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The effect of obesity on inflammatory cytokine and leptin production following acute mental stress

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Abstract

Obesity may contribute to cardiovascular disease (CVD) risk by eliciting chronic systemic inflammation and impairing the immune response to additional stressors. There has been little assessment of the effect of obesity on psychological stress, an independent risk factor for CVD. Therefore, it was of interest to examine interleukin-6, tumor necrosis factor-α, interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1Ra), and leptin following an acute mental stress task in nonobese and obese males. Twenty college-aged males (21.3 ± 0.56 years) volunteered to participate in a 20-min Stroop color-word and mirror-tracing task. Subjects were recruited for obese (body mass index: BMI > 30) and nonobese (BMI < 25) groups, and blood samples were collected for enzyme-linked immunosorbent assay analysis. The acute mental stress task elicited an increase in heart rate, catecholamines, and IL-1β in all subjects. Additionally, acute mental stress increased cortisol concentrations in the nonobese group. There was a significant reduction in leptin in obese subjects 30 min posttask compared with a decrease in nonobese subjects 120 min posttask. Interestingly, the relationship between the percent change in leptin and IL-1Ra at 120 min posttask in response to an acute mental stress task was only observed in nonobese individuals. This is the first study to suggest that adiposity in males may impact leptin and inflammatory signaling mechanisms following acute mental stress.

Descriptors: Acute mental stress, Obesity, Leptin, Cortisol, Inflammation, Cytokines

Cardiovascular disease (CVD) is the leading global cause of death, accounting for approximately 30% of all reported deaths (CDC, 2011). Among traditional CVD risk factors, obesity is a major target of research and clinical importance due to its epidemic prevalence; approximately 35.7% of adults in the United States are currently categorized as obese (body mass index; BMI ≥ 30) and an additional 32 million Americans are predicted to become obese by 2030 (CDC, 2011; Flegal, Carroll, Kit, & Ogden, 2012). Obesity contributes to the risk for CVD by acting as a chronic systemic stressor and eliciting inflammation, which includes elevations in basal levels of hormones, immune cells, and cytokines (Ippoliti, Canitano, & Businaro, 2013). Interestingly, whereas an acute stressor may elicit a protective inflammatory response, a chronic stressor such as obesity can be maladaptive and impair an individual's ability to produce a beneficial immune response to an additional stressor (Dragos & Tanasescu, 2010).

Considerable evidence has suggested that psychological stress is an independent risk factor for CVD (Gu, Tang, & Yang, 2012; Rozanski, Blumenthal, & Kaplan, 1999). Acute mental stress tasks have been used as a laboratory model to examine the psychological stress response, inducing the release of catecholamines, cortisol, and inflammatory cytokines into circulation (Benson et al., 2009; Rohleder et al., 2006; Steptoe, Hamer, & Chida, 2007). Interestingly, combined stressors, concurrent physical stress (physical activity) with acute mental stress, have been shown to elicit a greater physiological response relative to either stressor alone (Webb et al., 2010). In addition, diseases known to have an elevated inflammatory profile, such as CVD and rheumatoid arthritis, have demonstrated an enhanced inflammatory response to mental stress (Kop et al., 2008; Veldhuijzen van Zanten, Ring, Carroll, & Kitas, 2005). Accordingly, it seems plausible that obesity, acting as a chronic physical stressor, may augment the inflammatory response to a psychological stressor. Although studies have failed to observe differences in the catecholamine response of epinephrine (EPI) and norepinephrine (NE) to acute mental stress between obese and nonobese individuals (Huang, Franco, Evans, Mari, & Acevedo, 2014; Sothmann, Hart, & Horn, 1995), there is equivocal evidence that blunted cortisol responses following an acute mental stress task are associated with increased adiposity levels (Benson et al., 2009; Jones et al., 2012; Phillips, Roseboom, Carroll, & deRooij, 2012). In addition, there has been little examination of the effect of obesity on inflammatory cytokine production following acute mental stress. To date, only circulating interleukin-6 (IL-6; Benson et al., 2009) and lipopolysaccharide-induced IL-6 and tumor necrosis factor-α (TNF-α) responses (Huang et al., 2011) have been examined in obese and nonobese participants following

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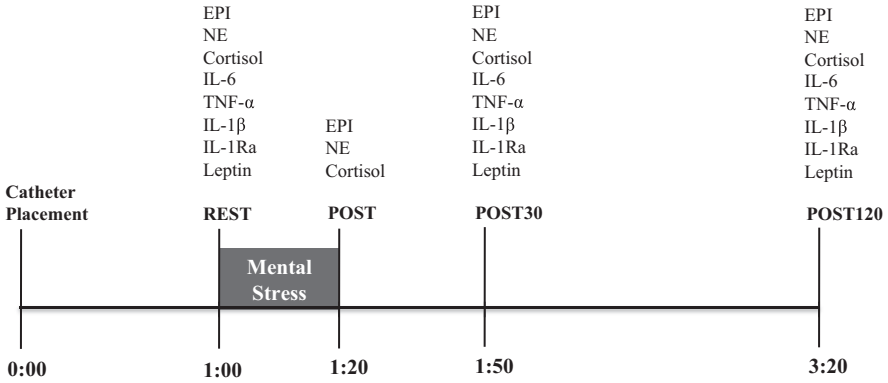

Figure 1. Schematic representation of the acute mental stress protocol.

an acute mental stress task. Therefore, it was of interest to examine the effect of obesity on the physiological response to an acute mental stress task in the context of additional immune signaling messengers.

