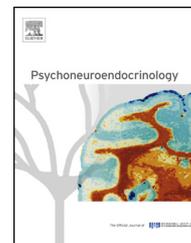




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# Chronic stress increases vulnerability to diet-related abdominal fat, oxidative stress, and metabolic risk

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Received 16 October 2013; received in revised form 5 March 2014; accepted 2 April 2014

## KEYWORDS

Psychological stress;  
Obesity;  
Abdominal adiposity;  
Metabolic syndrome;  
Pre-diabetes

## Summary

**Background:** In preclinical studies, the combination of chronic stress and a high sugar/fat diet is a more potent driver of visceral adiposity than diet alone, a process mediated by peripheral neuropeptide Y (NPY).

**Methods:** In a human model of chronic stress, we investigated whether the synergistic combination of highly palatable foods (HPF; high sugar/fat) and stress was associated with elevated metabolic risk. Using a case-control design, we compared 33 post-menopausal caregivers (the chronic stress group) to 28 age-matched low-stress control women on reported HPF consumption (modified Block Food Frequency Questionnaire), waistline circumference, truncal fat ultrasound, and insulin sensitivity using a 3-h oral glucose tolerance test. A fasting blood draw was assayed for plasma NPY and oxidative stress markers (8-hydroxyguanosine and F2-Isoprostanes).

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<http://dx.doi.org/10.1016/j.psyneuen.2014.04.003>

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**Results:** Among chronically stressed women only, greater HPF consumption was associated with greater abdominal adiposity, oxidative stress, and insulin resistance at baseline (all  $p$ 's  $\leq .01$ ). Furthermore, plasma NPY was significantly elevated in chronically stressed women ( $p < .01$ ), and the association of HPF with abdominal adiposity was stronger among women with high versus low NPY. There were no significant predictions of change over 1-year, likely due to high stability (little change) in the primary outcomes over this period.

**Discussion:** Chronic stress is associated with enhanced vulnerability to diet-related metabolic risk (abdominal adiposity, insulin resistance, and oxidative stress). Stress-induced peripheral NPY may play a mechanistic role.

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## 1. Introduction

Metabolic syndrome has reached epidemic proportions, affecting 20–30% of adults worldwide (Grundey, 2008). The implications for the future burden of chronic disease are grave, as metabolic syndrome doubles the risk of cardiovascular disease and increases the risk for type 2 diabetes by five-fold (Grundey, 2008). Although metabolic syndrome is defined as a cluster of medical conditions, abdominal adiposity and insulin resistance are core features, even among those of normal weight (Abbasi et al., 2004; Voulgari et al., 2011). Chronic psychological stress is an emerging risk factor that prospectively predicts metabolic syndrome (Pyykkonen et al., 2010), abdominal fat (Marniemi et al., 2002), and obesity (Brunner et al., 2007). Furthermore, stress-reduction can improve glycemic control in type 2 diabetics (Ismail et al., 2004). Yet, stress reduction has remained essentially an afterthought within the Western medical model, representing a lost opportunity to improve prevention and management.

Stress may promote overeating and physical inactivity, thereby contributing to metabolic risk (Epel et al., 2004a). In addition, there appears to be another important, but under-explored, physiological pathway. Preclinical studies find that chronic stress activates peripheral mechanisms within adipose tissue, which augment the adverse effects of sugar and fat on visceral tissue accumulation (Kuo et al., 2007). In mice fed a high fat/high sugar diet, those mice exposed to chronic stress developed visceral adiposity and metabolic syndrome at a considerably faster rate than their non-stressed counterparts (Kuo et al., 2007). A key biological mechanism for this synergistic interaction is the peripheral action of neuropeptide Y (NPY), released from sympathetic nerve terminals innervating visceral adipose tissue, which stimulates adipocyte growth and rapid expansion of visceral fat mass in response to stress (Kuo et al., 2007).

Excess intake of fat and sugar leads to oversupply of energy substrate (Picard and Turnbull, 2013), which increases the production of reactive oxygen species (ROS) by mitochondria and causes oxidative stress (Anderson et al., 2009). One rodent study has shown that the combination of chronic stress and a high fat/high sugar diet led to greater oxidative stress markers of fatty liver disease (Fu et al., 2010). Elevated markers of oxidative stress are associated with human obesity (Keaney et al., 2003), diabetes (Keaney et al., 2003), and cardiovascular mortality (Roest et al., 2008). Further,

8-hydroxyguanosine (8-oxoG), a marker of RNA oxidation, prospectively predicts long-term mortality among patients with diabetes (Broedbaek et al., 2011). Chronic psychological stress (e.g., caring for a spouse with dementia) has also been associated with heightened levels of both 8-oxoG and the oxidative byproduct F2-Isoprostanes (Epel et al., 2004b; Aschbacher et al., 2013). In turn, oxidative stress can induce insulin resistance (Ceriello and Motz, 2004; Hoehn et al., 2009), fomenting the development of metabolic syndrome (Bremer et al., 2012). However, as yet, no study has assessed the synergistic effects of psychological stress and diet on oxidative stress, insulin resistance and adiposity in humans.

