

Original Research Article

High-Protein Meal Challenge Reveals the Association Between the Salivary Cortisol Response and Metabolic Syndrome in Police Officers

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Objectives: Policing is considered a high-stress occupation and officers have elevated cardiovascular morbidity and mortality. To investigate a potential connection, we evaluated the association between salivary cortisol response to a high-protein meal challenge and the metabolic syndrome (MetSyn), a subclinical disorder associated with increased cardiovascular risk.

Methods: Cross-sectional data were from the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) Study (2004–2009). MetSyn was defined as having ≥ 3 components: abdominal obesity, hypertension, elevated triglycerides, reduced high-density lipoprotein cholesterol, and glucose intolerance. Officers provided five saliva samples for cortisol analysis, one before challenge (high-protein shake) and four at 15-min intervals thereafter, where the usual response is increase. Regression models were used to examine trends in mean number of MetSyn components across quartiles of area under the curve (AUC) salivary cortisol. Patterns of mean cortisol response were assessed by MetSyn status using repeated-measures analysis of covariance.

Results: Prevalence of MetSyn was 25.7% among 373 officers (74.0% male). The mean count of MetSyn components decreased (1.89, 1.75, 1.55, 1.37; $P < 0.01$) across increasing quartiles of AUC salivary cortisol. Mean salivary cortisol decreased from baseline (5.55, 4.58, 4.47, 4.79, 4.75 nmol/l) in officers with MetSyn and increased (5.08, 5.82, 5.92, 5.82, 5.60 nmol/l) in their counterparts. The test for interaction between MetSyn status and time of saliva collection was statistically significant ($P < 0.001$).

Conclusions: Reduced cortisol response to a high-protein meal challenge may be associated with MetSyn. Future longitudinal studies could provide useful evidence for planning intervention studies on cardiovascular risk among police officers. *Am. J. Hum. Biol.* 28:138–144, 2016. © 2015 Wiley Periodicals, Inc.

Work stress can be reflected in deleterious physical and emotional responses to job requirements (i.e., stressors) that differ from the capabilities, resources, or needs of the worker. In the United States, 40% of workers have reported that their job is “very or extremely stressful” (National Institute for Occupational Safety and Health, 1999). Effort-reward imbalance at work is related to increased cardiovascular risk (Peter et al., 1998; Siegrist et al., 1997; Vrijkotte et al., 1999). Policing is considered to be an inherently stressful occupation. Police officers have higher cardiovascular disease (CVD) morbidity and mortality than expected and have more adverse levels of traditional (e.g., dyslipidemia) and nontraditional (e.g., sudden physical exertion) CVD risk factors compared to the general population (Franke et al., 2002; Gershon et al., 2002; Hartley et al., 2011b; Joseph et al., 2009, 2010; Marmar et al., 2006; Zimmerman, 2012). CVD accounts for 7% of on-duty fatal events, a problem recognized by the Hometown Heroes Act of 2003 that provides death benefits for fatal heart attack or stroke associated with strenuous or stressful activities performed in the line of duty (Zimmerman, 2012). With 780,000 active-duty police officers in the United States, and an expected increase to 821,000 by 2022 (U.S. Bureau of Labor Statistics), there may be significant potential for public health impact through prevention of work-related illness.

The relationship between stress and CVD may develop in physiologic responses that can occur when an individual is exposed to a stressor and can involve activation of the hypothalamic-pituitary adrenal (HPA) axis. This axis is a major part of the neuroendocrine system that controls the reactions to stress or exposure and regulates many body processes. Functionally, this axis involves a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. Activation of the HPA axis results in a release of the glucocorticoid, cortisol, which can have metabolic consequences among its many effects. Responses to a stressor may also involve activation of the sympathetic nervous

Conflicts of interest and source of funding: No conflicts of interest are declared.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Contract grant sponsor: National Institute for Occupational Safety and Health; Contract grant number: 200-2003-01580.

