# Basal, Circadian, and Acute Inflammation in Normal vs. Overweight Men

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**ABSTRACT**

Increased inflammation is present in obese compared with normal weight individuals, but inflammation characteristics of nonobese, overweight individuals are less clear. Purpose: The objective of this study was to determine whether basal, circadian, and posteccentric exercise inflammation levels differ between normal and overweight men. **Methods:** Men (18–35 yr old) classified as normal weight (body mass index $\leq$2 5 kg·m$^{-2}$, n = 20) and overweight (body mass index = 25–30 kg·m$^{-2}$, $n$ = 10) completed exercise (EX) and control (CON) conditions in random order. Maximal voluntary effort and eccentric actions (3 X 15) using the elbow flexor muscles of one arm were performed, and blood was collected preexercise and 4, 8, 12, and 24 h postexercise at 7:00 a.m., 12:00 p.m., 4:00 p.m., 8:00 p.m., and 7:00 a.m. Blood was collected on a time-matched schedule without exercise for CON. Soluble tumor necrosis factor receptor-1, interleukin-6, C-reactive protein (CRP), and cortisol responses (EX value j time-matched CON value) were measured. **Results:** Basal CRP was higher in the overweight compared with normal weight group (mean $\pm$ SD, 0.542 $\pm$ 0.578 vs 1.395 $\pm$ 1.041 mg·L$^{-1}$). Soluble tumor necrosis factor receptor-1 increased ($P < 0.05$) 8 h postexercise in both groups, and the response was greater 12 and 24 h postexercise in the overweight compared with normal weight groups. Interleukin-6 increased ($P < 0.05$) 8 h postexercise, with a trend ($P = 0.09$) to be greater in the overweight group. CRP and cortisol responses were not detected. **Conclusions:** The low-grade inflammation state in overweight compared with normal weight men includes both higher basal CRP concentrations and enhanced acute inflammation, but not in changes to the circadian patterns of cortisol and inflammation variables.

There is a shift toward a chronic, low-level inflammatory state of the body that occurs as body mass index (BMI) and adiposity increase (33,34,37). As a result, obesity is considered a pro-inflammatory state, and individuals who are obese often have chronic low-grade inflammation, sometimes called microinflammation or metainflammation. Inflammatory processes contribute to the development of insulin resistance, CAD, neurodegen-erative diseases, and cancer (30); thus, it is important to understand when and how the pro-inflammatory state develops. C-reactive protein (CRP) is a biomarker indica-tive of the acute phase response portion of inflammation, and basal levels of this biomarker have been stratified to identify risk for inflammation-based chronic diseases, par-ticularly cardiovascular disease (29). Development of a pro-inflammatory state and higher disease risk may occur long before an obese BMI is reached, and evaluation of bio-markers other than CRP may be important to understanding the progression of inflammation as weight increases. The

development of effective inflammation and disease risk re-duction strategies will be more effective if it is determined whether low-grade inflammation occurs as simply an in-crease in selected biomarkers or as a fundamental change in inflammation regulation and responses.

In addition to CRP, several mediators of inflammation with a range of functions may have particular relevance to inflammation in younger populations. In addition to CRP, soluble tumor necrosis factor receptor-1 (sTNFR1) and interleukin (IL)-6 are among the inflammation markers pre-dictive of metabolic syndrome extent in young adults (15). sTNFR1 modulates tumor necrosis factor (TNF)-> bio-availability and may promote increases in adiposity by de-creasing thermogenesis (35). This may be an example of a mediator of inflammation actually participating in the mechanism underlying increased adiposity. IL-6 has a wide range of effects on inflammation and metabolism. Elevations in resting IL-6 occur with increasing adiposity and physical inactivity, and in this context, IL-6 plays a role in the pathology of cardiovascular disease development (13,30). Glycoprotein 130 (gp130) is an IL-6 receptor, and the soluble form of this receptor (sgp130) can block biological activity of IL-6. sgp130 also associates with development of metabolic syndrome (41). IL-10 is an anti-inflammatory cy-tokine, and it has been proposed that the increased expression of IL-10 in visceral adipose tissue of children may be a re-sponse to dampen inflammation in the early stages of devel-opment of the inflammation state (39). Soluble intercellular adhesion molecule-1 (sICAM-1) is a marker of cell adhesion and migration considered to be an early indicator of athero-sclerotic processes within arterial walls in young adults (9). Although there is evidence supporting a progression of in-flammation characteristics over time and across lean, over-weight, and obese groups, the details of this progression have not been adequately identified. These details may be useful in advancing the use of inflammation measurements to assess disease risk, the disease process, and the efficacy of inter-ventions to reduce inflammation.