IL-6, TNF-α, and interleukin-1β (IL-1β) are potent proinflammatory mediators that contribute to the activation and migration of immune cells, enhanced production of cytokines, and alteration of the vascular endothelium (Juge-Aubry, Henrichot, & Meier, 2005). In contrast, interleukin-1 receptor antagonist (IL-1Ra) is an antiinflammatory cytokine that competitively inhibits IL-1β, attenuating further proinflammatory cytokine production. Although increases in IL-6 (Brydon et al., 2005; Steptoe, Willemson, Owen, Flower, & Mohamed-Ali, 2001), TNF-α (Steptoe et al., 2001), IL-1β (Brydon et al., 2005; Heinz et al., 2003), and IL-1Ra (Rohleder et al., 2006; Steptoe at al., 2001) have been reported following various acute mental stress tasks, no studies to date have investigated these markers concurrently following acute mental stress in obese and nonobese individuals. Additionally, there is evidence that leptin, a hormone released from adipocytes and associated with obesity, increases relative to adiposity following acute mental stress (Brydon, 2011; Brydon et al., 2008). Leptin signaling can augment cytokine production (Iikuni, Lam, Lu, Matarese, & La Cava, 2008) and may provide a mechanism linking obesity and cytokine production following acute mental stress. Therefore, the purpose of this study was to examine changes in IL-6, TNF-α, IL-1β, IL-1Ra, and leptin following an acute mental stress task in obese and nonobese participants. It was hypothesized that the combined impact of an acute mental stress task and obesity would elicit a greater proinflammatory response than the mental stress task alone (i.e., enhanced IL-6, TNF-α, IL-1β, and leptin with reduced IL-1Ra), despite similar hormonal activation (EPI, NE, and cortisol).

Method

Subjects

Twenty apparently healthy male subjects between the ages of 18–28 were recruited from the general population to participate in this study. Subjects were categorized by their BMI into either a nonobese ($BMI < 25$) or obese ($BMI \geq 30$) group. Subjects were excluded from the study if they had known or suspected cardiovascular, metabolic, rheumatologic, or other inflammatory diseases/conditions or if they were taking medication, using tobacco products, consuming an average of more than 10 alcoholic beverages per week, or actively participating in at least moderate physical activity. Subjects were also excluded if they had a history of psychological disorders or if they had experienced a major life event (e.g., death in family, divorce, or wedding) within 30 days of participation. Ten nonobese and ten obese subjects met the criteria stated above and completed the study. Written informed consent was obtained from each subject before participation in the study. All procedures were approved by Virginia Commonwealth University's Institutional Review Board.

Testing Procedures

Subjects were initially screened by completing the International Physical Activity Questionnaire to determine participation in moderate and vigorous physical activity (Craig et al., 2003). Additionally, subjects completed a medical history questionnaire and had percent body fat (%BF) assessed with dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare, Madison, WI) to confirm subject grouping (nonobese: $\%BF < 25$, obese: $\%BF \geq 30$). All subjects remained in the same group following the determination of %BF by DXA. Subjects who met study criteria were instructed to report to the Exercise Physiology Research Laboratory on two different occasions.

The first day, participants arrived at 7:00 am. Subjects participated in a 4-min mental stress task to familiarize themselves with the psychological stressor. Subjects were instructed to refrain from exercise for 48 h and caffeine and alcohol for 24 h prior to visiting the laboratory for the second session. Within 48 h, participants returned to the laboratory at 7:00 am following an overnight fast (at least 9 h) and completed a Seven-Day Physical Activity Questionnaire (Blair et al., 1985). An intravenous catheter was inserted by a certified phlebotomy technician. Subjects rested for 1 h and blood was drawn immediately before the initiation of the acute mental stress task (REST). Blood was drawn immediately post (POST), 30 (POST30), and 120 (POST120) min following the stress task as indicated in Figure 1.

Acute Mental Stress Task

A computer-based mental task was used, alternating between 2-min cycles of a Stroop color-word and mental arithmetic tasks for a total of 20 min. This protocol has been previously shown to elicit a stress response as demonstrated by increases in State Anxiety Index, heart rate (HR), and NE (Acevedo et al., 2006; Huang, Franco, Evans, & Acevedo, 2010). As briefly described, subjects were presented with a color-word on the screen, which was written in a different color font. Simultaneously, the computer said a third color, while the subjects were required to identify the font color in

which the word was presented. A new color-word was given every second for 2 min. Following the Stroop color-word task, subjects were presented with a three-digit number from which they were required to randomly subtract either 3, 7, 8, or 13. Auditory feedback was given by the program when participants entered an incorrect answer. The mental arithmetic task continued for 2 min. This 4-min cycle of two stressors occurred five times for a total of 20 min. Prior to the start of the task, subjects were instructed to work as accurately and quickly as possible and informed that their scores would be recorded. In addition, an investigator stayed in the room, inducing a socioevaluative component by providing bogus critical feedback to the subject regarding the number of incorrect answers, the speed of their reactions, and their performance compared to their peers. HR was collected using a polar heart rate monitor (Polar Co., Port Washington, NY) prior to stress and every minute during the mental stress task. Maximum HR (HRmax) was calculated as the highest HR recorded during the task, and HR reactivity was calculated as HRmax − HR REST.