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Received 20 March 2015; Accepted 13 May 2015

DOI: 10.1002/ajhb.22748

Published online 19 June 2015 in Wiley Online Library (wileyonlinelibrary.com).

system (SNS) which is constantly active at a basic level to maintain homeostasis. The SNS is best known for mediating the neuronal and hormonal stress response commonly known as the fight-or-flight response, and the products of this activation act on the cardiovascular system by resulting in increases in blood pressure and heart rate (Anagnostis et al., 2009; McEwen, 1998). Extended and/or repeated exposure to stressors resulting in dysregulation of the HPA axis and SNS, along with the physiologic processes they control, may be associated with the development of the metabolic syndrome (MetSyn) (Bjorntorp and Rosmond, 2000a; Brunner et al., 2002; Chandola et al., 2006), a clustering of risk factors that may progress to subclinical CVD, and ultimately to clinical presentation of CVD (Gade et al., 2010). For example, constant excessively high levels of cortisol such as those found in Cushing's disease result in a deposition of abdominal fat, one of the components of MetSyn (Lee et al., 2014). Although the relationship between stress and CVD develops gradually over time, an intermediate assessment of the functioning of the HPA axis, using a metabolic challenge and subclinical metabolic and CVD indices, may prove valuable for workers in an occupation more likely to develop CVD.

Food intake can be used as a challenge to assess the functioning of the HPA axis. Quigley and Yen (1979) described a novel observation of a midday surge of cortisol levels connected with food intake. The magnitude and time course of the midday peak were markedly attenuated by food deprivation (Quigley and Yen, 1979). Follenius and colleagues evaluated how diurnal status may play a role in food stimulated cortisol secretion. At midday, but not evening, a reproducible, rapidly increasing meal-related peak appeared in fasting and nonfasting subjects. The midday peak was observed in subjects with differing dietary schedules (Follenius et al., 1982). Gibson and colleagues then demonstrated that an increased cortisol response after a protein-rich meal could be measured in saliva and that this response was repeatable (Gibson et al., 1999). Salivary cortisol can offer a noninvasive measurement of stress, is an excellent indicator of the amount of biologically active (e.g., unbound) serum concentration of cortisol (Kirschbaum and Hellhammer, 1994). Thus, salivary cortisol levels in response to various conditions (e.g., time of day) and challenges (e.g., high-protein meal at midday) can provide information about the status and functioning of the HPA axis.

The components of the MetSyn: (1) abdominal obesity, (2) hypertension, (3) elevated triglycerides, (4) reduced high-density lipoprotein cholesterol, and (5) glucose intolerance provide an established method to identify risk for subclinical metabolic and CVD (Bjorntorp and Rosmond, 2000a). The connection between cortisol and abdominal obesity has been studied widely. Hypercortisolism and abdominal obesity induced by exposure to stressors are associated with a two to threefold increase in all-cause mortality risk and reduced life expectancy by several years (Chrousos, 2000). Dysregulation of the HPA axis has been observed with abdominal obesity (Duclos et al., 2005) and elevated waist-to-hip ratio, independent of body mass index (Ljung et al., 2000). Cortisol levels collected during perceived stressful events along with abdominal obesity have been associated with endocrine, metabolic, and hemodynamic complications (Rosmond et al., 1998). In contrast, little evidence was found that the MetSyn or its components were associated with cortisol output or

patterns in a population-based study of subclinical CVD (DeSantis et al., 2011). As exposure to frequently repeated or chronic environmental stress challenges is often associated with HPA axis abnormality (Rosmond and Bjorntorp, 2000), our study examined associations of HPA axis function with measures of subclinical metabolic and CVD among workers in the high-stress occupation of policing. This was accomplished by evaluation of the association between cortisol response to a high-protein challenge and MetSyn status and also by identification of which MetSyn components are most strongly associated with the cortisol response.