The shift toward a pro-inflammatory state measured in basal or resting inflammation markers may include or be influenced by shifts in the circadian profile of cytokines and cortisol. A variety of research findings support the involve-ment of circadian rhythms in the development of meta-bolic syndrome including the demonstration of circadian rhythmicity in gene expression of adipocytes, glucose control, insulin action, inflammation, and disruption of feed-back loops between inflammation and stress hormones (noradrenaline and cortisol) (7,17,20,21,32). There is also a substantial body of evidence linking obesity-associated chronodisruption (disturbance of circadian rhythms, such as change in rhythm amplitude), increased incidence of meta-bolic syndrome, type 2 diabetes mellitus, and cardiovascular diseases (7,21). Cortisol is the mainneuroendocrine modulator of circadian variation in immune function (31). We previously reported that plasma concentrations of both sTNFR1 and IL-6 parallel the circadian rhythm of cortisol with levels that decrease from morning highs to evening lows (23). The rate of decrease in cortisol is a variable of growing interest because a flattened cortisol profile is influenced by BMI and may associate with health status and outcomes (16). Thus, the circadian patterns of both cortisol and inflammatory mediators are important elements to be examined to characterize the progression of inflammation with increases in body mass.

Differences in acute inflammation responses related to BMI may occur within and contribute to the chronic, low-level inflammatory state. We and others have found that acute inflammation is enhanced in overweight or obese individuals under either high carbohydrate intake or hyperglycemic conditions (4,14,25). For example, TNF-$\alpha$ is a potent pro-inflammatory cytokine that interferes with insulin signaling, and its production is suppressed under hypergly-cemic conditions. Kirwan et al. (14) found that the suppression of TNF-$\alpha$ was lost as BMI and waist circumference increased. In addition, we previously reported a positive association of BMI and waist-to-hip ratio with the magnitude of the increase in another pro-inflammatory cytokine, IL-1$\beta$, following eccentric exercise with high carbohydrate intake (25). Thus, there is evidence to suggest that inhibition of inflammation is diminished and acute inflammation may be enhanced in overweight individuals; however, research comparing acute inflammation responses across weight groups is limited.

High-force eccentric exercise elicits inflammation and is a useful model to study acute inflammatory responses. The sequence of inflammation events and regulation is for the eccentric exercise to elicit focal disruptions and tissue damage in muscle, followed by initiation of inflammation including local production of TNF-$\alpha$ and IL-1$\beta$, a robust increase in plasma IL-6 approximately 8 h postexercise that associate with perceived severity of delayed onset muscle soreness, and finishing with an increase in CRP if the systemic response is of sufficient magnitude to elicit an acute phase response (23). The damage to muscle tissue can be verified via measure-ment of creatine kinase (CK) activity in serum (24). Using this model of exercise-induced muscle damage, investigation of acute inflammation responses between normal and overweight individuals may help to determine whether the magnitude of or regulation of inflammation is altered or impaired as individuals become overweight.

The purpose of this study was to determine whether basal, circadian, or posteccentric exercise inflammation levels differ between normal and overweight men. Basal samples were fasting blood samples collected first thing in the morning, whereas circadian samples included basal and additional samples collected throughout the course of a nonexercise day. To achieve this purpose, we measured a mediator of the early activation of the inflammatory response (sTNFR1), a mediator in