Quantification of EPI, NE, Cortisol, IL-6, TNF-α, IL-1β, IL-1Ra, and Leptin

Blood samples were collected for analysis of plasma EPI, NE, IL-6, TNF-α, IL-1β, and IL-1Ra into EDTA tubes and immediately centrifuged for 15 min at ∼1000×g at 4°C. Blood samples for the analysis of serum cortisol and leptin were collected in serum separator tubes and allowed to clot for 30 min at room temperature prior to being centrifuged for 15 min at ∼1000×g at 4°C. All samples were immediately aliquoted into microtubes and stored at −80°C until analyzed. Sample concentrations were determined through enzyme-linked immunosorbent assays according to manufacturer's specifications (EPI and NE: Labor Diagnostika Nord, Nordhorn, Germany, and cortisol, IL-6, TNF-α, IL-1β, IL-1Ra, and leptin: R&D Systems, Minneapolis, MN). All samples were analyzed in duplicate, and the mean concentration of each sample was used for statistical analysis. Interassay coefficient of variations (CVs) for the measured samples were 2% (EPI), 8% (NE), 11% (cortisol), 12% (IL-6), 12% (TNF-α), 14% (IL-1β), 10% (IL-1Ra), and 10% (leptin). Intraassay CVs were 4% (EPI), 4% (NE), 7% (cortisol), 4% (IL-6), 5% (TNF-α), 7% (IL-1β), 3% (IL-1Ra), and 5% (leptin).

Statistical Analysis

Subject demographics, HR reactivity, and mean stress HR were compared using descriptive statistics and one-way analyses of variance (ANOVAs). The effect of the acute mental stress task on HR was examined using a repeated measures one-way ANOVA (HR REST vs. HRmax and HR REST vs. mean stress HR). Additionally, HR reactivity was compared between both tasks with a one-way ANOVA to examine any differences in task. Q-Q plots were examined for all blood markers, and log transformations were used to approximate normal distributions for IL-6, TNF-α, IL-1Ra, and leptin for all analyses. The assumption of sphericity was not met for all blood measures; therefore, a multivariate analysis of variance (MANOVA) was used to determine the impact of the acute mental stress task on concentrations of EPI, NE, and cortisol between the two groups (nonobese and obese) and across four different time points (REST, POST, POST30, POST120). Furthermore, a MANOVA was used to examine IL-6, TNF-α, IL-1β, IL-1Ra, and leptin concentrations in the two groups (nonobese and obese) across three different time points (REST, POST30, POST120). Effect size is reported as η_ρ^2. If a significant main effect was observed for time, a one-way repeated measures ANOVA with a Bonferroni post hoc analysis was used to examine at which time points significant differences were found. If a significant effect for both group and time were observed, Bonferroni post hoc analyses were used in each group individually. A linear regression was utilized to examine relationships between the percent change (%Δ) in leptin and cytokine concentrations following the acute mental stress task. %Δ from REST were assessed to minimize the impact of significant differences in baseline values between groups. Additionally, if a significant association was observed within the linear regression model, a hierarchical multiple regression was used to determine the impact of REST cytokine values. All analyses were run using SPSS (V21, Chicago, IL). Data are expressed as mean ± standard error of mean (*SEM*) with statistical significance set at $p \leq .05$.

Table 1. *Subject Characteristics*

Variable	Nonobese ($N = 10$)	Obese ($N = 10$)	p value	η_ρ^2
Age (yrs)	21.2 ± 0.83	21.4 ± 0.79	.863	.002
Height (cm)	176.4 ± 2.06	180.7 ± 2.64	.220	.082
Weight (kg)	67.2 ± 2.04	122.1 ± 6.86	<.001*	.766
BMI (kg/m^2)	21.8 ± 0.55	37.2 ± 1.44	<.001*	.911
BF (%)	16.7 ± 1.31	40.0 ± 1.12	<.001*	.846
REST EPI (pg/mL)	56.5 ± 5.56	53.0 ± 2.49	.566	.019
REST NE (pg/mL)	359.0 ± 57.30	294.8 ± 41.45	.376	.044
REST cortisol (ng/mL)	64.4 ± 7.36	49.1 ± 5.94	.045*	.204
REST IL-6 (pg/mL)	0.7 ± 0.14	1.9 ± 0.34	.006*	.353
REST TNF-α (pg/mL)	1.3 ± 0.10	2.1 ± 0.31	.033*	.228
REST IL-1β (pg/mL)	0.2 ± 0.02	0.2 ± 0.02	.145	.117
REST IL-1Ra (pg/mL)	158.7 ± 12.99	409.5 ± 40.17	<.001*	.662
REST leptin (ng/mL)	1.7 ± 0.33	26.2 ± 4.71	<.001*	.598
REST HR (bpm)	61.8 ± 4.22	65.0 ± 3.29	.558	.019
Max HR (bpm)	86.0 ± 4.65	88.9 ± 4.74	.668	.010
HR reactivity (bpm)	24.2 ± 1.93	23.9 ± 3.18	.937	.001
Mean stress HR (bpm)	72.5 ± 3.86	76.0 ± 3.22	.494	.026

Note. Data are shown as mean ± *SEM*. η_ρ^2 = effect size; BF = body fat; BMI = body mass index; REST = baseline measures; EPI = epinephrine; NE = norepinephrine; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; IL-1β = interleukin 1-beta; IL-1Ra = interleukin-1 receptor antagonist.
*$p \leq .05$.