METHODS

Subjects were from the BCOPS Study, a cross-sectional study conducted during 2004–2009. All 710 police officers in the Buffalo, New York Police Department were invited to participate and 464 (65%) officers accepted. Of the 431 officers who were currently employed, 377 provided all needed salivary cortisol samples, and MetSyn status was known for 414 officers. A total of 373 police officers met both criteria and were included in the analytic sample. All subjects were sworn officers and willingly participated. The study protocol was approved by The State University of New York at Buffalo Institutional Review Board and the National Institute for Occupational Safety and Health Human Subjects Review Board.

Salivary cortisol

Prior to the clinic visit, officers were asked to refrain from use of stimulants or smoking on the day of the challenge and received a 290 calorie standardized breakfast (protein, 12 g; carbohydrate, 46 g; fat, 6 g) for a nonfasting cortisol sample. A standardized high-protein challenge was delivered in the clinic at midday, a time of day when this challenge provokes a response in the HPA axis similar to that from a stress stimulus (Follenius et al., 1982; Gibson et al., 1999; Quigley and Yen, 1979). The shake consisted of 55 g of a commercially-available protein powder mixed with carbohydrate powder in milk, corresponding to protein intake equivalent to two tuna sandwiches. The shake contained 710 calories (protein, 65 g; carbohydrate, 77 g; fat, 15 g).

Five saliva samples were collected using a commercial device, a Salivette®, consisting of an absorbent cotton roll, a plastic container with a perforated bottom, and a centrifuge tube that holds the plastic container and roll. A baseline sample was taken prior to ingestion of the high-protein shake, and four additional samples were taken beginning 15 min after the shake, and then 3 others at 15-min intervals thereafter. This approach of sequential sampling over time is more informative than a single measure in assessing how well the HPA axis is functioning because it shows the pattern of the response to the challenge. The usual response is a twofold–fourfold increase after a high-protein meal. In comparison, the cortisol response is diminished for an individual experiencing chronic or prolonged stressor exposure (Gibson et al., 1999; Rosmond and Bjorntorp, 1998; Rosmond et al., 1998).

Following collection, samples were stored at 4°C and later shipped to the National Institute for Occupational Safety and Health where they were centrifuged to provide a nonviscous saliva sample. Prepared samples were stored at –20°C until assayed for cortisol by a time-resolved

immunoassay with fluorescence detection (DELFA) (Dressendorfer et al., 1992) at the University of Dresden, Dresden, Germany. For quality control purposes, each batch contained samples of sufficient number to include a low, medium, and high concentration quality control sample on each assay plate to allow assessment of laboratory drift. Also included were 5% blind replicate samples of participants' saliva for general quality control purposes. Samples with values outside of normal range were re-assayed. These procedures are a modified version of those employed by Gibson and colleagues (Gibson et al., 1999). The protein shake was a substitute for an actual meal for convenience of administration during the officers' daylong clinic visit that involved several other clinical measurements.

Metabolic syndrome

The MetSyn criteria were based on the National Cholesterol Education Program Adult Treatment Panel guidelines with recent modifications from the American Heart Association and the National Heart, Lung, and Blood Institute (Grundey et al., 2005). The five MetSyn components include: (1) elevated waist circumference (≥ 102 cm in men, ≥ 88 cm in women), (2) elevated triglycerides (≥ 150 mg/dl or reported treatment with nicotinic acid or fibrates), (3) reduced high-density lipoprotein cholesterol (HDL-C < 40 mg/dl in men, < 50 mg/dl in women, or reported treatment with nicotinic acid or fibrates), (4) elevated glucose (≥ 100 mg/dl or reported treatment for diabetes), and (5) elevated blood pressure (BP $\geq 130/85$ mm Hg or reported physician-diagnosed hypertension and antihypertensive treatment). An individual was considered to have this syndrome if three or more of the five components were present.