The current study utilized a dementia caregiving model of chronic stress exposure among post-menopausal women and age-matched, non-caregiving low-stress control women in a case-control design. We hypothesized that the synergistic combination of exposure to chronic stress (defined as being a caregiver) and greater highly palatable food (HPF) consumption would be associated with significantly higher waistline circumference and truncal fat (abdominal adiposity), markers of oxidative damage and insulin resistance. These relationships were examined both cross-sectionally at baseline and prospectively over 1 year. In addition, we hypothesized that the chronic stress group would have higher NPY levels relative to low-stress controls, supporting the peripheral mechanism of sympathetic innervation of adipocytes established in rodent models.

## 2. Methods

### 2.1. Participants

Sixty-three non-smoking, post-menopausal women participated in a larger study of chronic stress, metabolism, and cellular aging, as described previously (Epel et al., 2010). Women 50–80 years old were recruited from within the larger San Francisco Bay area using flyers, and community advertisements in newspapers, online and on radio stations. Data for HPF were not available for two women, precluding their inclusion in this investigation. Of the remaining 61 women, the chronic stress group (CS) consisted of 33 women providing care for a spouse or parent with dementia (average years of care = 4.7; range: 0.5–16.5 years). Antidepressant use was permitted among CS women, because excluding it would have biased the sample toward highly resilient individuals, whereas the overarching study sought to understand

both the mental and physical effects of chronic stress (e.g., previous publications in this sample have explored the prospective relations between CS and depressive symptoms (Aschbacher et al., 2012)). The low stress group (LS) consisted of 28 women of similar age (within a 4-year range) who were not caregiving, who scored below the national mean on a well-validated measure of chronic stress, the 10-item Perceived Stress Scale (PSS < 17) (Cohen and Janicki-Deverts, 2012), and were not taking antidepressants. Table 1 provides group comparisons on eligibility PSS scores, demographics, and other relevant health factors. General study exclusion criteria included chronic medical conditions (cardiovascular disease, cancer, diabetes, and autoimmune diseases), current smoking, or use of medications known to affect stress-responsive biomarkers. The mean age was 62 years (range: 51–79), the median income was \$70,000–79,000, and 66% of participants had completed a Bachelor's or advance degree. The sample was 80% Caucasian, 3% African American, 2% Latina/Hispanic, 10% Asian/Pacific Islander, 2% other, and 3% declined to answer. The study protocol was approved by the Committee for Human Research of the University of California, San Francisco.

## 2.2. Highly palatable food (HPF)

Self-reported dietary consumption was assessed at baseline using a modified version of the previously published 29-item version of the Block Food Frequency Questionnaire (FFQ) (Groesz et al., 2012). The 29-item FFQ is an abbreviated version of the 100-item FFQ, which has been validated against nutrient consumption (Block et al., 1990). HPF was assessed using a four-item subscale, inquiring about the

frequency of similarly categorized foods – e.g., chocolate, candy bars, cakes, cookies and brownies, etcetera. The response options ask “how often” a food item was consumed and response categories range from “never” (0) to “more than once a day” (5). The scale is deemed appropriate for comparing *relative individual differences* in HPF consumption, but not absolute caloric intake.

## 2.3. Blood draw procedure

Between 0730 and 0800 h, participants came into the Clinical Research Center at University of California San Francisco. A venous forearm catheter was inserted, participants rested for 20 min, and a fasting blood sample was drawn, which was assayed for peripheral NPY and oxidative stress measures.