the middle of the inflammatory cascade with both feedback and feedforward roles (IL-6), an acute phase protein to identify inflammation at the systemic level (CRP), and an immunoregulatory hormone that influences whole body metabolism and circadian rhythms (cortisol). While TNF-$\alpha$ is a cytokine of interest, it is difficult to measure changes in this cytokine in the plasma after eccentric exercise involving a small muscle mass. This difficulty may be related to the short, approximately 6-min half-life of TNF-$\alpha$ in the circulation (2). However, an increase TNF-$\alpha$ in the circulation elicits shedding of TNF receptors that are more stable in the circulation (1). Given the potential roles of TNFR1 in promoting obesity and as a marker of changes in the less stable TNF-$\alpha$, we measured sTNFR1 rather than TNF-$\alpha$. High-force eccentric exercise is being used as a tool to induce inflammation so that the characteristics of the response may be compared between normal weight and overweight individuals. We hypothesized that differences in inflammation markers and cortisol would be consistent with a shift toward enhanced inflammation in the over-weight individuals.

## MATERIALS AND METHODS

### Participants.

Individuals age 18–35 yr were recruited to participate in this investigation. The men included in the analysis for the present study were extracted from a larger pool of both men (n = 30) and women (n = 21) for which a summary of findings has been published previously (23). Individuals who reported performing activities in which lifting and lowering of heavy objects were performed or who recalled experiencing muscle soreness in the arm muscles at any time in the 6 months preceding the investi-gation were excluded from participation. The aim of these criteria was to eliminate confounding influences of the re-peated bout effect, in which muscle that has been exposed to high-force eccentric exercise will have a blunted muscle damage response to subsequent eccentric exercise bouts that occur within the next several months. Additional ex-clusion criteria included known anemia, musculoskeletal limitations, known inflammatory conditions, diabetes, heart disease, known kidney problems (excluding kidney stones), smoking, chronic use of anti-inflammatory medications (including over-the-counter nonsteroidal anti-inflammatory drugs), lipid-lowering medications, and regular performance of physical activity in which muscle soreness or bruising occurs. The research protocol and informed consent docu-ment for this investigation were approved by the Human Subjects Committee at Montana State University. Partici-pants were informed of the procedures and potential risks associated with the study and gave written informed consent before participation in this investigation. Data for the men were then grouped as normal weight (BMI = 18–24.9 kg·m$^{-2}$, n = 20) and overweight (BMI = 25–30 kg·m$^{-2}$, n =10) for the analysis in the present study. CRP levels above 10 mg·L$^{-1}$ are considered to reflect the presence of acute inflammation (29), and data from one man were excluded because of high initial CRP concentrations throughout both conditions (>10 mg·L$^{-1}$).

### Protocol.

All participants performed both an exercise (EX) and a control (CON) protocol in randomized order with equal numbers beginning in each of the two conditions to avoid a confounding effect of order (Table 1). Several restrictions were placed on participants to minimize vari-ability in physiological status. Standardized conditions for blood collections in the morning included an overnight fast and minimal physical activity before reporting to the labo-ratory at 7:00 a.m. for blood collection and assessments. Strenuous physical exercise that was judged to be near maximal in intensity or longer than 60 min in duration was not allowed while participants were active in the EX and CON protocols, i.e., not on the day of or the day before research activities took place. To avoid the influence of ill-ness on inflammatory parameters, participants were only tested if they were free of known infection for at least 1 wk. The EX protocol consisted of baseline assessments at 7:00 a.m., followed by a bout of high-force eccentric resistance exer-cise using the elbow flexor muscles of the nonpreferred limb (according to self-reported handedness), and follow-up assessments 4, 8, 12, 24, 48, 96, and 120 h postexercise. Assessments included muscle soreness, blood collection for blood-borne variables, and maximal force production. During the EX condition, the nonexercised arm was measured as a CON for maximal force production. A time-matched CON condition identical with the experimental condition but with-out the high-force eccentric exercise was performed for blood-borne variables. The experimental and control protocols were separated by at least 3 and no more than 6wk.

