age based on values ±1 SD from the mean [72]. No significant collinearity was observed.

Results

The women in our sample were slightly older than the men due to the oversampling of menopausal women for purposes not examined here. Men consumed significantly more alcoholic drinks per week than women but less caffeinated beverages, and engaged in more weekly exercise. Please refer to Table 2 for a description of participant characteristics.

Examination of the Composite Scores

As the efficacy of the stress protocol has already been reported elsewhere [46, 48], we concentrate here on potential sex differences in reactivity and recovery. Men exhibited significantly greater DBP reactivity (F(1, 135)=6.37, p=0.01) and HF-HRV reactivity (F(1,135)=4.65, p=0.03) compared to

women. Men also displayed significantly less HF-HRV recovery compared to women ($F(1, 135)=8.20, p<0.001$). No other differences emerged across the other parameters. Refer to Table 3 for details.

Table 2 Participant characteristics (mean±SD) at initial recruitment (time 1)

	Men ($n=78$)	Women ($n=115$)
Age (years)*	39.1 (11.23)	42.8 (11.46)
Body Mass Index (kg/m^2)	24.9 (4.11)	25.3 (5.61)
Years of schooling	15.9 (3.39)	16.0 (3.43)
Marital status *n* (%)		
Single	37 (47.4)	49 (42.6)
Married/living with someone	33 (42.3)	45 (39.2)
Separated/divorced/widowed	8 (10.3)	21 (18.3)
Annual family income *n* (%)		
≤$29,999	26 (33 %)	39 (34 %)
$30,000–59,999	25 (32 %)	47 (41 %)
≥$60,000	27 (35 %)	29 (25 %)
Smoker *n* (%)	13 (17 %)	28 (24 %)
Cups of coffee or tea/week*	11.6 (11.41)	15.0 (11.98)
Glasses of alcohol/week***	5.1 (6.10)	2.7 (4.18)
Hours of exercise/week***	4.7 (5.26)	2.6 (3.20)
Beck Depression Inventory-II Score*	7.2 (7.13)	9.4 (7.40)
Beck Anxiety Inventory Score	4.9 (6.07)	6.6 (6.48)
Anxiety Sensitivity Index Score	16.7 (10.61)	18.1 (10.31)
Baseline cardiovascular and autonomic measures		
SBP (mmHg)***	114.4 (9.60)	106.5 (12.84)
DBP (mmHg)**	71.7 (7.13)	67.6 (9.23)
HR (bpm)	64.0 (9.13)	66.2 (8.30)
HF-HRV (ms^2)	405.2 (824.2)	568.4 (950.0)
LF-HRV (ms^2)	612.5 (864.1)	537.5 (734.4)
LF/HF**	2.3 (2.18)	1.6 (1.54)
HF_{nu}	17.3 (19.39)	22.5 (26.60)
Medications (time 2), *n* (%)		
Psychotropic medication	2 (1 %)	5 (4 %)
Cardiovascular agents	1 (0.7 %)	2 (1 %)
Dyslipidemics	2 (1 %)	0
Aspirin	0	1 (0.7 %)

Both HF-HRV and LF-HRV were log transformed to increase normality for purposes of analyses, though raw data are presented in this table. Medications refer to those taken by participants at follow-up. Psychotrophics included selective serotonin reuptake inhibitors, benzodiazepines, atypical antipsychotics, and other antidepressants. Cardiovascular agents included angiotension II receptors antagonists, diuretic thiazide, and calcium channel blocking agents.

SBP systolic blood pressure, *DBP* diastolic blood pressure, *HDL* high-density lipoprotein cholesterol, *HR* heart rate, *HF-HRV* high frequency heart rate variability, *LF-HRV* low frequency heart rate variability, *HF_{nu}* high frequency heart rate variability in normalized units

*$p<0.05$; **$p<0.01$; ***$p<0.001$

The internal consistency of the composite stress and recovery scores was excellent, with Cronbach's alpha reliability coefficients well above 0.90 across all measures of reactivity and recovery, except LF/HF, that nonetheless showed adequate internal consistency, substantiating the use of reactivity and recovery aggregate scores in the primary analyses. For details, please see Electronic Supplementary Material (ESM), Table 1.

Use of Metabolic Burden as a Proxy for Metabolic Syndrome

Participants with metabolic syndrome in our sample showed significantly more metabolic burden at time 2 (3.25±1.18) than those without metabolic syndrome (1.14±1.27; $p<0.001$).

Metabolic Burden and Its Univariate Associations with Stress Responses

HF-HRV reactivity predicted increased metabolic burden at time 2. Refer to Table 4.

Metabolic Burden as a Function of Stress Responses, Sex, and Age

Covariates (age, sex, marital status, and metabolic burden at time 1) explained 33 % of the variance.

Reactivity to Stress

A significant main effect emerged for HF-HRV (beta=−0.14, $p=0.047$), but not for any of the other reactivity change scores: SBP (beta=−0.02, $p=0.75$), DBP (beta=−0.07, $p=0.37$), HR (beta=0.06, $p=0.45$), HF_{nu} (beta=0.03, $p=0.48$), and LF/HF (beta=−0.06, $p=0.38$).

However, significant interactions with sex emerged for HF_{nu} and LF/HF (see Table 5 for details). Simple slope analyses indicated that women who did not show the expected decrease in HF_{nu} during stress compared to baseline tended to show an increase in metabolic burden over follow-up ($b=0.26, p<0.06$). In men, an opposite association was observed ($b=-0.23, p=0.16$) (Fig. 1a). Similarly, blunted LF/HF reactivity predicted increased metabolic burden at follow-up in women ($b=-0.35, p=0.03$) but not in men ($b=0.07, p=0.60$) (Fig. 2a). No significant interactions emerged with age.

Recovery from Stress

No main effects emerged for any of the recovery change scores: SBP (beta=0.08, $p=0.23$), DBP (beta=0.10, $p=0.18$), HR (beta=0.11, $p=0.11$), HF-HRV (beta=−0.02, $p=0.77$), HF_{nu} (beta=0.10, $p=0.15$), and LF/HF (beta=−0.04, $p=0.60$).

Table 3 Sex differences in reactivity and recovery change scores

	Reactivity			Recovery		
	Mean (SD)			Mean (SD)		
	Men	Women	*p*	Men	Women	*p*
SBP	11.5 (5.89)	12.3 (9.02)	0.57	3.2 (4.80)	3.8 (6.83)	0.52
DBP	10.2 (6.09)	7.6 (5.80)	0.01	2.8 (3.87)	1.8 (4.11)	0.15
HR	5.5 (4.25)	5.7 (4.73)	0.83	2.3 (2.70)	2.1 (2.94)	0.71
HF(log)	31.2 (7.21)	28.6 (6.58)	0.03	1.2 (0.01)	1.2 (0.03)	<0.001*
LF/HF	1.7 (1.91)	1.2 (1.92)	0.15	1.7 (1.88)	1.1 (1.88)	0.09
HF_{nu}	−15.1 (16.22)	−17.9 (17.8)	0.35	−13.6 (17.78)	−15.3 (15.06)	0.52

SBP systolic blood pressure (mmHg), *DBP* diastolic blood pressure (mmHg), *HR* heart rate (beats per minute), *HF* high frequency heart rate variability (ms^2) in log units, HF_{nu} high frequency in normalized units, *LF/HF* sympathovagal balance

*The sex difference for HF(log) recovery becomes evident when comparing the values at two decimal points (1.20 in men versus 1.18 in women)

However, significant interactions with sex emerged for HF_{nu} and LF/HF (see Table 5 for details). Women whose HF_{nu} values post-stress were closer to baseline values showed increased metabolic burden at time 2 (b=0.41, p<0.01). In men, the opposite was true (b=−0.11, p=0.47). See Fig. 1b. For the LF/HF measure, greater LF/HF recovery predicted an increased risk of metabolic burden at time 2 in women (b=−0.34, p=0.04) but not in men (b=0.11, p=0.36) (Fig. 2b). No significant interactions emerged with age.