Results

Subject Characteristics

Demographic characteristics for both nonobese and obese groups are presented in Table 1. Weight, $F(1,18) = 58.853$, $p < .001$, $\eta_\rho^2 = .766$; BMI, $F(1,18) = 99.025$, $p < .001$, $\eta_\rho^2 = .911$; %BF, $F(1,18) = 184.395$, $p < .001$, $\eta_\rho^2 = .846$; REST cortisol, $F(1,18) = 4.621$, $p = .045$, $\eta_\rho^2 = .204$; IL-6, $F(1,18) = 9.820$, $p = .006$, $\eta_\rho^2 = .353$; TNF-α, $F(1,18) = 5.315$, $p = .033$, $\eta_\rho^2 = .228$; IL-1Ra, $F(1,18) = 35.287$, $p < .001$, $\eta_\rho^2 = .662$, and leptin, $F(1,18) = 26.797$, $p < .001$, $\eta_\rho^2 = .598$, were significantly different between the groups, while age, height, EPI, NE, and IL-1β were not different between groups. It should be noted that BMI and %BF had a strong positive association, $r(19) = .914$, $p < .001$, further supporting the categorization of subjects within each group.

HR, Catecholamine, and Cortisol Responses

HR REST, HRmax, HR reactivity, and mean HR are reported in Table 1. HR significantly increased from REST to HRmax during

Table 2. *Physiological Stress Markers in Nonobese and Obese Subjects Following Acute Mental Stress*

Group	Variable	REST	POST	POST30	POST120
Nonobese	EPI (pg/mL)	56.5 ± 5.57	66.2 ± 4.67*	64.8 ± 6.17*	59.9 ± 2.36
($N = 10$)	NE (pg/mL)	359.0 ± 57.30	668.3 ± 93.98*	582.4 ± 80.21*	621.6 ± 97.53*
	Cortisol (ng/mL)	69.4 ± 7.36	102.4 ± 6.41*	96.3 ± 7.91	60.2 ± 6.75
	IL-6 (pg/mL)	0.7 ± 0.14	–	0.9 ± 0.11	1.1 ± 0.22
	TNF-α (pg/mL)	1.3 ± 0.10	–	1.2 ± 0.10	1.1 ± 0.10
	IL-1β (pg/ml)	0.2 ± 0.02	–	0.2 ± 0.03	0.3 ± 0.07**
	IL-1Ra (pg/ml)	158.7 ± 12.99	–	158.9 ± 11.54	146.0 ± 7.32
	Leptin (ng/ml)	1.7 ± 0.33	–	1.5 ± 0.28	1.4 ± 0.22*
Obese	EPI (pg/mL)	53.0 ± 2.49	59.7 ± 2.83*	57.4 ± 3.57*	59.3 ± 3.02
($N = 10$)	NE (pg/mL)	294.8 ± 41.45	576.4 ± 99.88*	457.9 ± 73.88*	506.5 ± 52.27*
	Cortisol (ng/mL)	49.1 ± 5.94	83.7 ± 9.46	72.4 ± 8.54	44.6 ± 6.97
	IL-6 (pg/mL)	1.9 ± 0.34	–	2.0 ± 0.39	2.1 ± 0.59
	TNF-α (pg/mL)	2.1 ± 0.32	–	2.0 ± 0.35	2.0 ± 0.34
	IL-1β (pg/ml)	0.2 ± 0.02	–	0.2 ± 03	0.3 ± 0.04**
	IL-1Ra (pg/ml)	409.5 ± 40.17	–	398.9 ± 35.43	393.7 ± 35.75
	Leptin (ng/ml)	26.2 ± 4.71	–	23.8 ± 4.12*	24.1 ± 4.49

Note. Data are shown as mean ± *SEM*. EPI = epinephrine; NE = norephinephrine; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; IL-1β = interleukin 1-beta; IL-1Ra = interleukin-1 receptor antagonist.
*$p \leq .05$ different from REST.
**$p < .05$ different from POST30.

the task, $F(1,19) = 176.550$, $p < .001$, $\eta_\rho^2 = .908$, and from REST to mean stress HR, $F(1,19) = 16.045$, $p < .001$, $\eta_\rho^2 = .458$. However, there was no significant difference in HR reactivity, $F(1,19) = .007$, $p = .937$, $\eta_\rho^2 = .001$, or mean stress HR between groups, $F(1,19) = .488$, $p = .494$, $\eta_\rho^2 = .026$. Additionally, there was no significant difference in HR reactivity between the Stroop color-word and mental arithmetic tasks, $F(1,38) = .006$, $p = .938$, $\eta_\rho^2 = .000$.