### High-force eccentric exercise.

The protocol for inducing muscle damage in the flexor muscles (primarily *m. biceps brachii* and *m. brachialis*) of the nonpreferred arm was performed using a computer-controlled, isokinetic dynamometer (Kin Com125 E+; Chattecx Corporation, Chattanooga, TN). The dynamometer was adjusted to the body height and limb length of the individual. The arm was supported by a padded bench at approximately 0.79 rad of shoulder abduction, the axis of rotation of the dyna-

mometer was aligned with the axis of rotation of the elbow, and the forearm was secured to the lever arm of the dynamometer with padded support just proximal to the wrist joint. Three sets of 15 repetitions of eccentric elbow flexion were per-formed with maximal effort at a rate of one repetition per 15 s and 5 min of rest between sets. Repetitions began with the elbow fully flexed and ended with the elbow fully ex-tended. Using maximal effort, participants attempted to keep the elbow in the fully flexed position as the dynamometer pulled the arm to a fully extended position at an angular velocity of 0.79 radIsj1. The dynamometer returned the arm to the fully flexed position and paused for 10 s before beginning the next repetition. Participants were verbally encouraged to give a maximal effort with each repetition.

TABLE 1. Characteristics and resting serum or plasma concentrations of blood lipid and inflammation variables for normal and overweight BMI groups.

| | Normal BMI Group | Overweight BMI Group | $P$-Value |
|---|---|---|---|
| Age (yr) | $20.6 \pm 3.9$ | $19.9 \pm 1.9$ | 0.588 |
| BMI (kg·m$^{-2}$) | $22.4 \pm 1.3$ | $27.1 \pm 5.1$* | <0.001 |
| TGs (mg·dL$^{-1}$) | $94.9 \pm 31.0$ | $113.9 \pm 46.0$ | 0.196 |
| Cholesterol (mg·dL$^{-1}$) | $154.6 \pm 25.4$ | $161.5 \pm 25.0$ | 0.493 |
| sTNFR1 (pg·mL$^{-1}$) | $1210.1 \pm 144.8$ | $1244.2 \pm 193.2$ | 0.596 |
| IL-6 (pg·mL$^{-1}$) | $1.310 \pm 0.760$ | $2.053 \pm 1.568$ | 0.185 |
| sgp130 (ng·mL$^{-1}$) | $251.0 \pm 38.3$ | $268.9 \pm 50.3$ | 0.238 |
| CRP (mg·L$^{-1}$) | $0.542 \pm 0.593$ | $1.395 \pm 1.040$* | 0.020 |
| IL-10 (pg·mL$^{-1}$) | $1.27 \pm 0.78$ | $1.21 \pm 0.86$ | 0.860 |
| sICAM-1 (ng·mL$^{-1}$) | $202.3 \pm 44.9$ | $197.4 \pm 29.3$ | 0.760 |
| Cortisol ($\mu$g·dL$^{-1}$) | $33.3 \pm 8.2$ | $31.9 \pm 6.1$ | 0.642 |
| CK activity (IU·L$^{-1}$) | $155.1 \pm 59.4$ | $201.9 \pm 114.7$ | 0.263 |
| CON/EX condition 1st | 11/8 | 4/6 | 0.359 |

Values are presented as mean $\pm$ SD unless stated otherwise.
* $P < 0.05$ compared with normal weight BMI group.

## Maximal force production.

Maximal isometric force production for elbow flexion at an enclosed elbow angle of 1.57 rad was measured before exercise, immediately after exercise (to identify the amount of fatigue induced by the exercise), and 24, 48, 96, and 120 h postexercise (to identify prolonged strength loss, which is an indicator of muscle damage [24]). To perform the isometric strength measurement, the dynamometer and subject position were adjusted as for the high-force eccentric exercise, and the lever arm was fixed such that the elbow was positioned with a 1.57 rad angle. Participants were instructed to pull (flex) for 3 s using maximal effort. Three maximal efforts were performed with 30-s rest between repetitions. To ensure uniformity of measurements from day to day, all dynamometer position settings for each subject were recorded and reproduced at each testing session. To eliminate variation due to initial strength levels, data were converted to percentages of the initial strength measurement before analysis.

## Muscle soreness.

A subjective assessment of muscle soreness was made by participants using a 100-mm visual analog scale anchored at one end with ''no soreness'' and at the other end with ''very, very sore.'' Participants were instructed to fully flex and extend the elbow while holding a 1-kg weight and gently squeezing the elbow flexor muscles and then to place a tick mark on the analog scale that rep-resented the degree of soreness. Participants also were instructed to think of their ratings in terms of muscle soreness, not of fatigue or relative to other types of pain, e.g., a broken bone.