The specific laboratory and clinic procedures were conducted by trained laboratory personnel. The procedures were measurement of waist circumference (abdominal girth at the highest point of the iliac crest and the lowest point of the costal margin in the mid-axillary line), collection of a 12-h fasting blood sample by a certified phlebotomist, and blood pressure using the average of the second and third of three separate measurements of resting systolic and diastolic blood pressure obtained with a standard sphygmomanometer while in the clinic. Blood parameters were measured by standard laboratory techniques on the Beckman Coulter LX20 clinical chemistry analyzer and included a blood lipid panel for HDL-C and triglycerides and chemistry panels for glucose. Medication use was ascertained through self-report and by inventory of current medications brought to the clinic.

Statistical methods

We used analysis of variance (ANOVA) to characterize the trend in the mean number of MetSyn components across quartiles of the total salivary cortisol response, expressed as area under the curve (AUC) relative to the baseline salivary cortisol measurement (Fekedulegn et al., 2007). Linear regression was used to assess the statistical significance of this relationship. In this analysis, the overall cortisol response was conceptualized as the independent variable. In contrast, we also conceptualized the salivary cortisol response as the dependent variable and used repeated-measures ANOVA to assess the mean salivary cortisol response over time by MetSyn status and

TABLE 1. Demographic, lifestyle, and physiological characteristics

Characteristics ^{a,b}	Total (N = 373) N (%)
Male	276 (74.0)
MetSyn components	
Abdominal obesity	113 (30.3)
Elevated blood pressure	142 (38.1)
Elevated fasting glucose	86 (23.1)
Reduced HDL cholesterol	156 (41.8)
Elevated triglyceride	116 (31.1)
MetSyn	96 (25.7)
Mean (S.D.)	
Age (years)	41.0 (7.1)
Police service (years)	14.5 (7.5)
Alcohol (drinks/week)	5.0 (8.0)
Body mass index (kg/m ²)	29.0 (4.5)
Systolic blood pressure (mm Hg)	120.7 (12.3)
Diastolic blood pressure (mm Hg)	77.3 (10.2)
Physical activity/week (METS)	284.2 (46.3)
Abdominal height (cm)	20.7 (3.4)
Waist circumference (cm)	93.8 (14.1)
Fasting glucose (mg/dl)	92.6 (13.3)
HDL cholesterol (mg/dl)	46.3 (14.7)
Triglycerides (mg/dl)	136.7 (130.7)
Mean number of components	1.6 (1.4)

^aFor categorical variables, the values are numbers and percentages.

^bFor continuous variables, the values are means and standard deviations.

by the status of individual MetSyn components. Repeated-measures ANOVA accounted for the lack of independence between the repeated salivary cortisol measurements and modeled the within-subject correlation using an unstructured covariance model. We used this approach to test the null hypothesis that the two time courses were parallel by assessing the significance of the interaction term between the timing of the cortisol sample and MetSyn status. The cortisol concentration data were log-transformed due to its skewed nature and to approximate a normal distribution for the analyses. The reported results are back-transformed values. All models were adjusted for age and sex, except the unadjusted models that estimated the effect of each MetSyn component.

RESULTS

Table 1 presents characteristics of the 373 police officers of which 276 were men and 97 were women. On average, officers were 41 years of age and had 14.5 years of police service. The average number of MetSyn components was 1.6 overall and 25.7% of the officers met the criteria for the MetSyn. The most prevalent component was reduced HDL-C (41.8%) and the least prevalent was elevated fasting glucose (23.1%). Several officer characteristics were identified from the literature as potential confounders and evaluated by their associations with MetSyn and salivary cortisol (Table 2). Based on these associations, the final models were only age and sex-adjusted. Table 3 presents the mean salivary cortisol concentration by number of MetSyn components. The mean salivary cortisol concentration increased following the high-protein shake for officers with fewer than three components, while officers with three or more components experienced a decline in mean salivary cortisol concentration. On average, an increasing number of components showed a more diminished cortisol response.