## 2.4. Adiposity measures

Dual-energy X-ray absorptiometry (DXA), a gold-standard method for assessing abdominal fat depots, was used to assess truncal fat (kg). Truncal fat and leg fat have been shown to have opposite relations with fasting and post-load glucose, suggesting differences in the underlying fat tissue (Snijder et al., 2004); therefore, we focused on truncal fat, which is most likely to contribute to or correlate positively with insulin resistance. Subjects underwent whole-body DXA scans on a Lunar Prodigy densitometer. Post hoc manual analysis of fat in the arms, legs, and trunk was performed using skeletal and soft-tissue landmarks, as first described by Lo et al. (1998). The trunk region was defined by an upper horizontal border at the lower edge of the chin, lateral borders formed by vertical lines which bisected each axilla

**Table 1** Group mean comparisons on demographics, psychosocial factors, and metabolic risk.

	Chronic stress	Low stress	Statistical test	p-Value
<b>Demographic characteristics</b>				
Age, years <sup>a</sup>	62 (7)	62 (6)	$t(59) = -0.50$	.62
Non-Caucasian ethnicity <sup>b</sup>	6 (19%)	4 (15%)	FET	.74
College or higher education <sup>b</sup>	20 (63%)	19 (70%)	$\chi^2(1) = 0.41$	.53
Below median income <sup>b</sup>	15 (50%)	14 (54%)	$\chi^2(1) = 0.08$	.77
<b>Lifestyle/health factors</b>				
Perceived stress scale <sup>a</sup>	20.27 (5.71)	5.30 (3.84)	$t(53.15) = -11.56$	.001 <sup>*</sup>
Highly palatable food <sup>a</sup>	1.42 (0.75)	1.39 (0.65)	$t(59) = -0.16$	.87
Low physical activity <sup>b</sup>	22 (69%)	11 (41%)	$\chi^2(1) = 4.66$	.03 <sup>*</sup>
NSAIDs <sup>b</sup>	14 (44%)	4 (15%)	FET	.02 <sup>*</sup>
<b>Cardiometabolic risk</b>				
Body mass index <sup>a</sup>	26.35 (4.93)	26.29 (5.33)	$t(59) = -0.50$	.96
Triglycerides <sup>a</sup>	99.67 (42.36)	102.96 (41.98)	$t(57) = 0.30$	.77
HDL <sup>a</sup>	66.87 (19.66)	65.29 (15.92)	$t(57) = -0.34$	.74
LDL <sup>a</sup>	117.71 (29.90)	106.61 (35.40)	$t(57) = -1.30$	.20
Fasting glucose (mg/dl) <sup>a</sup>	94.34 (8.82)	97.43 (10.50)	$t(58) = 1.24$	.22
Systolic blood pressure <sup>a</sup>	133.58 (18.81)	131.25 (19.07)	$t(56) = -0.47$	.64
Diastolic blood pressure <sup>a</sup>	77.12 (8.70)	75.82 (11.37)	$t(56) = -0.49$	.63

Critical alpha = .05. FET = Fisher's exact test, 2-sided. College or higher education was categorized as participants holding a Bachelor's or an advanced educational degree.

<sup>a</sup> Mean (SD).

<sup>b</sup> n (%).

<sup>\*</sup>  $p \leq .05$ .

and which were oriented obliquely to include the waist, hip, buttock and thigh tissue, and a lower border formed by the intersection of oblique lines extending from the level of the superior aspect of the iliac crest and passing through the hip joint. The coefficients of variation for repeated analyses of the same scans are 0.8, and 1.3% for total and trunk fat, respectively. For comparison, we also included a simpler measure that can be easily integrated into large studies: waistline circumference (cm) at the narrowest point, which was measured twice by trained research assistants and averaged.

## 2.5. Neuropeptide Y (NPY) assay

NPY levels in plasma obtained from peripheral blood were measured by ELISA (EMD Millipore, St. Charles MO). This assay is a Sandwich ELISA based on: (1) capture of NPY in the sample by anti-human NPY IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies, (2) binding of a second biotinylated antibody to NPY after brief washings, (3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, (4) wash away of free enzyme, and (5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically at 450 nm, and corrected from the absorbency at 590 nm after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured NPY in the unknown sample, the concentration of NPY is derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human NPY. The intra-assay and inter-assay coefficients of variation were 5 and 14% respectively.

## 2.6. Oxidative damage assays

Blood serum from fasting blood samples were assayed for 8-hydroxyguanosine (8-OxoG) and 8-iso-prostaglandin F<sub>2α</sub> (IsoP) using the API 4000™ QTRAP® LC/MS/MS System (Kronos Science Laboratory) per a previously published protocol (Aschbacher et al., 2013). Serum samples were combined with an internal standard of either O<sup>18</sup>-8-hydroxy-2'-deoxyguanosine (for 8-OxoG) or 8-isoprostaglandin F<sub>2α</sub>-d4 (for IsoP) and an organic solvent was added to precipitate proteins. 8-OxoG (ng/mL) and IsoP (ng/mL) were quantified using the ion pairs of *m/z* 300/168 and 353/193 (respectively) investigated under multiple reaction-monitoring (MRM) detection mode. The coefficients of variation for ranged from 4 to 8% (intra) and 8 to 12% (inter) for 8-OxoG and from 4 to 7% (intra) and 6 to 11% (inter) for IsoP.