Table 4 Partial correlations between residualized reactivity and recovery change scores and metabolic burden obtained at time 2

	Metabolic burden time 2
Reactivity change scores	
SBP	−0.008
DBP	−0.069
HR	−0.092
HF-HRV	*−0.170**
HF_{nu}	−0.079
LF/HF	0.027
Recovery change scores	
SBP	0.112
DBP	0.130
HR	0.154
HF-HRV	−0.025
HF_{nu}	−0.048
LF/HF	0.106

Partial correlation analysis controls for metabolic burden at time 1.

SBP systolic blood pressure, *DBP* diastolic blood pressure, *HR* heart rate, *HF-HRV* high frequency heart rate variability, HF_{nu} high frequency in normalized units, *LF/HF* sympathovagal balance

*p<0.05

Post Hoc Analyses

Supplemental analyses were performed in order to better understand the results obtained. First, to increase our confidence in the findings regarding metabolic burden, we performed a series of logistic regression analyses using metabolic syndrome instead of metabolic burden as the outcome variable. Note that only eight men and nine women met metabolic syndrome criteria at follow-up, but results using metabolic syndrome are highly consistent with those obtained with metabolic burden. More specifically, a significant interaction between sex and HF_{nu} reactivity (O.R.= 17.30, p=0.03, 95 % C.I.=1.30–229.43) and recovery (O.R.= 8.04, p=0.01, 95 % C.I.=1.57–41.23) emerged. While non-significant, similar trends were nonetheless observed for the interaction terms between sex and LF/HF reactivity (O.R.= 0.29, p=0.10, 95 % C.I.=0.06–1.27) and recovery (O.R.=0.17, p=0.09, 95 % C.I.=0.02–1.28). No associations or trends emerged with the cardiovascular measures.

The sample was then categorized into three equal groups (low, moderate, or high reactivity) according to their continuous HF_{nu} reactivity scores. HF_{nu} reactivity group (low, moderate, high reactivity) by period (baseline, stress, post-stress) repeated measures ANOVAs were performed to determine whether the low HF_{nu} reactivity group was showing low stress responsiveness across all physiological parameters or whether these were limited to HRV parameters. In these analyses, LF-HRV was also examined to further comprehend the pattern of ANS activity contributing to the LF/HF ratio. The period effects were all significant (p<0.002) as expected. No group or group by period interactions emerged for SBP, DBP, and HR, suggesting that the groups did not differ in their CVR to stress. However, a significant main effect of group and a significant group by period interaction emerged for HF-HRV, LF-HRV, and LF/HF (all p values <0.002). The low HF_{nu} reactivity group showed higher overall HF-HRV and LF-HRV activity across the three periods (all p values <0.001), but

Table 5 Summary of significant results

Block 1	*β*	*t*	*p*
Age	−0.019	−0.246	0.806
Sex	−0.026	−0.366	0.715
Marital status	0.110	1.485	0.140
Metabolic burden at time 1	0.573	7.697	<0.001
F_{model} (4, 131)=17.930, p<0.001			
R^2_{model}=0.354, R^2_{adj}=0.334			
HF_{Log} reactivity			
Block 2	*β*	*t*	*p*
HF_{Log}	−0.145	−2.002	0.047
F_{model} (1, 120)=4.009, p=0.047			
R^2_{model}=0.373, R^2_{adj}=0.349			
HF_{nu} reactivity			
Block 2	*β*	*t*	*p*
HF_{nu}	0.035	0.484	0.629
F_{model} (1, 130)=0.234, p=0.629			
R^2_{model}=0.355, R^2_{adj}=0.330			
Block 3	*β*	*t*	*p*
HF_{nu} × sex	0.159	2.224	0.028
F_{model} (1, 129)=4.945, p=0.028			
R^2_{model}=0.379, R^2_{adj}=0.350			
LF/HF reactivity			
Block 2	*β*	*t*	*p*
LF/HF	−0.065	−0.872	0.385
F_{model} (1, 130)=0.760, p=0.385			
R^2_{model}=0.358, R^2_{adj}=0.333			
Block 3	*β*	*t*	*p*
LF/HF × sex	−0.153	−2.085	0.039
F_{model} (1, 129)=4.348, p=0.039			
R^2_{model}=0.378, R^2_{adj}=0.350			
HF_{nu} recovery			
Block 2	*β*	*t*	*p*
HF_{nu}	0.105	1.444	0.151
F_{model} (1, 130)=2.084, p=0.151			
R^2_{model}=0.364, R^2_{adj}=0.340			
Block 3	*β*	*t*	*p*
HF_{nu} × sex	0.169	2.378	0.019
F_{model} (1, 129)=5.653, p=0.019			
R^2_{model}=0.391, R^2_{adj}=0.362			
LF/HF recovery			
Block 2	*β*	*t*	*p*
LF/HF	−0.040	−0.527	0.599
F_{model} (1, 130)=0.278, p=0.599			
R^2_{model}=0.355, R^2_{adj}=0.330			
Block 3	*β*	*t*	*p*
LF/HF × sex	−0.170	−2.263	0.025
F_{model} (1, 129)=5.121, p=0.025			
R^2_{model}=0.380, R^2_{adj}=0.351			

LF/HF=sympathovagal balance. Reactivity=Stress−Baseline; Recovery =Recovery−Baseline

HF-HRV high frequency heart rate variability, *HF_{nu}* high frequency heart rate variability in normalized units

showed less or no change in LF/HF and HF-HRV activity in response to stress (see Fig. 3). A similar series of analyses was repeated on self-reported arousal, negative affect, and positive affect to verify that the protocol was experienced as equally stressful by all groups. No significant group or group by period interactions emerged for any of these measures.

A Chi Squared analysis was performed to determine whether the sex differences reflected an insufficient number of men or women among extreme HF_{nu} responders. No significant sex difference was observed (χ^2=3.375, p=0.185). However, t tests indicated a sex difference in self-reported arousal and negative affect during the stress period, with women reporting significantly more arousal (t(134)=1.76, p<0.01)), and higher negative affect (t(134)=1.57, p<0.01)) compared to men.