Table 4 presents the ANOVA and ANCOVA results. A statistically significant decrease was identified in the mean number of MetSyn components across increasing

TABLE 2. Demographic, lifestyle, and physiological characteristics by number of MetSyn components and AUC_i in salivary cortisol

Characteristics	N	%	MetSyn Components		Salivary Cortisol AUC _i	
			Mean	S.D.	Mean	S.D.
Age group (years)						
< 40	158	42.4	1.35	1.32	21.66	284.89
40–49	167	44.8	1.72	1.46	12.86	267.51
≥ 50	48	12.9	2.35	1.47	43.62	323.66
P-value ^a			<.0001		0.6232	
Sex						
Male	276	74.0	1.92	1.44	1.28	288.39
Female	97	26.0	0.85	1.10	75.36	202.50
P-value ^b			<.0001		0.0201	
Race						
White	281	76.8	1.63	1.42	20.34	272.63
Black	79	21.6	1.63	1.47	17.15	276.08
Hispanic	6	1.6	1.83	1.17	–46.25	81.30
P-value ^b			0.9400		0.8371	
Education						
≤ High school/GED	38	10.2	1.87	1.34	30.00	277.73
College < 4 yrs	202	54.5	1.62	1.43	34.18	286.60
College ≥ 4 yrs	131	35.3	1.63	1.48	–3.81	241.57
P-value ^a			0.3771		0.4981	
Years of service						
0–9 yrs	104	28.0	1.23	1.25	41.00	300.86
10–14 yrs	84	22.6	1.49	1.48	–7.13	266.37
15–19 yrs	83	22.4	1.78	1.35	17.53	309.53
≥ 20 yrs	100	27.0	2.11	1.53	25.87	199.95
P-value ^a			<.0001		0.8646	
Smoking status						
Current	62	16.8	1.55	1.47	30.35	303.27
Former	83	22.5	1.76	1.57	78.53	282.95
Never	224	60.7	1.65	1.39	–1.39	255.39
P-value ^b			0.6785		0.0695	
Rank						
Police officer	274	73.9	1.53	1.40	21.44	265.31
Sergeant/Lieutenant	48	12.9	1.96	1.56	13.67	303.07
Captain/Detective	49	13.2	2.02	1.48	24.00	273.90
P-value ^b			0.6330		0.9795	
Alcohol drinks/week						
0	71	19.4	1.32	1.27	64.66	258.13
< 1	61	16.7	1.67	1.50	1.33	290.94
1–6	143	39.1	1.79	1.40	2.97	262.74
≥ 6	91	24.9	1.58	1.51	21.84	272.77
P-value ^a			0.2091		0.3459	
Physical activity intensity						
0–1.50	93	24.9	1.78	1.50	–31.47	249.38
1.51–6.50	90	24.1	1.57	1.37	45.63	261.77
6.51–14.50	93	24.9	1.80	1.42	20.51	279.43
≥ 14.51	97	26.0	1.43	1.46	47.18	285.43
P-value ^a			0.2112		0.0898	

^aTest for trend.^bANOVA (test of differences in means).AUC_i (area under the curve increase).

TABLE 3. Mean salivary cortisol concentration (nmol/l) by number of MetSyn components

Number of components	N	Baseline	+15 min	+30 min	+45 min	+60 min
0	101	4.76 (1.82)	5.42 (1.84)	5.47 (1.86)	5.64 (1.90)	5.75 (1.86)
1	91	6.42 (1.95)	7.10 (1.92)	7.61 (1.75)	7.10 (1.92)	6.62 (1.90)
2	85	5.21 (2.01)	6.23 (1.70)	6.05 (1.75)	5.99 (1.75)	5.53 (1.82)
3	49	6.49 (2.05)	5.93 (2.18)	5.58 (2.36)	5.58 (1.88)	5.64 (1.72)
4	30	5.31 (2.03)	4.53 (1.97)	4.39 (1.79)	5.31 (1.68)	4.95 (1.82)
5	17	7.32 (1.45)	4.22 (3.16)	4.66 (2.69)	4.95 (2.41)	5.21 (1.77)

Standard deviation values in parentheses.

quartiles of AUC salivary cortisol. The mean number of MetSyn components was 2.03 (S.D. 1.54) for officers in the first quartile of AUC salivary cortisol and 1.35 (S.D. 1.36) for officers in the fourth quartile. The trend differed very little after adjustment for age and sex.