## 2.7. Insulin sensitivity

Participants underwent a 3-h oral glucose tolerance test (OGTT), conducted immediately following the fasting blood draw, and the composite insulin sensitivity index was determined from multiple glucose and insulin measures taken over this 3-h period, per the following standard formula: 10,000/

$\sqrt{[(\text{fasting glucose} \times \text{fasting insulin})(\text{mean glucose} \times \text{mean insulin})]}$  (Matsuda and DeFronzo, 1999). A higher score indicates higher insulin sensitivity.

## 2.8. Health factors

Participants reported whether they had previously been diagnosed by a physician with any of the following health conditions relevant to metabolic syndrome and cellular aging: hypertension, high blood cholesterol, arthritis/osteoarthritis, or chronic pain. Participants reported use of medications such as non-steroidal anti-inflammatory medications (NSAIDs), statins, antidepressants, and vitamin supplements that might contain anti-oxidants. Using a previously published method (Puterman et al., 2011), we assessed participants' self-reports of whether they were sedentary or active, based on the Center for Disease Control's recommendations of 75 min of vigorous activity per week.

## 2.9. Data analysis

Variable distributions were inspected graphically, and a few outliers were winsorized to 2.6 SD to prevent biasing of the regression results.<sup>1</sup> The primary hypotheses were tested with regression, in which the main effect of HPF was z-scored so the corresponding coefficient would reflect the change in the outcome attributable to a one-standard deviation change in HPF. The interaction term represented the product of the standardized HPF score by Group. Two-tailed *p*-values and a critical alpha of .05 were used as the criteria for statistical significance. Longitudinal analyses were conducted by first examining predictions of the year 1 outcomes, uncorrected for baseline, and then examining predictions of delta change variables (e.g., outcome at year 1 – outcome at baseline). Age was used as a covariate in all analyses, as was antidepressant use (an exclusion criteria for the LS), and other demographic, health or medical factors that significantly different between the groups were additionally included as covariates in all full model analyses (see results). All final model analyses (i.e., the group by HPF interaction regression models including covariates presented in Table 3) were bootstrapped using residual resampling with 1000 iterations and bias-corrected confidence intervals using Matlab R2013b for Macintosh, which enhances the accuracy of these estimates. The following variables were missing data at baseline (number of participants missing data in parentheses) – waist circumference (4), truncal fat (1), insulin sensitivity (5), 8-OxoG (2), IsoP (2), and NPY (4). The following variables were missing data at year 1 – waist circumference (7), truncal fat (6), insulin sensitivity (7), IsoP (9), and 8-OxoG (20; 8-OxoG

<sup>1</sup> One point for IS at baseline and one for IS at 1 year with respective z-scores of 4.3 and 4.7 were winsorized. One point for NPY with a z-score of 4.1 was winsorized. We additionally confirmed that regression analyses of the full model using the raw IS outcomes and all covariates (as specified in Table 3) did not change the pattern of significance. Winsorizing was not necessary to obtain the reported results, merely desirable because conclusions from regression are less subject to potential bias by extreme points.

was analyzed in a subset of the full sample at year 1). Analyses have varied 'n' dependent on the outcome.

### 3. Results

#### 3.1. Group differences on demographics, psychological factors and cardiometabolic risk

T-tests or Chi-Squared tests were used to compare the chronic stress group (CS) and low stress group (LS) on demographics, psychological factors, and cardiometabolic risk profiles at the study onset (Table 1). As per the inclusion criteria, the CS group self-reported significantly higher levels of chronic stress on the PSS than did the LS group (Table 1). The mean hours of care provided per day by the CS group was 13.6 h (range: 1–24, mode: 24), and all LS women, by definition, provided 0 h of care. The CS group endorsed significantly lower physical activity, and was more likely to take NSAIDs (Table 1). Hence, the final statistical model controlled for the following covariates: age, antidepressant use, NSAID use, and reduced levels of physical activity. The CS and LS groups were compared on the mean levels of the primary metabolic outcomes at baseline and one year later, and significantly differed only on basal 8-OxoG, which, as previously reported (Aschbacher et al., 2013), was significantly higher among the CS group ( $p < .01$ ; Table 2). Waistline circumference, truncal fat and insulin sensitivity exhibited high stability over 1 year ( $.78 < r's < .97$ ), indicating that little change occurred on these outcomes from baseline through year 1.