Catecholamine concentrations are reported in Table 2, and the changeover time is shown in Figure 2. MANOVAs revealed a significant main effect for time in EPI, $F(3,16) = 6.76$, $p = .004$, $\eta_\rho^2 = .559$, and NE, $F(3,16) = 8.43$, $p = .001$, $\eta_\rho^2 = .612$. However, the main effects for group were not significant (EPI, $F(1,18) = 0.866$, $p = .365$, $\eta_\rho^2 = .046$, and NE, $F(1,18) = 1.16$, $p = .295$, $\eta_\rho^2 = .061$). The Bonferroni pairwise analyses revealed that EPI significantly increased at POST ($p = .001$) and POST30 ($p = .044$) as compared to REST, before returning to similar values to REST at POST120. Likewise, NE concentrations were significantly increased at POST ($p < .001$), POST30 ($p = .003$), and POST120 ($p = .003$) as compared to REST. Interestingly, NE concentrations were significantly lower at POST30 compared to POST ($p = .011$); however, the poststress NE concentrations did not return to baseline levels at POST120.

Additionally, cortisol concentrations were examined following the acute mental stress task, as shown in Table 2 and Figure 2. A MANOVA revealed a significant main effect for time, $F(3,16) = 12.700$, $p < .001$, $\eta_\rho^2 = .704$, and group, $F(1,18) = 8.288$, $p = .010$, $\eta_\rho^2 = .315$. In the nonobese group, the Bonferroni pairwise analyses revealed a significant increase in cortisol at POST as compared to REST ($p = .013$), with concentrations at POST30 and POST120 significantly lower than POST ($p = .001$ and $p = .002$, respectively). The main effect of time was not significant in the one-way ANOVA for the obese group ($p = .080$).

IL-6, TNF-α, IL-1β, IL-1Ra, and Leptin Responses

Concentrations of IL-6, TNF-α, IL-1β, IL-1Ra, and leptin were examined at REST, POST30, and POST120 and are reported in Table 2 and Figure 2. A MANOVA for IL-6 concentrations did not produce a significant main effect for time, $F(2,17) = 0.831$, $p = .453$, $\eta_\rho^2 = .089$; however, there was a significant main effect for group, $F(1,18) = 12.330$, $p = .002$, $\eta_\rho^2 = .407$, with concentrations greater in obese individuals compared to nonobese at all time points. A MANOVA for TNF-α concentrations revealed a significant main effect for time, $F(2,17) = 4.260$, $p = .032$, $\eta_\rho^2 = .334$, and a significant main effect for group, $F(1,18) = 6.870$, $p = .017$, $\eta_\rho^2 = .276$, with concentrations greater in obese individuals at each time point. However, when a one-way ANOVA was performed to determine the differences in time within each group, statistical differences were not detected. A MANOVA for IL-1β demonstrated a significant main effect for time, $F(2,17) = 4.520$, $p = .027$, $\eta_\rho^2 = .347$, but not group, $F(1,18) = 0.037$, $p = .849$, $\eta_\rho^2 = .002$. Since there was not a main effect for group, all subjects were combined to determine the differences in time. A Bonferroni pairwise comparison revealed a significant increase ($p = .017$) in IL-1β at POST120 as compared with POST30. In addition, a MANOVA for IL-1Ra did not reveal a significant main effect for time, $F(2,17) = 0.899$, $p = .425$, $\eta_\rho^2 = .096$; however, there was a significant main effect for group, $F(1,18) = 54.680$, $p < .001$, $\eta_\rho^2 = .752$, with IL-1Ra elevated in obese individuals at all time points. A MANOVA for leptin concentrations produced a significant main effect for time, $F(2,17) = 6.400$, $p = .008$, $\eta_\rho^2 = .430$, and a significant main effect for group, $F(1,18) = 95.330$, $p < .001$, $\eta_\rho^2 = .841$, for leptin concentrations. A Bonferroni pairwise comparison in the nonobese group revealed a significant decrease in leptin at POST120 as compared to REST ($p = .037$). In the obese group, there was a significant decrease in leptin at POST30 as compared to REST ($p = .020$). Leptin concentrations were greater in the obese group at each time point.

Associations Between Hormonal Responses and Subsequent Cytokine Responses

Since leptin has been shown to influence the secretion of inflammatory cytokines (Iikuni et al., 2008), the relationships between %Δ in leptin and cytokines were further examined. These