Figure 1 displays results from the repeated-measures ANOVA where we assessed the age and sex-adjusted

mean cortisol response following the high-protein shake by MetSyn status. Individuals with the MetSyn had a diminished response following the shake, while individuals without the MetSyn showed an initial increase after the shake followed by a gradual decline. Effect modification between MetSyn status and time since the high-protein shake was statistically significant ($P = 0.0002$).

TABLE 4. Mean number of MetSyn components by quartiles of AUC_i in salivary cortisol (nmol/l)

Salivary Cortisol AUC _i	N	Unadjusted		Age-adjusted		Age- and sex-adjusted	
		Mean	S.D.	Mean	S.E.	Mean	S.E.
Q1	93	2.03	1.54	2.00	0.14	1.89	0.14
Q2	93	1.63	1.41	1.72	0.14	1.75	0.14
Q3	92	1.54	1.37	1.50	0.14	1.55	0.14
Q4	94	1.35	1.36	1.34	0.14	1.37	0.13
P-value		0.0007		0.0004		0.0030	

Q1: (−1,265.87 to −106.775), Q2: (−106.776 to 19.51), Q3: (19.52 to 143.565), Q4: (143.566 to 1,067.70).
AUC_i (area under the curve increase).

Table 5 presents the age- and sex-adjusted results for the repeated measures ANOVA by MetSyn status and unadjusted results for the status of the individual MetSyn components. Effect modification was statistically significant between time since the high-protein shake and the individual components of abdominal obesity ($P = 0.0167$) and hypertension ($P = 0.0356$). Effect modification was not statistically significant between time since the high-protein shake and elevated triglycerides ($P = 0.0504$), reduced HDL-C ($P = 0.0797$), or with glucose intolerance ($P = 0.5410$). Although for elevated triglycerides, there is little difference following the sample at 15 min after the shake, but a slight shift appeared in the two response patterns.

DISCUSSION

This study demonstrates a link between HPA axis functioning and the MetSyn in subjects who are members of an occupational group that is subjected to a variety of stressors and has an increased risk of developing CVD (Gade et al., 2010; Zimmerman, 2012). Subjects from this cohort have previously been shown to have more subclinical CVD, increased levels of atherosclerosis (Joseph et al., 2009) and decreased endothelial function, as compared with a similarly aged civilian population sample from the same geographical region (Joseph et al., 2010). After the protein shake challenge, HPA axis functioning was assessed by the total salivary cortisol response above the baseline measurement using AUC (Fekedulegn et al., 2007), and as a cortisol response over time across the individual cortisol measurements. Associations between these responses and MetSyn and several of its components were statistically significant and in the expected direction (Bjorntorp and Rosmond, 2000a; Brunner et al., 2002; Chandola et al., 2006). Overall, the total salivary cortisol response was inversely associated with an increasing number of MetSyn components present, an indicator of the severity of subclinical disease. The cortisol response over time was associated with MetSyn and subsequently with two of the five MetSyn components (abdominal obesity and hypertension) individually. Individuals with the MetSyn had a diminished response following the protein shake, while individuals without the MetSyn had a more typical response. The early cortisol response to the high-protein challenge in these models resembles the results of Gibson and colleagues (Gibson et al., 1999) and earlier studies involving other types of food challenges (Follenius et al., 1982; Quigley and Yen, 1979). Our results differ from those of the Multi-Ethnic Study of Atherosclerosis (MESA) that found little evidence that the MetSyn or its components were associated with cortisol output or patterns in a group of subjects without clinical diabetes