To better understand what may be driving the relationship between autonomic stress responses and metabolic burden, we conducted two-way ANOVAs with HF_{nu} grouping and sex entered as independent variables, and the difference in each of the individual metabolic burden parameters (time 2−time 1) entered as dependent variable. A significant sex by HF_{nu} grouping interaction emerged for glucose (F(2,133)=3.519, p=0.003). More specifically, in women, low responders exhibited an increase in glucose over time, whereas high responders showed a decrease. In men, the opposite was observed. A significant sex by HF_{nu} grouping interaction also emerged for waist circumference (F(2, 130)=3.586, p= 0.031). In women, low responders exhibited an increase in waist circumference, whereas high responders showed a decrease. Again, the opposite was observed in men.

Finally, Pearson correlations between the continuous HF_{nu} reactivity change score and various sociodemographic and psychological variables at time 1 were performed to better characterize individuals with low HF_{nu} reactivity. Blunted HF_{nu} reactivity was associated with significantly higher scores on the Anxiety Sensitivity Index (r=0.20, p<0.05). No other significant associations emerged.

Discussion

The goal of the present research was to examine the prospective relationships between physiological responses to stress and metabolic burden, and to verify whether these associations were moderated by sex and/or age. Blood pressure and heart rate responses to stress did not predict future metabolic dysfunction in either men or women. Greater parasympathetic withdrawal during stress, on the other hand, was associated with an increase in metabolic burden in the overall sample, though the overall pattern of results suggests that this was true mostly in men. In women, blunted autonomic responses to stress predicted increased metabolic burden over the 3-year follow-up period.

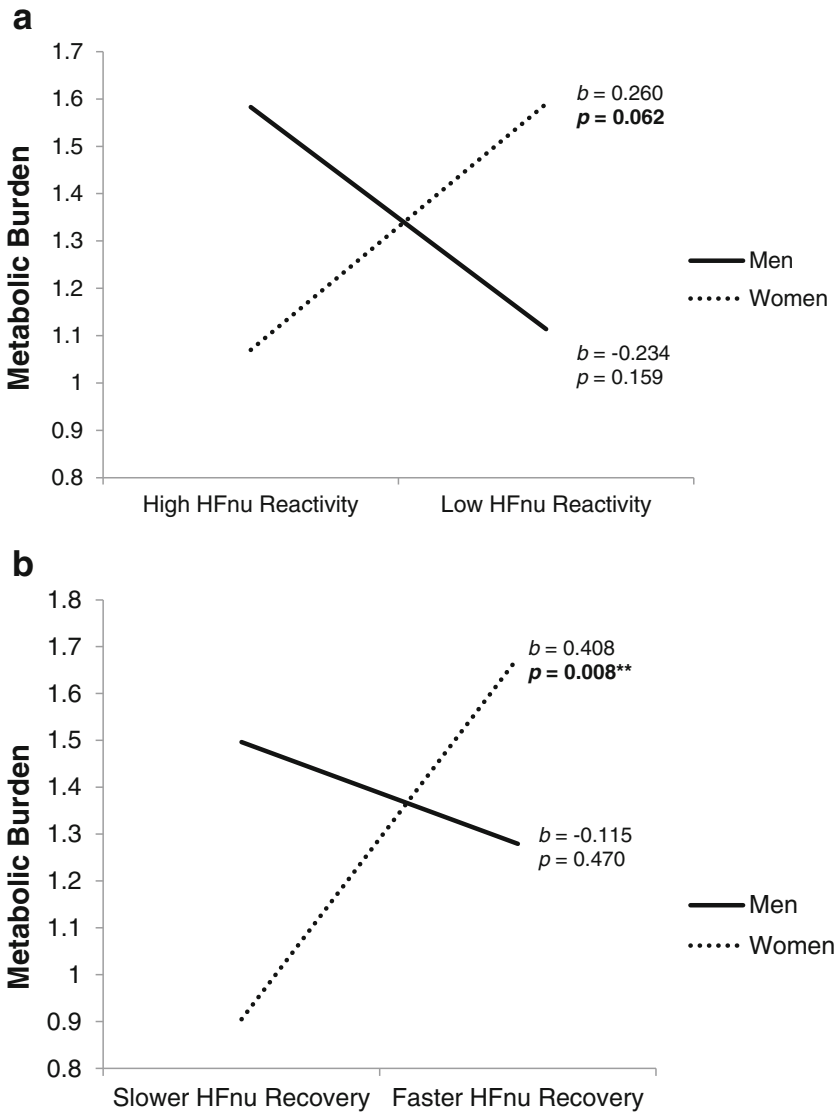

Fig. 1 **a** The prospective association between metabolic burden and HF_{nu} reactivity is moderated by sex. In women, there is a trend for low HF_{nu} reactivity to be associated with increased metabolic burden. An opposite association was observed in men. **b** The prospective association between metabolic burden and HF_{nu} recovery is moderated by sex. In women, faster HF_{nu} recovery was associated with increased metabolic burden. An opposite association was observed in men

Cross-sectional studies have previously reported greater cardiovascular responses during or following exercise [73–75], as well greater catecholamine levels in urine (suggestive of increased sympathetic nervous system activity) [76] in those with metabolic syndrome or a mathematical representation of metabolic syndrome versus healthy controls. However, in cross-sectional investigations, especially those comparing individuals with metabolic dysfunction with healthy controls, it has not been possible to determine whether the heightened physiological responses to exercise represent an outcome of metabolic syndrome, whether it contributed to its pathogenesis, or whether both reflect another underlying process. The fact that heightened parasympathetic reactivity predicted increased metabolic burden 3 years later in the current study is consistent with the notion that autonomic responses to psychological stress may be implicated in the worsening of metabolic burden over time in otherwise healthy individuals.

Surprisingly, in the current study, it was blunted responsiveness of the parasympathetic system that predicted increased metabolic burden in women. These findings were rather robust and consistent with concurrent analyses at study onset (data not presented in text due to space limitations; see ESM Figs. 1 and 2 for details). In other cross-sectional work, women with metabolic syndrome were recently shown to display reduced LF/HF responsiveness to a resistance training exercise session [77]. The reasons for these sex differences in the relationship between autonomic responses and metabolic dysfunction are unclear. Sex differences in the meaning and experience of life situations may be involved. Indeed, some research suggests that women tend to report higher levels of perceived stress and psychological distress [78], and view life events as being more serious, disruptive, and stressful than men do [79]. In the current study, there was limited evidence for this. Women reported slightly more depressive symptoms compared to men and reported greater