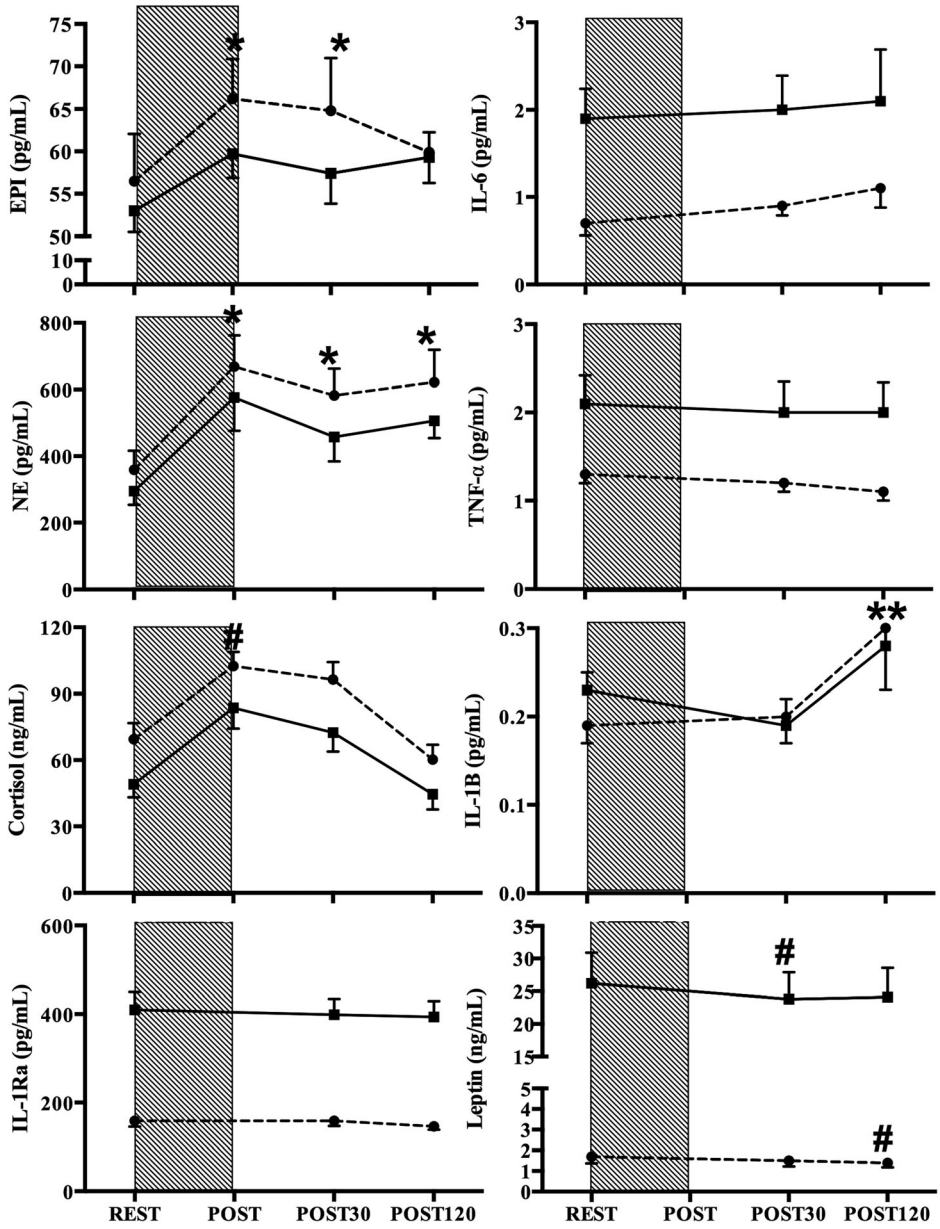

Figure 2. Effect of acute mental stress task on hormone and cytokine concentrations. The black line represents the obese group and the dotted line represents the nonobese group. The gray bars indicate the acute mental stress period. *$p \leq .05$ from REST in both groups. **$p \leq .05$ from POST30 in both groups. $^{\#}p \leq .05$ from REST in the group indicated.

relationships were examined independently within each group because leptin changes occurred at different time points following the acute mental stress task in the obese versus nonobese group. Additionally, the significant difference in baseline levels between the nonobese and obese group may have an impact on the percent change following acute mental stress. There was a significant association between the %Δ in leptin POST120 and %Δ IL-1Ra POST120 in the nonobese group only ($R^2 = .518$, $F(1,9) = 10.661$, $p = .011$), as shown in Figure 3. After entry of baseline leptin and IL-1Ra, the total variance explained by the model as a whole remained significant ($R^2 = .579$, $F(3,9) = 5.125$, $p = .043$). Importantly, the baseline measures did not significantly contribute (R^2 change = 0.148, F change $(2,6) = 1.582$, $p = .281$) to the association between the %Δ in leptin POST120 and %Δ IL-1Ra POST120. The response of leptin to an acute mental stress task was not significantly associated with any other cytokine response.

Discussion

This is the first study to examine changes in IL-6, TNF-α, IL-1β, IL-1Ra, and leptin following an acute mental stress task in obese and nonobese males. The acute mental stress task elicited a significant increase in HR, EPI, and NE in both groups, demonstrating physiological activation in response to the stressor. Additionally, there was a significant increase in IL-1β in both groups. Although IL-6, TNF-α, and IL-1Ra did not change following acute mental stress, they were elevated in the obese group at all time points. There was a significant increase in cortisol in the nonobese group