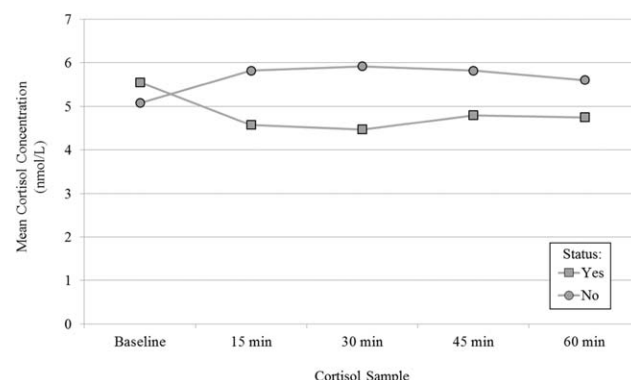


Fig. 1. Adjusted mean salivary cortisol response by metabolic syndrome status ($N = 373$, $P = 0.0002$) nmol/l.

(DeSantis et al., 2011). These contrasting results may be because subjects were from a population-based study who may not experience the repeated or chronic environmental stress challenges often associated with HPA axis abnormality (Rosmond and Bjorntorp, 2000). Likely most important, the cortisol awakening response, cortisol decline across the waking day, and total cortisol output were used in the MESA study (DeSantis et al., 2011) as opposed to a high-protein challenge.

Among the individual MetSyn components, irregularities in cortisol levels have been linked with abdominal obesity most often (Bjorntorp and Rosmond, 2000b; Chrousos, 2000; Duclos et al., 2005; Ljung et al., 2000; Rosmond et al., 1998). Given that the cortisol response can reflect dysregulation in the HPA axis, an association would be expected with both abdominal obesity and fasting glucose as cortisol acts to increase the supply of blood glucose for tissues during exposure to stressors (Rosmond et al., 1998). Among the associations between the cortisol response across time and the individual components, abdominal obesity was most strongly associated with the cortisol response in our study. These results agree with a related study of variability in cortisol among these subjects in which visceral adiposity, as measured by lean trunk mass index, was consistently associated with within-subjects cortisol variability, but not with the within-subjects cortisol mean (Sharp et al., 2013).

Although fasting glucose was not associated with the cortisol response across time, subjects with glucose intolerance had a slightly more suppressed response overall compared with subjects without glucose intolerance. The lack of significant effect modification by glucose intolerance status may be due in part to the small percentage of officers having glucose intolerance, but sensitivity

TABLE 5. Mean salivary cortisol response (nmol/l) by MetSyn and component status

	Baseline	+15 min	+30 min	+45 min	+60 min	P-value ^a
MetSyn ^b						
Yes	5.55 (1.04)	4.58 (1.04)	4.47 (1.04)	4.79 (1.04)	4.75 (1.04)	0.0002
No	5.08 (1.07)	5.82 (1.07)	5.92 (1.07)	5.82 (1.07)	5.60 (1.07)	
Abdominal obesity						
Yes	6.05 (1.07)	5.39 (1.07)	5.34 (1.06)	5.43 (1.06)	5.31 (1.06)	0.0167
No	5.42 (1.04)	6.13 (1.04)	6.22 (1.04)	6.22 (1.04)	6.01 (1.04)	
Elevated blood pressure						
Yes	5.91 (1.06)	5.64 (1.06)	5.48 (1.06)	5.77 (1.06)	5.76 (1.05)	0.0356
No	5.42 (1.05)	6.06 (1.05)	6.24 (1.04)	6.09 (1.04)	5.81 (1.04)	
Elevated triglycerides						
Yes	6.30 (1.06)	5.77 (1.07)	5.75 (1.06)	6.06 (1.06)	5.77 (1.06)	0.0504
No	5.31 (1.04)	5.96 (1.04)	6.02 (1.04)	5.93 (1.04)	5.80 (1.04)	
Reduced HDL cholesterol						
Yes	6.14 (1.06)	5.90 (1.06)	5.85 (1.06)	5.96 (1.05)	5.63 (1.05)	0.0797
No	5.25 (1.05)	5.89 (1.05)	6.00 (1.05)	5.98 (1.04)	5.91 (1.04)	
Glucose intolerance						
Yes	5.24 (1.08)	5.08 (1.08)	5.25 (1.07)	5.37 (1.07)	5.02 (1.07)	0.5410
No	5.72 (1.04)	6.17 (1.04)	6.16 (1.04)	6.16 (1.04)	6.04 (1.04)	

^aThe P-value is for the interaction term between the timing of the cortisol sample and MetSyn or component status.