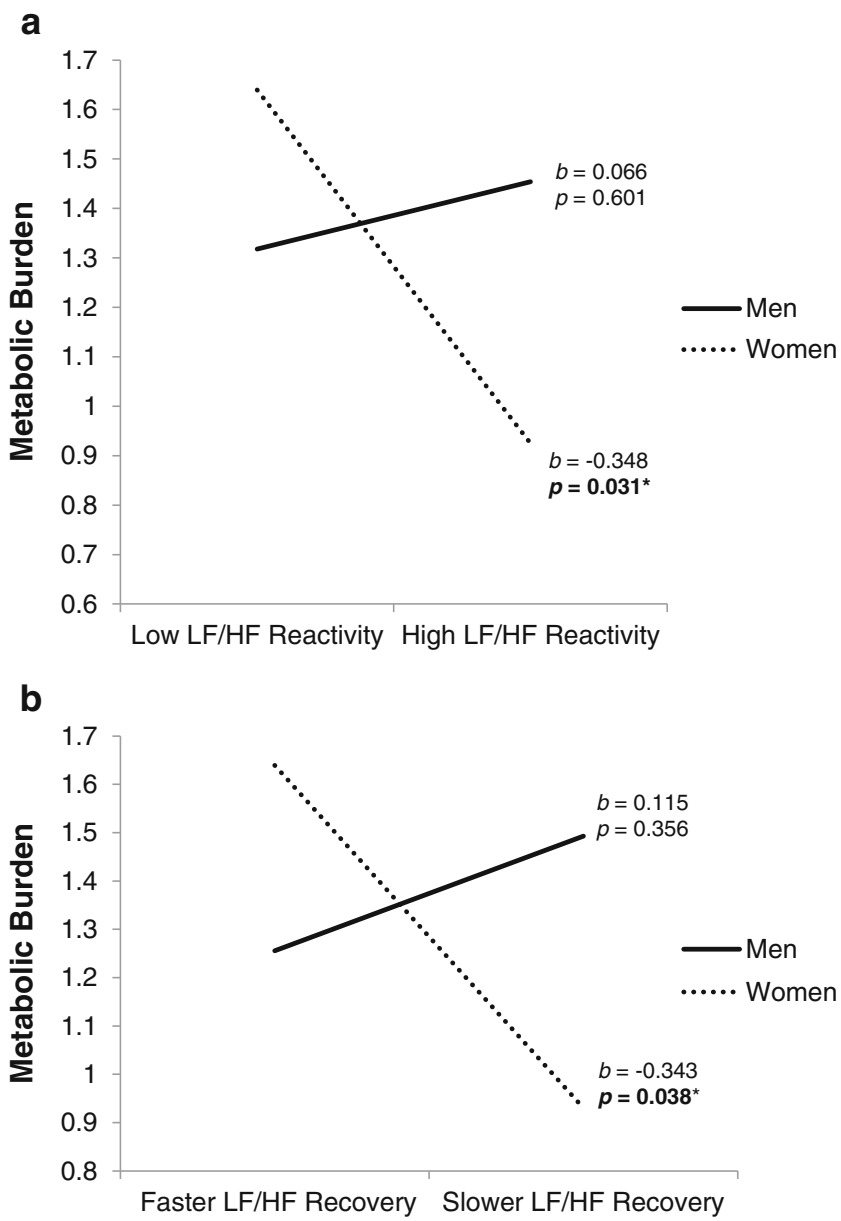

Fig. 2 **a** The prospective association between metabolic burden and LF/HF reactivity is moderated by sex. In women, blunted LF/HF reactivity was associated with increased metabolic burden. An opposite association was observed in men. **b** The prospective association between metabolic burden and LF/HF recovery is moderated by sex. Faster LF/HF recovery was associated with increased metabolic burden in women. An opposite association was observed in men

affect arousal and negative affect during stress compared to men. A positive association between blunted stress reactivity and higher levels of perceived stress or psychological distress has been reported, particularly in women [78]. That sex differences may exist in the regulation of emotions and cardiac autonomic control has some basis in the literature. Indeed, individuals who show blunted BP reactivity to a stressor have been shown to exhibit blunted neural reactions of the limbic system to the same task [80, 81]. However, this relation appears to differ in women (positive correlation) compared to men (no or negative associations) [82]. It has also been suggested that men and women may differ in their hormonal response to stress [83]. That is, the sympathetic nervous system "fight or flight" response may be inhibited in women as a result of their higher levels of oxytocin, favoring more affiliative "tend and befriend" behaviors [83]. In the current investigation, however, the pattern of physiological responses to stress suggested sex differences in parasympathetic rather than sympathetic control of the heart, with greater and more prolonged parasympathetic withdrawal in men compared to women. The origin of such differences remains to be elucidated but their significance to the development of metabolic abnormalities is suggested by our results.

In contrast to the stress reactivity hypothesis, which posits that heightened and/or prolonged stress responses increase risk for disease outcomes, there is a small but growing body of literature in support of our findings in women; i.e., that blunted responsiveness of various stress systems, including the ANS, may have adverse health effects [25, 37, 84–89]. For instance,

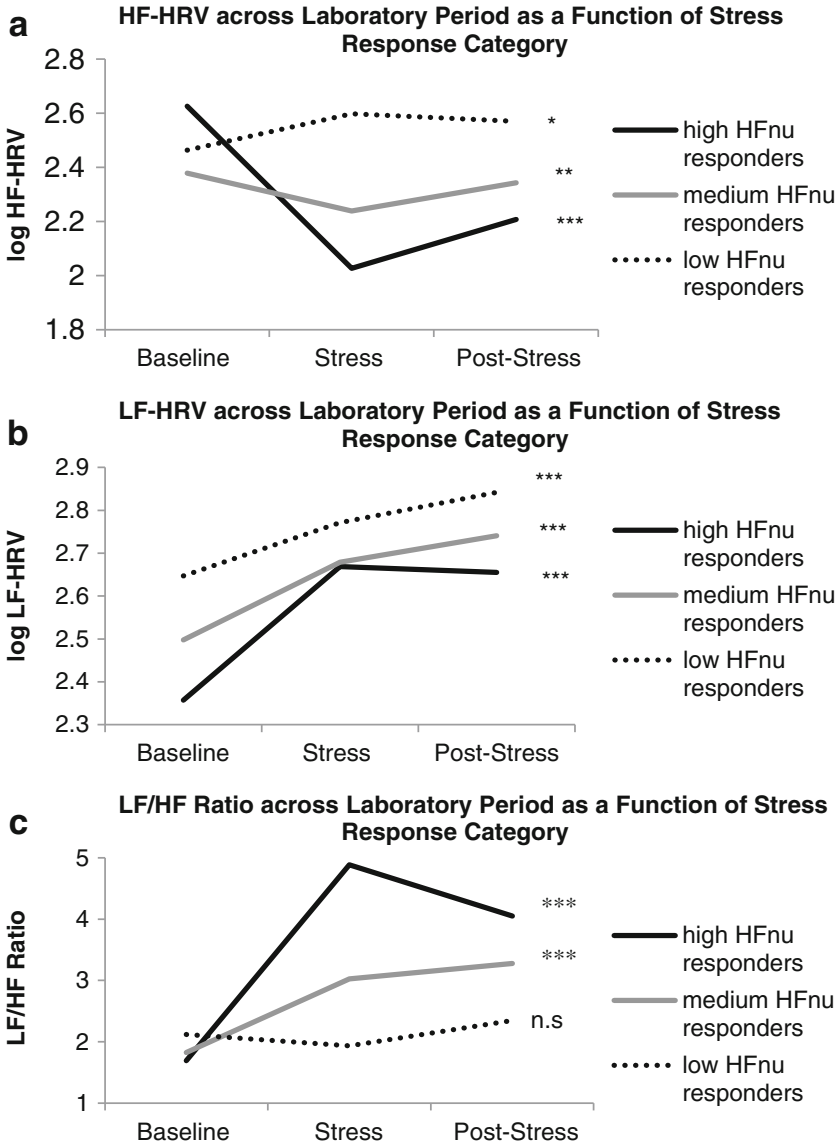

Fig. 3 **a** HF-HRV across laboratory period as a function of HF_{nu} stress response category. The low HF_{nu} responder group showed a slight significant increase in response to the stressor, whereas the medium and high responder groups showed significant decreases in response to stress. **b** LF-HRV across laboratory period as a function of stress response category. All groups showed a significant increase in response to stress. **c** LF/HF Ratio across laboratory period as a function of stress response category. The high and medium responders exhibited significant increases in LF/HF in response to stress, whereas the low responder group showed no change in LF/HF across the protocol. *n.s.* non-significant, *p<0.05, **p<0.01, ***p<0.001