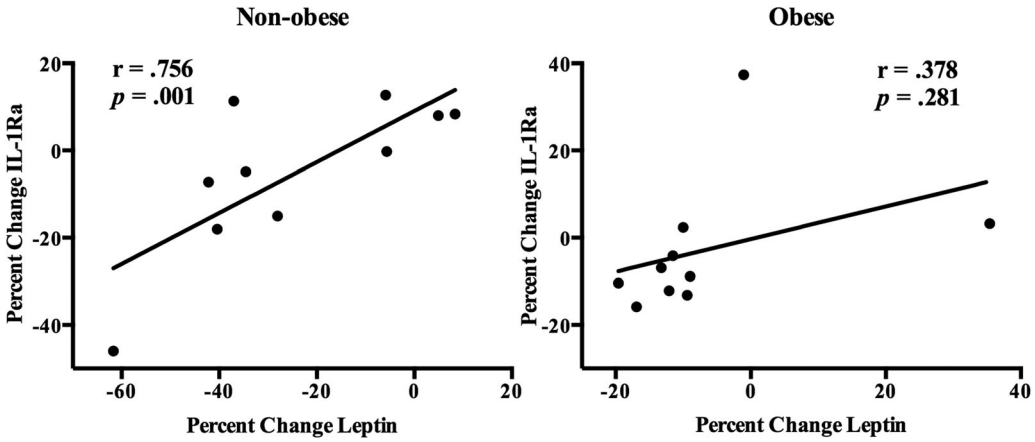

Figure 3. Relationship between the POST120 percent changes in leptin and IL-1Ra in the nonobese and obese groups.

only. Interestingly, a significant decrease in leptin was observed earlier in the obese group (30 min posttask) than in the nonobese group (120 min posttask). Furthermore, the association between percent change in leptin and IL-1Ra at 120 min following the acute mental stress task was shown to only be significant in the nonobese group. Since leptin has previously been shown to regulate the secretion of IL-1Ra (Gabay, Dreyer, Pellegrinelli, Chicheportiche, & Meier, 2011), it is plausible that obesity may impact the interaction between these two immune signaling messengers.

Sympathetic activation has been demonstrated to impact the inflammatory mechanisms of interest in this investigation (Johnson et al., 2005). Our results suggest that, in apparently healthy college-aged males, obesity does not affect HR or catecholamine production in response to acute mental stress. This is supported by similar findings in other studies of apparently healthy males following acute mental stress (Huang et al., 2014; Sothmann et al., 1995). Other chronic stressors, such as CVD and rheumatoid arthritis, have been shown to impact physiological responses to mental stress (Kop et al., 2008; Veldhuijzen van Zanten et al., 2005), and it is plausible that other comorbidities may be necessary to detect an effect of adiposity on the acute mental stress response. Additionally, we did not observe an effect of obesity on the increase in IL-1β concentrations. Together, these findings suggest that, in the absence of comorbidities and other CVD risk factors, obesity alone does not impact sympathetic markers of stress reactivity and IL-1β following an acute mental stress task. Conversely, our results suggest that obesity does impact cortisol concentrations in response to acute mental stress. While cortisol increased in nonobese individuals following our acute mental stress protocol, there was no significant change in obese individuals. Similar findings have been reported by other investigators who observed blunted cortisol responses with increased adiposity following acute mental stress (Jones et al., 2012; Phillips et al., 2012).

At rest, IL-6, TNF-α, and IL-1Ra were significantly elevated in obese individuals. These results support previous findings (Benson et al., 2009; Charles et al., 2011; El-Wakkad, Hassan, Sibaii, & El-Zayat, 2013; Ziccardi et al., 2002). It is important to note that IL-6, TNF-α, and IL-1Ra concentrations were not affected by the acute mental stress task. Previous findings consistently report increases in IL-6 following models of acute mental stress in both males and females (Brydon et al., 2005; Steptoe et al., 2001, 2007). While our results were not significant, IL-6 concentrations were similar to those reported by Steptoe, Owen, Kunz-Ebrecht, and Mohamed-Ali (2002) with a similar increase following acute mental stress. It is unclear why our protocol did not induce significant changes in IL-6; however, our small sample size or the time points of interest may have impacted the ability to determine significance. In a meta-analysis, the effect for TNF-α was not significant (Steptoe et al., 2007), and in individual study findings, equivocal results have been reported (Steptoe et al., 2001, 2002). Additionally, while many studies suggest that IL-1Ra increases following acute mental stress in women and both genders combined (Rohleder et al., 2006; Steptoe et al., 2001, 2002), there have been studies that have not observed a change in women (Brydon, 2011) or men (Steptoe et al., 2002). Furthermore, our results did not indicate an effect of obesity on the IL-1Ra response to the acute mental stress task. Only one study has reported a significant association between the change in IL-1Ra following acute mental stress and waist-hip circumference; however, subjects were healthy and nonobese females, with a reported mean BMI of 23.3 ($SD = 3.1$) and mean %BF of 25% ($SD = 5.4$; Brydon, 2011). One important consideration regarding differences observed between our data and others may be the differences in the acute mental stressors (Steptoe et al., 2007). Physiological responses may differ between public speaking, mirror tracing, mental arithmetic, and the Stroop color-word task. Furthermore, additional measures of stress and immune activation, such as C-reactive protein, would provide more clarity to the cytokine data reported in this study. Future studies should examine the effects of adiposity on other immune markers and other acute mental stress tasks.