#### 3.2. Synergistic effects of chronic stress and consumption of highly palatable food

The primary hypothesis that chronic stress increases vulnerability to diet-related metabolic risk was investigated with bias-corrected bootstrapped regression tests of each group-by-HPF interaction term on all five outcomes (waistline

circumference, truncal fat, insulin sensitivity, 8-OxoG, and IsoP) at baseline, 1-year, and the longitudinal change terms. In addition, we investigated the HPF main effect for each group (chronic versus low stress) to indicate the direction and magnitude of the association, and to investigate the pattern across outcomes, even when the interaction might be of borderline significance in this relatively small sample. Chronological age, NSAIDs, antidepressants, and exercise were included as covariates in all analyses (rationale described above). The group by HPF interaction effects were significant for insulin sensitivity at baseline and year 1, and for 8-OxoG at baseline (Table 3 and Figs. 1 and 2). The stability coefficients for waistline circumference, truncal fat, and insulin sensitivity were high (all  $r's > 0.78$ ; Table 3), which indicates that very little change from baseline occurred. Indeed, the full model did not predict significant change over 1-year for any outcome. Simple effect analyses were conducted to additionally examine the association of HPF with all outcomes separately in the CS and LS groups. Among the CS group at baseline, HPF was significantly associated with all outcomes in the expected direction (all  $p's \leq .05$ ; Table 3), whereas no significant relationships were present among the LS group. This pattern persisted at year 1, with similar effect sizes, smaller samples, and decreased significance.

#### 3.3. Investigation of potential explanatory factors

We investigated several non-exclusive pathways that might explain why chronic stress was associated with greater diet-related metabolic risk: (1) stress promotes heightened consumption of HPF, and/or (2) stress alters peripheral stress-arousal physiology, which augments adipocyte responses to HPF. As a group, the CS did not report significantly more HPF consumption than the LS ( $p = .87$ ; Table 1). However, providing more hours of care per day (an objective index of burden) was associated with significantly greater HPF consumption among the CS (Spearman's rho:  $r = .40$ ,  $p = .03$ ).

**Table 2** Group mean differences on metabolic risk indices.

	Chronic stress	Low stress	Statistical comparison	
			<i>t</i> ( <i>df</i> )	<i>p</i> -Value
<b>Baseline</b>				
Waistline circumference (cm)	86.91 (12.78)	86.63 (12.77)	−0.09 (55)	.93
Dex trunk fat (kg)	14.45 (6.09)	14.37 (5.95)	−0.05 (58)	.96
Insulin sensitivity (composite)	4.35 (2.72)	4.49 (2.12)	0.21 (54)	.83
8-oxoG (ng/mL)	0.04 (0.01)	0.02 (0.01)	−3.31 (57)	<.01**
F2-Isoprotanes (ng/mL)	0.05 (0.02)	0.04 (0.02)	−0.66 (55)	.51
<b>1 Year</b>				
Waistline circumference (cm)	87.17 (13.50)	84.56 (12.17)	−0.74 (52)	.46
Dex trunk fat (kg)	14.26 (6.38)	13.95 (6.34)	−0.18 (53)	.86
Insulin sensitivity (composite)	4.63 (3.20)	4.64 (2.15)	0.02 (52)	.99
8-oxoG (ng/mL)	0.05 (0.03)	0.03 (0.02)	−1.64 (36.67 <sup>a</sup> )	.11
F2-Isoprotanes (ng/mL)	0.04 (0.02)	0.04 (0.02)	−0.67 (50)	.51

Critical alpha = .05. Means and standard deviations given in table. FET = Fisher's exact test, 2-sided.

<sup>a</sup> Unequal variance assumed per significant Levine's test.

\*\*  $p \leq .01$ .