reduced HR reactivity has been associated with an increased likelihood of becoming obese over periods of up to 7 years [25, 85]. Similarly, we recently reported that patients with a blunted parasympathetic response to an autonomic challenge (valsalva maneuver) had higher rates of complications during and after cardiac surgery compared to patients with "normal" autonomic responses [90]. It has been hypothesized that blunted reactivity may reflect an overall deteriorating stress response [85] due to chronic exposure to stressful conditions. Phillips et al. [85] suggest that down regulation of beta-adrenergic stress receptors may be involved. However, blunted ANS responsiveness to psychological stress in the current study did not reflect overall low responsiveness of the stress systems in either men or women. Indeed, those with blunted parasympathetic reactivity were as reactive across the cardiovascular and LF-HRV measures as those who showed the expected decreases in parasympathetic activity in response to stress. Nonetheless, and consistent with the idea of chronic stress exposure, participants who showed blunted parasympathetic responses to stress also demonstrated significantly greater tonic or baseline levels of both sympathetic and parasympathetic activity. High tonic levels of parasympathetic activity may reflect the organism's attempt to limit the disruptions created by heightened sympathetic activity, the cost of which may be the inability of the parasympathetic system to adapt further as required by life circumstances. In a prior study in patients undergoing cardiac surgery, predictors of autonomic dysfunction had also included higher baseline parasympathetic activation and greater psychological distress [90]. Dampened HR reactivity has similarly been reported in more depressed individuals [91–93], while dampened LF/HF reactivity to passive head-up tilt testing was found in more anxious individuals [94]. The latter is consistent

with reports of more anxiety sensitivity (fear of fear) in individuals with blunted autonomic responses to stress in the current study.

While there was some limited evidence for moderating effects of age on the associations between stress responses and concurrent metabolic burden at time 1 (data not presented in text due to space limitations; refer to ESM Figs. 3 and 4 for details), these were not maintained prospectively. Whether this reflects lack of stability in the impact of age is unknown, though additional research may be warranted.

Certain limitations of the current investigation require consideration. The sample consisted primarily of francophone Caucasians, and results may not be generalizable to other cultural groups. Racial differences, particularly between African-Americans and Caucasians, have been reported with regard to cardiovascular reactivity to stress [95] and prevalence of metabolic syndrome [96]. There were a few notable differences in the characteristics of those who returned for follow-up versus those who did not, which may also affect the generalizability of our results. For instance, our results may be less applicable to those who are heavier smokers. In addition, while the use of a healthy sample enabled us to examine the impact of stress responses on metabolic burden progression, unencumbered by other known disease processes, it may also have dampened our ability to observe significant associations. This may explain why cardiovascular measures did not emerge as significant predictors of metabolic burden. Relatedly, both the men and women in our sample had rather low BP values and tended more towards overweight. Given the substantial literature showing increased hypertension risk with greater cardiovascular reactivity, and the smaller literature suggesting greater obesity with blunted stress reactivity, our sample characteristics may have biased our results towards increased metabolic burden with blunted ANS responsiveness. Post hoc analyses did indeed suggest that changes in waist circumference (and glucose) might be driving the metabolic burden findings in the current study.

Some have criticized the integrity of the metabolic syndrome construct, suggesting that it is the specific individual parameters that account for the increased risk of negative cardiovascular outcomes, rather than the syndrome itself [97–100]. The use of metabolic burden rather than the individual parameters could in that viewpoint be construed a limitation. However, recent large-scale studies have supported that the metabolic syndrome construct is a genuine predictor of adverse cardiovascular outcomes, independently of its individual parameters [39–43]. For example, in a 16-year longitudinal study (n=2,805), Simons et al. [41] showed that metabolic syndrome predicted negative cardiovascular outcomes and all-cause mortality, regardless of which individual components showed elevations. The use of metabolic burden as a proxy for metabolic syndrome in the main analyses may also be considered a limitation. However, we have shown that the two constructs are highly overlapping, and post hoc analyses with metabolic syndrome as outcome variable produced results that were highly consistent with those obtained with metabolic burden.

Finally, it is noteworthy that significant group differences emerged on the HF_{nu} and LF/HF, but not the HF-HRV variable. Reyes del Paso et al. [59] recently reported concern regarding the interpretation of LF/HF and showed that normalizing HRV indices may lead to artificially inflated correlations with variables. Replication of this study is therefore needed.

Nonetheless, the current study possesses several strengths that increase the confidence that can be had in the results obtained. This is the first investigation to examine the relationship between stress responses and a global representation of metabolic syndrome. The design was prospective and employed a rigorous methodology. Recruitment was performed such as to ensure a heterogeneous sample of healthy individuals (with respect to sex, age, sociodemographic, and work characteristics). Multiple stressors with an interpersonal component were chosen to be of relevance to women as well as men, and were aggregated to increase reliability and validity, compared to single tasks or purely physical or cognitive tasks. The blood pressure component of the metabolic burden construct was obtained from 24-h ambulatory monitoring, which has been shown to be more predictive of cardiovascular outcomes compared to clinic or laboratory measures [54, 55]. Autonomic measures of reactivity and recovery to psychological stress had been ignored to date in this literature. Yet, in the current study, these measures were of particular importance to the prediction of metabolic burden. The examination of sex differences was both novel and fortuitous, as results were significantly moderated by sex. Finally, analyses controlled for relevant characteristics of participants.

In conclusion, this study contributes to the small but growing body of evidence suggesting that both heightened and blunted stress responses may be detrimental for health. More specifically, blunted parasympathetic responses increased metabolic burden in women, while in men, greater and more prolonged parasympathetic responses to stress increased risk. While the reasons for these sex differences are unclear, they highlight the need to consider sex differences in such research. Future investigations may benefit from including more than one follow-up period to verify whether there are differences in the early versus later effects of stress responses on metabolic burden. Although stress management has been shown to be effective in reducing clinically significant metabolic abnormalities [101–103], their utility in preventing metabolic syndrome in healthy individuals is unknown. Moreover, these

strategies have typically focused on reducing exaggerated physiological reactivity to stress [104–106]. Our results suggest that developing interventions that also target blunted physiological responses to stress may be of importance.