## Blood collection and analysis.

Participants sat for 10–15 min before blood was collected from an antecubital vein into evacuated tubes using a standard venipuncture technique. Blood was collected in a vacuum tube without additive for analysis of CRP, cortisol, and CK, and containing ethylenediaminetetraacetic acid for IL-6 and sTNFR1. After clotting, serum was separated from cells using a refrigerated 21000R Marathon centrifuge (Fisher Scientific, Pittsburgh, PA). All samples were stored at j80-C until analysis. Fasting, basal, serum cholesterol, and triglyceride (TG) concentrations were measured in duplicate by standard laboratory techniques using an VitrosDT60 Ektachem analyzer (Eastman Kodak Co., Rochester, NY) and the procedures described by Lie et al.(19). Serum CRP (high sensitivity assay; MP Biomedicals, Irvine, CA), plasma IL-6 (high sensitivity assay; R&D Sys-tems, Minneapolis, MN), plasma sTNFR1 (R&D Systems), serum sICAM (R&D Systems), serum IL-10 (R&D Sys-tems), and serum cortisol (Diagnostic Systems Laboratories, Inc., Webster, TX) concentrations were measured using com-mercially available enzyme-linked immunosorbent assay kits according to the instructions of the manufacturers. Absor-bance of 96-well assay plates was read using a KQuant Uni-versal microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). All samples were run in duplicate. Average intraassay coefficients of variation for CRP, IL-6, sTNFR1, and cortisol were 11.0%, 7.6%, 3.8%, and 3.4%, respectively. On the basis of the anticipated time course for changes in these variables and the need to identify potential circadian variations throughout the day, CON samples were measured for the first 24 h postexercise only for these variables.

As an indirect marker of muscle damage to determine whether muscle damage responses were comparable be-tween normal and overweight groups, serum CK activity was measured using an ultraviolet, kinetic assay at 37-C (Thermo Scientific, Waltham, MA). The assay was modified for microplate analysis and read using a KQuant Univer-sal microplate spectrophotometer (Bio-Tek Instruments). Samples were run in duplicate, and all samples for a given participant were run in the same assay. The intraassay co-efficient of variation was 4.5%.

## Statistical analysis.

Data were analyzed using the Statistical Package for Social Sciences for Windows (version 20.0; IBM Corporation, Somers, NY). Variables were tested for normal distribution using the Kolmogorov–Smirnov test. Nonnormally distributed variables CRP and IL-6 were log transformed before statistical analyses. Base-line measures were compared between BMI groups using an independent t-test for continuous variables and chi-square analysis for categorical variables (condition order). Baselines for inflammatory variables were the mean of the initial values for the EX and CON conditions. Delta scores for inflammation variables of sTNFR1, IL-6, CRP, and cortisol were calculated by subtracting each value from the CON condition from the time-matched value from the EX condition. A general linear model two-way repeated-measures ANOVA was used to compare CON condition values (circadian variation) and delta scores (acute inflammation responses) over time and between groups. Post hoc analysis to determine the location of differences when significant main effects or interactions were detected was performed using paired t-tests to detect time differences and independent samples t-tests to detect differences between groups. The Bonferroni correction to alpha was used for multiple comparisons. Statistical significance was set at the alpha = 0.05 level.

## RESULTS

Basal CRP was the only variable that was higher in the overweight compared with normal weight group (Table 1). TGs, cholesterol, sTNFR1, IL-6, IL-10, sICAM-1, cortisol, and serum CK activity were similar between groups.

Circadian variation was similar between groups for cor-tisol, sTNFR1, and IL-6 (Table 2). Cortisol was highest at 7:00 a.m. and decreased (P G 0.05) at 12:00, 4:00, and 8:00 p.m., reaching a nadir at 8:00 p.m. in both groups. sTNFR1 followed a circadian pattern similar to that of cor-tisol with the highest value measured in the early morning and lower values throughout the rest of the day. IL-6 also was highest early in the morning; however, lower concen-trations were measured only at 12:00 p.m. compared with 7:00 a.m. Observed A values were calculated when trends were detected to determine the statistical power for detecting differences of the magnitude that occurred within the current data set. The observed A value for the group by time inter-action trend (P = 0.073) for sTNFR1 was less than 0.80. Thus, the potential for an interaction exists, but differences over time between groups were not sufficiently robust to achieve statistical significance.