**Table 3** Multivariate associations between highly palatable food consumption and metabolic outcomes by group.

Outcome	Chronic stress (CS)		Low stress (LS)		Group*HPF interaction	
	Unstandardized coefficient	95% CI	Unstandardized coefficient	95% CI	Unstandardized coefficient	95% CI
Basal waistline circumference (WC)	5.22**	(1.47, 8.73)	-0.24	(-5.34, 4.40)	-5.50***	(-12.03, 0.04)
1 Year WC	4.89*	(0.82, 9.23)	-1.89	(-7.24, 3.59)	-6.86*	(-13.74, -0.36)
Change in WC from baseline to 1 year	0.40	(-1.03, 1.75)	-1.14	(-2.81, 0.70)	-1.54	(-3.64, 0.63)
Basal trunk fat (TF), kg	2.54**	(0.58, 4.21)	0.07	(-2.12, 2.08)	-2.44***	(-5.07, 0.61)
1 Year TF	2.26*	(0.27, 4.61)	-0.92	(-3.50, 2.17)	-3.12***	(-6.54, 0.07)
Change in TF from baseline to 1 year	-0.25	(-0.81, 0.26)	-0.85*	(-1.51, -0.21)	-0.59	(-1.42, 0.21)
Basal insulin sensitivity (IS)	-1.21**	(-1.95, -0.44)	0.43	(-0.47, 1.41)	1.66**	(0.49, 2.88)
1 Year IS	-1.16**	(-1.90, -0.44)	0.60	(-0.35, 1.64)	1.78**	(0.56, 2.98)
Change in IS from baseline to 1 year	0.52	(-0.26, 1.23)	0.22	(-0.70, 1.20)	-0.30	(-1.58, 0.91)
Basal 8-hydroxyguanosine (8-OxoG)	0.007**	(0.004, 0.012)	-0.001	(-0.005, 0.004)	-0.008**	(-0.014, -0.002)
1 Year 8-OxoG	0.003	(-0.009, 0.013)	0.005	(-0.009, 0.019)	0.003	(-0.015, 0.019)
Change in 8-OxoG from baseline to 1 year	-0.004	(-0.015, 0.009)	0.008	(-0.006, 0.024)	0.011	(-0.007, 0.031)
Basal F2-isoprostanes (IsoP)	0.010*	(0.004, 0.017)	0.005	(-0.002, 0.013)	-0.005	(-0.015, 0.006)
1 Year IsoP	0.005***	(-0.001, 0.011)	0.001	(-0.007, 0.007)	-0.004	(-0.013, 0.004)
Change in IsoP from baseline to 1 year	-0.005	(-0.012, 0.002)	-0.001	(-0.010, 0.007)	-0.004	(-0.008, 0.014)

All models were adjusted for age, antidepressant use, NSAID use and exercise. All statistical estimates provided herein are based on bootstrapping using residual resampling with 1000 iterations and bias-corrected confidence intervals. Change outcomes were calculated using delta scores. The interaction term was formed by calculating the product of caregiver status and the standardized variable for highly palatable food (HPF).

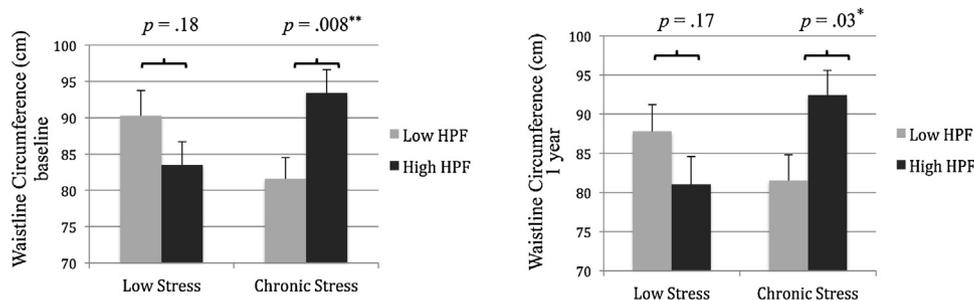
\*  $p \leq .05$ .

\*\*  $p \leq .01$ .

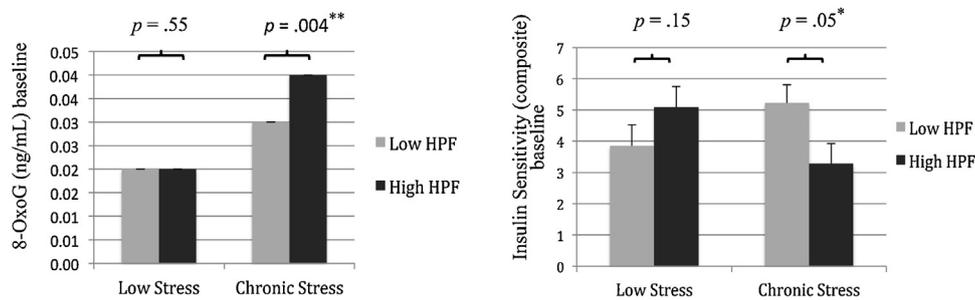
\*\*\*  $p \leq .10$ .