^bAge and sex-adjusted model. Models for individual components are unadjusted. Standard error values in parentheses.

analyses using tertiles or quartiles of fasting glucose did not indicate significant effect modification either. The lack of a significant result may be a consequence of studying active duty police officers. Type 2 diabetes is less prevalent among police officers than in the general population and more similar after retirement (Zimmerman, 2012), perhaps glucose intolerance follows a similar pattern. It was the least prevalent MetSyn component among these officers.

As a clustering of risk factors for CVD, the MetSyn was associated with the cortisol response across time, but the individual components of elevated triglycerides and reduced HDL-C were not. These two components could be working through the autonomic nervous system pathway; and while related to the other components as a syndrome, they were not individually related to the cortisol response. Hartley et al. (2011a) investigated sex differences in the associations between the perceived stress of police work, as measured by self-report using the Spielberger Police Stress Survey, and MetSyn in these subjects. Associations were identified with MetSyn, and specifically with abdominal obesity and reduced HDL-C, among female officers only (Hartley et al., 2011a; Spielberger et al., 1981). There are some similarities with our results, but sex differences in self-reporting of perceived stress may account for the lack of significant findings among male officers.

Among the strengths of this study are that a standardized laboratory protocol was used to gather information to assess salivary cortisol and MetSyn among the police officers (Bjorntorp and Rosmond, 2000a; Rosmond et al., 1998). Salivary cortisol is a physiologic measure of HPA axis function and a more quantitative marker of the response to a stressor or challenge than self-reported measures of stress. And, assessing the response to a high-protein challenge is fairly unique and use of a shake may be a more accessible protein source than a traditional meal. Even though salivary cortisol is a noninvasive method of assessing the functioning of the HPA axis and the values reflect biologically active cortisol, the relationship between salivary cortisol levels and the MetSyn following a specific food-related challenge has received little attention compared to those of more traditional

challenges such as a dexamethasone-suppression test (de Kloet et al., 2006; Gibson et al., 1999; Kirschbaum and Hellhammer, 1994).

Limitations of the study include the cross-sectional design that prevents assessment of a causal relationship. Lifestyle factors (alcohol intake, smoking, and physical activity) were self-reported. Selection bias may be present through exclusion of officers who did not provide complete cortisol samples or for whom MetSyn status was unknown. As compared to the 373 officers in the sample, the officers who were excluded had a higher average age, years of police service, abdominal height, fasting glucose, systolic and diastolic blood pressure, and alcohol consumption per week, and a lower percentage of never smokers. Exclusion of these officers may have biased the results toward the null and decreased associations. Use of a healthy, working population prevents generalization of results to the general population. Also, there are mixed results as to whether the percent of dietary calories from protein could be associated with the cortisol response to a high-protein challenge (Gibson et al., 1999). The percent of dietary calories from protein, as derived from food frequency questionnaire responses, was examined as a potential confounder in a post hoc analysis and did not influence our original results.

CONCLUSION

A reduced cortisol response to a high-protein challenge is associated with MetSyn and several of its components in Buffalo police officers. In the context of preventing sub-clinical CVD in police officers, these laboratory-based results redirect attention to development of activities that effectively reduce the physiological and psychological responses to stressor exposure and the prevalence of the various MetSyn components. A longitudinal study of these officers is underway that could provide additional useful evidence for the design of intervention studies on cardiovascular risk among police officers. In the meantime, general interventions to reduce exposure and the response to workplace stressors as well as the prevalence of MetSyn may benefit these police officers.

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