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Authors' Statement of Conflict of Interest and Adherence to Ethical Standards Authors Gentile, Dragomir, Solomon, Nigam, and D'Antono declare that they have no conflict of interest. All procedures, including the informed consent process, were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

References

1. Riediger ND, Clara I. Prevalence of metabolic syndrome in the Canadian adult population. *CMAJ*. 2011; 183: E1127-E1134.
2. Jorgensen RS, Nash JK, Lasser NL, Hymowitz N, Langer AW. Heart rate acceleration and its relationship to total serum cholesterol, triglycerides, and blood pressure reactivity in men with mild hypertension. *Psychophysiology*. 1988; 25: 39-44.
3. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009; 120: 1640-1645.
4. Dekker JM, Girman C, Rhodes T, et al. Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. *Circulation*. 2005; 112: 666-673.
5. McNeill AM, Rosamond WD, Girman CJ, et al. The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. *Diabetes Care*. 2005; 28: 385-390.
6. Lakka HM, Laaksonen DE, Lakka TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002; 288: 2709-2716.
7. Expert Panel on Detection E. Treatment of high blood cholesterol in A: Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001; 285: 2486-2497.
8. Cohen BE, Panguluri P, Na B, Whooley MA. Psychological risk factors and the metabolic syndrome in patients with coronary heart disease: Findings from the heart and soul study. *Psychiatry Res*. 2010; 175: 133-137.
9. Goldbacher EM, Matthews KA. Are psychological characteristics related to risk of the metabolic syndrome? A review of the literature. *Ann Behav Med*. 2007; 34: 240-252.
10. Roohafza H, Sadeghi M, Talaei M, Pourmoghaddas Z, Sarrafzadegan N. Psychological status and quality of life in relation to the metabolic syndrome: Isfahan cohort study. *Int J Endocrinol*. 2012; 2012: 380902.
11. D'Antono B, Moskowitz DS, Nigam A. The metabolic costs of hostility in healthy adult men and women: Cross-sectional and prospective analyses. *J Psychosom Res*. 2013; 75: 262-269.
12. Nelson TL, Palmer RF, Pedersen NL. The metabolic syndrome mediates the relationship between cynical hostility and cardiovascular disease. *Exp Aging Res*. 2004; 30: 163-177.
13. Chida Y, Steptoe A. Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: A meta-analysis of prospective evidence. *Hypertension*. 2010; 55: 1026-1032.
14. Muldoon MF, Herbert TB, Patterson SM, et al. Effects of acute psychological stress on serum lipid levels, hemoconcentration, and blood viscosity. *Arch Intern Med*. 1995; 155: 615-620.
15. Burker EJ, Fredrikson M, Rifai N, Siegel W, Blumenthal JA. Serum lipids, neuroendocrine, and cardiovascular responses to stress in men and women with mild hypertension. *Behav Med*. 1994; 19: 155-161.
16. Owens JF, Stoney CM, Matthews KA. Menopausal status influences ambulatory blood pressure levels and blood pressure changes during mental stress. *Circulation*. 1993; 88: 2794-2802.
17. McKinney ME, McIlvain HE, Hofschire PJ, et al. Cardiovascular changes during mental stress: Correlations with presence of coronary risk factors and cardiovascular disease in physicians and dentists. *J Hum Hypertens*. 1987; 1: 137-145.
18. Fredrikson M, Blumenthal JA. Serum lipids, neuroendocrine and cardiovascular responses to stress in healthy type A men. *Biol Psychol*. 1992; 34: 45-58.
19. van Doornen LJ, Snieder H, Boomsma DI. Serum lipids and cardiovascular reactivity to stress. *Biol Psychol*. 1998; 47: 279-297.
20. Waldstein SR, Burns HO, Toth MJ, Poehlman ET. Cardiovascular reactivity and central adiposity in older African Americans. *Health Psychol*. 1999; 18: 221-228.
21. Goldbacher EM, Matthews KA, Salomon K. Central adiposity is associated with cardiovascular reactivity to stress in adolescents. *Health Psychol*. 2005; 24: 375-384.
22. Davis MC, Twamley EW, Hamilton NA, Swan PD. Body fat distribution and hemodynamic stress responses in premenopausal obese women: A preliminary study. *Health Psychol*. 1999; 18: 625-633.
23. Ljung T, Holm G, Friberg P, et al. The activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. *Obes Res*. 2000; 8: 487-495.
24. Steptoe A, Wardle J. Cardiovascular stress responsivity, body mass and abdominal adiposity. *Int J Obes (Lond)*. 2005; 29: 1329-1337.
25. Carroll D, Phillips AC, Der G. Body mass index, abdominal adiposity, obesity, and cardiovascular reactions to psychological stress in a large community sample. *Psychosom Med*. 2008; 70: 653-660.
26. Phillips AC, Roseboom TJ, Carroll D, de Rooij SR. Cardiovascular and cortisol reactions to acute psychological stress and adiposity: Cross-sectional and prospective associations in the Dutch Famine Birth Cohort Study. *Psychosom Med*. 2012; 74: 699-710.
27. Villegas R, Perry IJ, Creagh D, Hinchion R, O'Halloran D. Prevalence of the metabolic syndrome in middle-aged men and women. *Diabetes Care*. 2003; 26: 3198-3199.
28. Allen MT, Stoney CM, Owens JF, Matthews KA. Hemodynamic adjustments to laboratory stress: The influence of gender and personality. *Psychosom Med*. 1993; 55: 505-517.