We analyzed whether, in humans, as in rodents (Kuo et al., 2007), peripheral NPY might be a mechanism that could explain how chronic stress enhances vulnerability to diet-related adiposity. Consistent with this hypothesis, the CS group had significantly higher levels of basal NPY relative to the LS group ( $p = .001$ ; Fig. 3).

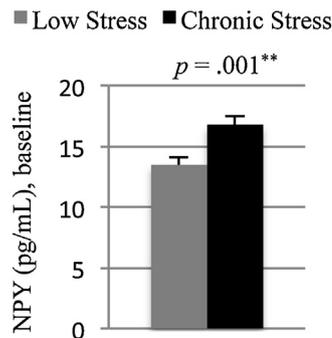
Further, we reasoned that if NPY is one of the key stress-arousal mechanisms contributing to the accumulation of fat in the CS group, then the strength of the association between HPF and indices of abdominal adiposity should be greatest among women with high NPY levels. This hypothesis was tested by assessing the HPF by NPY interaction, which was



**Fig. 1** Synergistic effects of chronic stress and highly palatable food on waistline circumference. \*\* $p \leq .01$ , \* $p \leq .05$ . Note: For the sake of data visualization,  $p$ -values given here are derived from  $t$ -tests; however, Table 3 provides bootstrapped regression coefficients adjusted for the covariates, which provide the more appropriate, final statistical tests of synergistic (interaction) and main effects.



**Fig. 2** Synergistic effects of chronic stress and highly palatable food on oxidative stress and insulin sensitivity.  $**p \leq .01$ ,  $*p \leq .05$ . *Note:* For the sake of data visualization,  $p$ -values given here are derived from  $t$ -tests; however, Table 3 provides bootstrapped regression coefficients adjusted for the covariates, which provide the more appropriate, final statistical tests of synergistic (interaction) and main effects.



**Fig. 3** Peripheral neuropeptide Y (NPY) is elevated under chronic stress.  $**p \leq .01$ . Mean + SEM depicted. An independent  $t$ -test using a critical alpha of .05 was conducted to obtain the  $p$ -value for the group comparison.

significantly associated with basal waistline circumference ( $p = .02$ ), truncal fat ( $p = .01$ ; supplementary figure), and insulin sensitivity ( $p = .04$ ), but not 8-OxoG ( $p = .40$ ), or IsoP ( $p = .12$ ), while controlling for age, physical activity, NSAIDs and antidepressant use (as in previous final models).

Supplementary figure related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2014.04.003>.

#### 4. Discussion

Traditionally, the maintenance of body weight is thought to be determined by the balance of food intake and expenditure. However, psychological stress has been associated with both weight gain and weight loss (Nyberg et al., 2012), and may therefore alter energy homeostasis. There is a missing link in the current understanding of how and when stress contributes to obesity and metabolic dysfunction. In animal studies, when stress becomes chronic (defined by duration and intensity), sympathetic nerve activity upregulates peripheral expression of NPY, which promotes diet-induced abdominal adiposity and metabolic dysregulation (Kuo et al., 2007). Our data are the first to demonstrate in humans that the synergistic combination of chronic stress along with consuming more high fat/high sugar foods was associated with significantly worse metabolic outcomes and greater

waist circumference. This study underscores the importance of chronic stress as a modifier of peripheral metabolic and adipose physiology. If confirmed in interventional studies, these data suggest the possibility that increasing stress-resilience skills could improve the efficacy of interventions to treat metabolic syndrome and obesity, at least for patients with chronically stressful life circumstances.

Our data, collected among a group of post-menopausal women who are overweight on average, demonstrate that more frequent HPF consumption (foods high in sugar and fat) significantly predicted increased waistline, truncal fat, and insulin resistance, but only among the group of women exposed to chronic stress. This association was observed both at the time of initial assessment and 1 year later (unadjusted for baseline), while statistically controlling for physical activity and other possible confounders. In addition, at baseline, the combination of psychological stress and HPF was significantly associated with higher basal levels of 8-OxoG, a marker of oxidative damage to the genome that predicts long-term mortality among patients with type 2 diabetes (Broedbaek et al., 2011). However, no prediction of changes in metabolic outcomes over 1-year was found, which may be due to the high stability of these measures across the study. One previous rodent study also reported synergistic stress-HPF effects on oxidative stress markers in the context of fatty liver disease (Fu et al., 2010). While that study did not show changes in visceral fat (Fu et al., 2010) in contrast to the study by Kuo et al. (2007), the diet used was considerably lower in fat and the chronic stressor model did not involve social threat, a key factor in human stress hormone reactivity. Although the stress by HPF synergism (i.e., the interaction term) did not reach statistical significance for every outcome in this clinical study, there was a highly consistent pattern whereby HPF consumption significantly predicted all metabolic markers in the chronically stressed group, controlling for age, physical activity, and medical factors (Table 3). Whereas high HPF consumption and a hypercaloric diet may lead to obesity regardless of stress, these data suggest that chronic stress may decrease the threshold of metabolic resilience, so that even low to moderate levels of HPF consumption proffer greater risks.