This is the first study to examine leptin as a mechanism that may distinguish the inflammatory response in obese and nonobese individuals. At rest, leptin was significantly elevated in obese males as compared to their nonobese counterparts. This is well supported in the literature (Juge-Aubrey et al., 2005; Meier et al., 2002). Following acute mental stress, leptin concentrations decreased in both groups. Equivocal responses have been reported in female populations (Brydon et al., 2008; Tomiyama et al., 2012); however, no known studies have utilized males. Additionally, catecholamines are known to attenuate leptin production (Rayner & Trayhurn, 2001), which may have contributed to our findings. Interestingly, the decrease in leptin from resting values at different time points in the nonobese and obese groups suggests a potential impact of obesity on the production and clearance of leptin. A significant decrease in leptin at 30 min posttask was observed in the obese group, whereas the nonobese group demonstrated a decrease at 120 min. The influence of testosterone and cortisol may provide possible explanations for the varying time point changes observed in our leptin concentrations following acute

mental stress. Testosterone is known to inhibit leptin secretion from adipocytes in vitro (Wabitsch et al., 1997) and has been shown to increase following acute mental stress (Lennartsson, Kushnir, Bergquist, Billig, & Jonsdottir, 2012). Circulating testosterone concentrations are lower with increased adiposity (Camacho et al., 2013; Mogri, Dhindsa, Quattrin, Ghanim, & Dandona, 2013), and it is plausible that adiposity influences testosterone and therefore leptin following acute mental stress. Additionally, glucocorticoids are known to increase leptin production (Leal-Cerro, Soto, Martinez, Dieguez, & Casanueva, 2001). In the current study, the increase in cortisol in the nonobese group may have stimulated leptin production to counterbalance an inhibitory effect of testosterone and catecholamines, potentially delaying any reduction observed in leptin following acute mental stress.

Our findings suggest a significant relationship between the percent change in leptin and IL-1Ra at 120 min following an acute mental stress task in nonobese subjects. This relationship existed while controlling for BMI, percent BF, and baseline concentrations of leptin and IL-1Ra. Monocyte and adipocyte in vitro stimulation by leptin has been shown to increase IL-1Ra secretion, although this has not been examined in cells taken from obese individuals (Gabay et al., 2011; Perrier, Caldefie-Chezet, & Vasson, 2009). Obese individuals display greater leptin concentrations, which appear to contribute to reduced central leptin sensitivity in obesity (Zhang & Scarpace, 2006). Impaired leptin signaling, due to reduced leptin receptor sensitivity or saturated leptin signaling, may impact the relationship between leptin and IL-1Ra in obese individuals both at rest and in response to acute mental stress. Additionally, IL-1Ra may be primarily regulated by different hormones and cytokines following acute mental stress in obese individuals as compared to their nonobese counterparts. Examination of leptin receptor sensitivity could provide additional information about the effects of leptin on antiinflammatory cytokine production following acute stress.

Several limitations to this study, including a relatively small sample size, lack of a nonstress control condition, and precise time points examined, may have influenced the interpretations to cellular responses of acute mental stress. Although our sample size is relatively small, the comprehensive exclusion criteria resulted in a fairly homogenous sample population that aimed to differentiate the effect of adiposity in young males. The responses observed following acute mental stress are similar to those reported by other investigators (Phillips et al., 2012; Sothmann et al., 1995; Steptoe et al., 2002). Additionally, while changes due entirely to mental stress cannot be elucidated, experimental designs utilizing nonstress control groups have observed no changes in HR, blood pressure, and cytokines in the control group or in the nonstress condition in a within-subjects design (Brydon, Edwards, Mohamed-Ali, & Steptoe, 2004; Brydon et al., 2005; Heinz et al., 2003). It should be noted that leptin has not been examined in a nonstress control group and although leptin has a known diurnal variation, the daily fluctuations are linked to meal timing and not one's circadian clock (Schoeller, Cella, Sinha, & Caro, 1997). This meal-dependent variation has been completely abolished in mice following a period of fasting (Ahren, 2000). Our subjects reported to the Exercise Physiology Research Lab in a fasted state (~9 h postprandial), and both groups completed the study at the same time each day. Future examination should consider a within-subject design and a larger sample size in order to improve the detection of the effects of stress with the model used here. Lastly, the exact time course for the secretion and degradation of leptin and cytokines is unknown following acute mental stress, and our results were limited to concentrations observed 30 and 120 min following our 20-min stressor. It is possible that more robust increases and decreases occurred at time points that were not assessed, and future studies should examine additional time points.

In conclusion, obesity appears to influence the effect of acute mental stress on cortisol and leptin in apparently healthy college-aged males, potentially impacting the inflammatory response to an acute mental stress task. A significant relationship between the percent change in leptin and IL-1Ra following the acute mental stress task was only observed in the nonobese individuals. Additional research is warranted to examine the effects of obesity on acute mental stress responses and their association to CVD risk. Although our findings were observed following a 20-min acute mental stress protocol, it is believed that many individuals may experience longer or repeated stress bouts. It is of interest to investigate additional models of acute mental stress that would allow for comparisons that may be more closely related to real-life events. The results of this study suggest that, while leptin and leptin resistance are often examined in relation to hunger and satiety in obese individuals, consideration should be given to other physiological consequences related to leptin, including relationships to cytokine activity following acute stressors.

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