29. Matthews KA, Davis MC, Stoney CM, Owens JF, Caggiula AR. Does the gender relevance of the stressor influence sex differences in psychophysiological responses? *Health Psychol*. 1991; 10: 112-120.
30. Uchino BN, Uno D, Holt-Lunstad J, Flinders JB. Age-related differences in cardiovascular reactivity during acute psychological stress in men and women. *J Gerontol B Psychol Sci Soc Sci*. 1999; 54: P339-P346.
31. Brame AL, Singer M. Stressing the obvious? An allostatic look at critical illness. *Crit Care Med*. 2010; 38: S600-S607.
32. Logan JG, Barksdale DJ. Allostasis and allostatic load: Expanding the discourse on stress and cardiovascular disease. *J Clin Nurs*. 2008; 17: 201-208.
33. Steptoe A, Marmot M. Impaired cardiovascular recovery following stress predicts 3-year increases in blood pressure. *J Hypertens*. 2005; 23: 529-536.
34. Carnethon MR, Craft LL. Autonomic regulation of the association between exercise and diabetes. *Exerc Sport Sci Rev*. 2008; 36: 12-18.
35. Tsuji H, Larson MG, Venditti FJ Jr, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation*. 1996; 94: 2850-2855.
36. Liao D, Cai J, Barnes RW, et al. Association of cardiac autonomic function and the development of hypertension: the ARIC study. *Am J Hypertens*. 1996; 9: 1147-1156.
37. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol*. 2010; 141: 122-131.
38. Tentolouris N, Argyrakopoulou G, Katsilambros N. Perturbed autonomic nervous system function in metabolic syndrome. *Neuromol Med*. 2008; 10: 169-178.
39. Scuteri A, Najjar SS, Morrell CH, Lakatta EG, Cardiovascular Health S. The metabolic syndrome in older individuals: Prevalence and prediction of cardiovascular events: The Cardiovascular Health Study. *Diabetes Care*. 2005; 28: 882-887.
40. Reilly MP, Rader DJ. The metabolic syndrome: More than the sum of its parts? *Circulation*. 2003; 108: 1546-1551.
41. Simons LA, Simons J, Friedlander Y, McCallum J. Is prediction of cardiovascular disease and all-cause mortality genuinely driven by the metabolic syndrome, and independently from its component variables? *Dubbo Stud Heart Lung Circ*. 2011; 20: 214-219.
42. Scuteri A, Najjar SS, Muller DC, et al. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol*. 2004; 43: 1388-1395.
43. Gupta AK, Dahlof B, Sever PS, Poulter NR. Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm I: Metabolic syndrome, independent of its components, is a risk factor for stroke and death but not for coronary heart disease among hypertensive patients in the ASCOT-BPLA. *Diabetes Care*. 2010; 33: 1647-1651.
44. Seplaki CL, Goldman N, Glei D, Weinstein M. A comparative analysis of measurement approaches for physiological dysregulation in an older population. *Exp Gerontol*. 2005; 40: 438-449.
45. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J*. 1996;17:354–381.
46. Gordon JL, Ditto B, D'Antono B. Cognitive depressive symptoms associated with delayed heart rate recovery following interpersonal stress in healthy men and women. *Psychophysiology*. 2012; 49: 1082-1089.
47. Levesque K, Moskowitz DS, Tardif JC, Dupuis G, D'Antono B. Physiological stress responses in defensive individuals: Age and sex matter. *Psychophysiology*. 2010; 47: 332-341.
48. Levesque K, Bureau S, Moskowitz DS, et al. Defensiveness and metabolic syndrome: Impact of sex and age. *Biol Psychol*. 2009; 80: 354-360.
49. Boisclair Demarble J, Moskowitz DS, Tardif JC, D'Antono B. The relation between hostility and concurrent levels of inflammation is sex, age, and measure dependent. *J Psychosom Res*. 2014; 76: 384-393.
50. Dragomir AI, Gentile C, Nolan RP, D'Antono B. Three-year stability of cardiovascular and autonomic nervous system responses to psychological stress. *Psychophysiology*. 2014. doi: 10.1111/psyp.12231
51. D'Antono B, Moskowitz DS, Miners C, Archambault J. Gender and communal trait differences in the relations among social behaviour, affect arousal, and cardiac autonomic control. *J Behav Med*. 2005; 28: 267-279.
52. Rejeski WJ, Gagne M, Parker PE, Koritnik DR. Acute stress reactivity from contested dominance in dominant and submissive males. *Behav Med*. 1989; 15: 118-124.
53. Hoffman R, Al'Absi M. The effect of acute stress on subsequent neuropsychological test performance (2003). *Arch Clin Neuropsychol*. 2004; 19: 497-506.
54. Linden W, Lenz JW, Con AH. Individualized stress management for primary hypertension: A randomized trial. *Arch Intern Med*. 2001; 161: 1071-1080.
55. Sherwood A, Gullette EC, Hinderliter AL, et al. Relationship of clinic, ambulatory, and laboratory stress blood pressure to left ventricular mass in overweight men and women with high blood pressure. *Psychosom Med*. 2002; 64: 247-257.
56. Hermida RC, Smolensky MH, Ayala DE, et al. 2013 Ambulatory blood pressure monitoring recommendations for the diagnosis of adult hypertension, assessment of cardiovascular and other hypertension-associated risk, and attainment of therapeutic goals (summary). Joint recommendations from the International Society for Chronobiology (ISC), American Association of Medical Chronobiology and Chronotherapeutics (AAMCC), Spanish Society of Applied Chronobiology, Chronotherapy, and Vascular Risk (SECAC), Spanish Society of Atherosclerosis (SEA), and Romanian Society of Internal Medicine (RSIM). *Clin Investig Arterioscler*. 2013; 25: 74-82.
57. O'Brien E, Waeber B, Parati G, Staessen J, Myers MG. Blood pressure measuring devices: Recommendations of the European Society of Hypertension. *BMJ*. 2001; 322: 531-536.
58. Tarvainen MP, Ranta-Aho PO, Karjalainen PA. An advanced detrending method with application to HRV analysis. *IEEE Trans Biomed Eng*. 2002; 49: 172-175.
59. del Paso GA R, Langewitz W, Mulder LJ, van Roon A, Duschek S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: A review with emphasis on a reanalysis of previous studies. *Psychophysiology*. 2013; 50: 477-487.
60. Russell JA, Weiss A, Mendelsohn G. Affect Grid: A single-item scale of pleasure and arousal. *J Pers Soc Psychol*. 1989; 57: 493-502.
61. Marchand L, Stephenson R, Feeney S, Marchand A. *Validation factorielle de l'adaptation canadienne-française de l'Anxiety Sensitivity Scale*. Poster presented at the "67e Congrès annuel de l'Association Francophone pour le Savoir (ACFAS). 1999.
62. Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency and the prediction of fearfulness. *Behav Res Ther*. 1986; 24: 1-8.
63. Beck ATSR, Steer RA, Brown GK. *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation; 1996.
64. Dozois DJA, Dobson KS, Ahnberg JL. A psychometric evaluation of the Beck Depression Inventory-II. *Psychol Assess*. 1998; 10: 83-89.
65. Beck AT, Steer RA. *Manual for the Beck Anxiety Inventory*. San Antonio: Psychological Corporation; 1993.
66. Freeston MH, Ladouceur R, Thibodeau N, Gagnon F, Rheaume J. The Beck Anxiety Inventory. Psychometric properties of a French translation. *Encéphale*. 1994; 20: 47-55.