Animal models suggest that one mechanism of action by which chronic stress exacerbates the adverse metabolic effects of HPF is via upregulation of stress-induced NPY secretion from sympathetic nerve terminals innervating

visceral adipose tissue (Kuo et al., 2007). In our data, chronically stressed women had significantly higher levels of plasma NPY, measured in peripheral blood, compared to low stress women. Furthermore, HPF consumption was a significantly better predictor of trunk fat and waistline circumference among those individuals with high levels of plasma NPY. Hence, these results are consistent with the animal models in which chronic stress increased NPY secretion, which in turn, altered adipose tissue sensitivity to HPF. This raises the intriguing question of whether reducing psychological stress or peripheral NPY signaling could potentially help individuals lose fat more successfully, even if diet and activity levels remained the same.

This study used a well-accepted and oft-studied human model of chronic stress, caring for a loved one with dementia, which has been previously associated with weight gain (Fredman and Daly, 1997) and with alterations in a host of cardiometabolic risk markers (Vitaliano et al., 2002; Aschbacher et al., 2008; Mausbach et al., 2012). As a group, chronically stressed women did not report greater HPF consumption than low stress women. However, within the high stress group, the objective indicator of the degree of chronic stress exposure (hours of care provided per day) was significantly related to greater HPF consumption. Future studies should investigate whether other socially relevant models of chronic stress (e.g., low socioeconomic status, unemployment, traumatic stress) moderate the effects of HPF on metabolic risk, as well as extending these findings to males, children, and other age groups.

As a limitation, this study used a case-control design, as it would be unethical to assign humans to chronically stressful conditions. Self-reports (such as our HPF measure) may be subject to social desirability and recall biases, and provide stronger indications of relative than absolute intake. Nonetheless, this study represents a critical step needed to justify considerably more resource-intensive studies involving feeding paradigms, multiple daily dietary assessments, and biomarkers for macronutrient consumption. Future studies might randomly assign participants with a range of stress levels to a feeding study comparing diets with relatively greater or lesser amounts of sugar/fat, and obtain absolute measures of overall caloric, macro- and micronutrient intake. Waistline and truncal fat are proxies but not precise measures of visceral fat; nonetheless, both visceral and subcutaneous fat are associated with heightened metabolic risk (Fox et al., 2007). There are limits to the generalizability of these findings, given our sample was composed of postmenopausal women who were predominantly Caucasian. Menopause is a risk factor for an increase in visceral fat specifically, as well as total body fat (Lovejoy et al., 2008). The high stability of metabolic outcomes over 1 year in this sample made it difficult to detect prospective changes in metabolic risk. Future studies should reexamine prospective relations in samples with greater longitudinal variability, over longer periods, or in which an intervention has been used to experimentally elicit variability.

People under chronic stress are already known to be at higher risk of metabolic disease, although the mechanisms underlying this link are unclear. This study illuminates an important pathway by which stress-induced changes in peripheral physiology increase the likelihood that consuming HPF will lead to the preferential accumulation of abdominal

fat and the worsening of metabolic risk factors. The implication of this work is that chronically stressed individuals are more *vulnerable* to a high fat/high sugar diet. If confirmed, these data invite the exciting possibility that increasing resilience skills could improve the efficacy of interventions to help individuals lose weight and manage metabolic syndrome, even if diet and activity levels remained the same.

## Role of funding source

NIH/NIA R01 AG030424-01A2, NIH/NHLBI grant K23 HL112955, NIH/NCRR UCSF-CTSI grant No. UL1 RR024131, NIH/NCATS, UCSF-CTSI UL1 TR000004, P01 AT005013, the Marchionne Foundation and The Institute for Integrative Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

## Conflict of interest

No authors have conflicts of interest to declare.

## Acknowledgments

We thank Aoife O'Donovan and Yali Su for their technical and intellectual assistance.

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