TABLE 2. Diurnal variation for cortisol and inflammation variables measured during the nonexercise control (CON) condition for normal and overweight groups.

| | Normal Weight Group | | Overweight Group | P |
|---|---|---|---|---|
| Cortisol ($\mu$g·dL$^{-1}$) | | | | |
| 7:00 a.m. | 33.5 ± 1.8 | | 31.7 ± 2.2 | <0.001 (T) |
| 12:00 p.m. | 20.3 ± 1.3 | † | 23.7 ± 2.2 | 0.977 (G) |
| 4:00 p.m. | 14.5 ± 1.5 | † | 16.7 ± 2.8 | 0.211 (T x G) |
| 8:00 p.m. | 10.7 ± 1.2 | † | 9.3 ± 1.4 | |
| 7:00 a.m. | 31.5 ± 1.5 | | 28.9 ± 2.6 | |
| sTNFR1 (pg·mL$^{-1}$) | | | | |
| 7:00 a.m. | 1213 ± 41 | | 1229 ± 55 | <0.001 (T) |
| 12:00 p.m. | 1095 ± 38 | † | 1129 ± 51 | 0.811 (G) |
| 4:00 p.m. | 1046 ± 36 | † | 1046 ± 49 | 0.073 (T x G) |
| 8:00 p.m. | 1114 ± 35 | † | 1021 ± 47 | |
| 7:00 a.m. | 1192 ± 39 | | 1169 ± 52 | |
| IL-6 (pg·mL$^{-1}$) | | | | |
| 7:00 a.m. | 1.165 ± 0.143 | | 1.663 ± 0.367 | 0.001 (T) |
| 12:00 p.m. | 0.944 ± 0.179 | † | 1.515 ± 0.439 | 0.519 (T) |
| 4:00 p.m. | 1.071 ± 0.155 | | 1.410 ± 0.397 | 0.126 (T x G) |
| 8:00 p.m. | 1.563 ± 0.531 | | 1.676 ± 0.420 | |
| 7:00 a.m. | 1.219 ± 0.206 | | 1.834 ± 0.457 | |

Values are presented as mean ± SEM unless stated otherwise. T indicates main effect for time; G, main effect for group, T x G, time by group interaction.
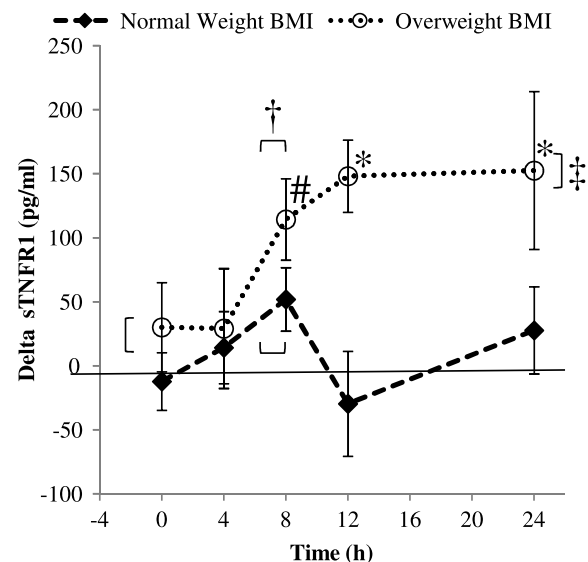† $P < 0.05$ compared with 7:00 a.m.



FIGURE 1—Delta (EX value − CON value) scores across time for the normal weight and overweight BMI groups for sTNFR1. Values = mean ± SEM. # $P < 0.05$ compared with preexercise within group. *$P < 0.05$ between groups. †$P < 0.05$ compared with preexercise. ‡$P < 0.05$ compared with normal weight group (group main effect).