67. Kabacoff RI, Segal DL, Hersen M, Van Hasselt VB. Psychometric properties and diagnostic utility of the Beck Anxiety Inventory and the State-Trait Anxiety Inventory with older adult psychiatric outpatients. *J Anxiety Disord*. 1997; 11: 33-47.
68. Kamarck TW, Debski TT, Manuck SB. Enhancing the laboratory-to-life generalizability of cardiovascular reactivity using multiple occasions of measurement. *Psychophysiology*. 2000; 37: 533-542.
69. Schwartz AR, Gerin W, Davidson KW, et al. Toward a causal model of cardiovascular responses to stress and the development of cardiovascular disease. *Psychosom Med*. 2003; 65: 22-35.
70. Kamarck TW, Lovallo WR. Cardiovascular reactivity to psychological challenge: Conceptual and measurement considerations. *Psychosom Med*. 2003; 65: 9-21.
71. Llabre MM, Spitzer SB, Saab PG, Ironson GH, Schneiderman N. The reliability and specificity of delta versus residualized change as measures of cardiovascular reactivity to behavioral challenges. *Psychophysiology*. 1991; 28: 701-711.
72. Preacher KJ, Curran PJ, Bauer DJ. Computational tools for probing interactions in multiple linear regression, multilevel modeling, and latent curve analysis. *J Educ Behav Stat*. 2006; 31: 437-448.
73. Miyai N, Shiozaki M, Yabu M, et al. Increased mean arterial pressure response to dynamic exercise in normotensive subjects with multiple metabolic risk factors. *Hypertens Res*. 2013; 36: 534-539.
74. Deniz F, Katircibasi MT, Pamukcu B, Binici S, Sanisoglu SY. Association of metabolic syndrome with impaired heart rate recovery and low exercise capacity in young male adults. *Clin Endocrinol (Oxf)*. 2007; 66: 218-223.
75. Sung J, Choi YH, Park JB. Metabolic syndrome is associated with delayed heart rate recovery after exercise. *J Korean Med Sci*. 2006; 21: 621-626.
76. Brunner EJ, Hemingway H, Walker BR, et al. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: Nested case–control study. *Circulation*. 2002; 106: 2659-2665.
77. Tibana RA, Boullosa DA, Leicht AS, Prestes J. Women with metabolic syndrome present different autonomic modulation and blood pressure response to an acute resistance exercise session compared with women without metabolic syndrome. *Clin Physiol Funct Imaging*. 2013; 33(5):364-372
78. Allen MT, Bocek CM, Burch AE. Gender differences and the relationships of perceived background stress and psychological distress with cardiovascular responses to laboratory stressors. *Int J Psychophysiol*. 2011; 81: 209-217.
79. Carroll D, Phillips AC, Ring C, Der G, Hunt K. Life events and hemodynamic stress reactivity in the middle-aged and elderly. *Psychophysiology*. 2005; 42: 269-276.
80. Gianaros PJ, May JC, Siegle GJ, Jennings JR. Is there a functional neural correlate of individual differences in cardiovascular reactivity? *Psychosom Med*. 2005; 67: 31-39.
81. Gianaros PJ, Sheu LK, Matthews KA, et al. Individual differences in stressor-evoked blood pressure reactivity vary with activation, volume, and functional connectivity of the amygdala. *J Neurosci*. 2008; 28: 990-999.
82. Nugent AC, Bain EE, Thayer JF, Sollers JJ, Drevets WC. Sex differences in the neural correlates of autonomic arousal: A pilot PET study. *Int J Psychophysiol*. 2011; 80: 182-191.
83. Taylor SE, Klein LC, Lewis BP, et al. Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. *Psychol Rev*. 2000; 107: 411-429.
84. Lovallo WR. Do low levels of stress reactivity signal poor states of health? *Biol Psychol*. 2011; 86: 121-128.
85. Phillips AC, Roseboom TJ, Carroll D, de Rooij SR. Cardiovascular and cortisol reactions to acute psychological stress and adiposity: Cross-sectional and prospective associations in the Dutch Famine Birth Cohort Study. *Psychosom Med*. 2012; 74(7):699-710
86. Thayer JF, Lane RD. The role of vagal function in the risk for cardiovascular disease and mortality. *Biol Psychol*. 2007; 74: 224-242.
87. Weinberg A, Klonsky ED, Hajcak G. Autonomic impairment in borderline personality disorder: A laboratory investigation. *Brain Cogn*. 2009; 71: 279-286.
88. Cohen H, Kotler M, Matar MA, et al. Analysis of heart rate variability in posttraumatic stress disorder patients in response to a trauma-related reminder. *Biol Psychiatry*. 1998; 44: 1054-1059.
89. Singh K, Shen BJ. Abdominal obesity and chronic stress interact to predict blunted cardiovascular reactivity. *Int J Psychophysiol*. 2013; 90: 73-79.
90. Deschamps A, Denault A, Rochon A, et al. Evaluation of autonomic reserves in cardiac surgery patients. *J Cardiothorac Vasc Anesth*. 2013; 27: 485-493.
91. Phillips AC, Hunt K, Der G, Carroll D. Blunted cardiac reactions to acute psychological stress predict symptoms of depression five years later: Evidence from a large community study. *Psychophysiology*. 2011; 48: 142-148.
92. Salomon K, Clift A, Karlsdottir M, Rottenberg J. Major depressive disorder is associated with attenuated cardiovascular reactivity and impaired recovery among those free of cardiovascular disease. *Health Psychol*. 2009; 28: 157-165.
93. York KM, Hassan M, Li Q, et al. Coronary artery disease and depression: Patients with more depressive symptoms have lower cardiovascular reactivity during laboratory-induced mental stress. *Psychosom Med*. 2007; 69: 521-528.
94. Piccirillo G, Elvira S, Bucca C, et al. Abnormal passive head-up tilt test in subjects with symptoms of anxiety power spectral analysis study of heart rate and blood pressure. *Int J Cardiol*. 1997; 60: 121-131.
95. Knox SS, Hausdorff J, Markovitz JH. Coronary artery risk development in young adults S: Reactivity as a predictor of subsequent blood pressure: Racial differences in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Hypertension*. 2002; 40: 914-919.
96. Smith SC Jr, Clark LT, Cooper RS, et al. Discovering the full spectrum of cardiovascular disease: Minority Health Summit 2003: Report of the Obesity, Metabolic syndrome, and Hypertension Writing Group. *Circulation*. 2005; 111: e134-e139.
97. Iribarren C, Go AS, Husson G, et al. Metabolic syndrome and early-onset coronary artery disease: Is the whole greater than its parts? *J Am Coll Cardiol*. 2006; 48: 1800-1807.
98. Sundstrom J, Vallhagen E, Riserus U, et al. Risk associated with the metabolic syndrome versus the sum of its individual components. *Diabetes Care*. 2006; 29: 1673-1674.
99. Kahn R, Buse J, Ferrannini E, et al. The metabolic syndrome: Time for a critical appraisal: Joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*. 2005; 28: 2289-2304.
100. Guembe MJ, Toledo E, Barba J, et al. Association between metabolic syndrome or its components and asymptomatic cardiovascular disease in the RIVANA-study. *Atherosclerosis*. 2010; 211: 612-617.
101. Paul-Labrador M, Polk D, Dwyer JH, et al. Effects of a randomized controlled trial of transcendental meditation on components of the metabolic syndrome in subjects with coronary heart disease. *Arch Intern Med*. 2006; 166: 1218-1224.
102. Innes KE, Bourguignon C, Taylor AG. Risk indices associated with the insulin resistance syndrome, cardiovascular disease, and possible protection with yoga: A systematic review. *J Am Board Fam Pract*. 2005; 18: 491-519.
103. Nolan RP, Floras JS, Harvey PJ, et al. Behavioral neurocardiac training in hypertension: A randomized, controlled trial. *Hypertension*. 2010; 55: 1033-1039.

104. Palomba D, Ghisi M, Scozzari S, et al. Biofeedback-assisted cardiovascular control in hypertensives exposed to emotional stress: A pilot study. *Appl Psychophysiol Biofeedback*. 2011; 36: 185-192.
105. Nolan RP, Jong P, Barry-Bianchi SM, Tanaka TH, Floras JS. Effects of drug, biobehavioral and exercise therapies on heart rate variability in coronary artery disease: A systematic review. *Eur J Cardiovasc Prev Rehabil*. 2008; 15: 386-396.
106. Nyklicek I, Mommersteeg PM, Van Beugen S, Ramakers C, Van Boxtel GJ. Mindfulness-based stress reduction and physiological activity during acute stress: A randomized controlled trial. *Health Psychol*. 2013; 32: 1110-1113.