Some elements of the acute inflammation response were greater in the overweight compared with normal weight men. The response of each variable was calculated as the value from the EX condition minus the value from the CON condition. Significant (P < 0.05) time, group, and time-by-group interactions were measured for sTNFR1. Compared with preexercise, there was an increased sTNFR1 response (P < 0.05, time main effect) at 8 h postexercise. *Post hoc* analysis identified that the sTNFR1 response was greater (P < 0.0125) at 8 h postexercise in the overweight group, and the sTNFR1
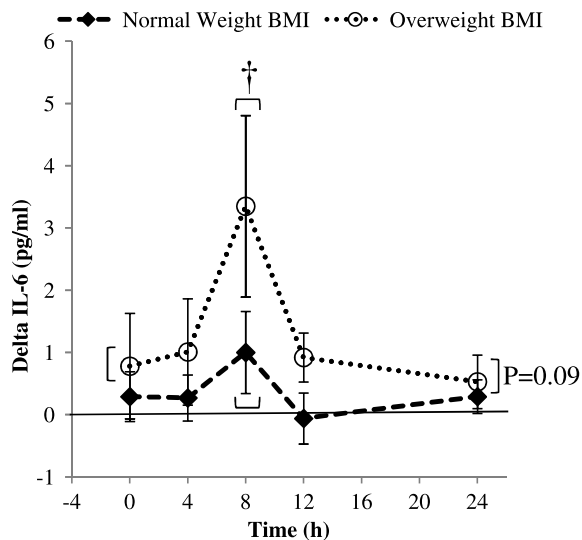
FIGURE 2—Delta (EX value − CON value) scores across time for the normal weight and overweight BMI groups for IL-6. Values = mean ± SEM. †P < 0.05 compared with preexercise. P = 0.09 compared with normal weight group (group main effect).

response was greater in the overweight compared with the normal weight group 12 and 24 h postexercise (Fig. 1). A significant time effect (P < 0.05) and trends for group (P = 0.09) and the time-by-group interaction (P = 0.10) were measured for IL-6. The IL-6 response increased (P < 0.05) 8 h postexercise and tended (P = 0.09) to be higher in the overweight group (Fig. 2). An effect for time (P < 0.05) was detected for the CRP (Fig. 3) and cortisol (data not shown) responses, but post hoc comparisons were not significant. Observed b for the group main effects that were trends for IL-6 and CRP were less than 0.80. Thus, the potential for group differences exists, but the differences between groups were not sufficiently robust to achieve statistical significance.

The increase in markers of muscle damage was similar between the normal and overweight groups (Table 3). Decreased (P < 0.05) strength was measured immediately through 120 h postexercise in both groups. Similarly, mus-cle soreness and serum CK activity were increased at all postexercise time points but did not differ between groups.

## DISCUSSION

Whether low-grade inflammation occurs as a change in selected biomarkers reflecting aspects of inflammation, as a change in the underlying neuroendocrine regulation (e.g., circadian variation), as a change in responses to acute stimuli (e.g., muscle damage), or as a combination of these is important to determine. This information may provideinsights to understand disease risk and to develop inflammation reduction interventions. The purpose of this study was to determine whether basal, circadian, or posteccentric exercise inflammation levels differ between normal and overweight men. The primary findings of this investigation were that there is evidence of basal, low-grade inflammation and enhanced acute inflammation responses in nonobese, overweight, young men when compared with normal weight men. This is consistent with our hypothesis and provides evidence that acute inflammation differences may play a role in the shift toward the pro-inflammatory state that coincided with increased CRP. Circadian variations in mediators of inflammation and cortisol were similar between groups.

Low-grade, basal inflammation is predictive of cardio-vascular disease risk connected to metabolic syndrome and considered to be part of the mechanism for the development of a variety of disease conditions including neurodegenerative diseases, cancer, type 2 diabetes mellitus, and cardiovascular diseases (10,29,30). The positive association between basal inflammation, particularly CRP, and proxies for adiposity such as BMI, waist circumference, or waist to hip ratio is well established (33,34,37). Our finding that overweight men with an average BMI of about 27 kg·m$^{-2}$ had higher CRP concentrations compared with normal weight men with a BMI of about 22 kg·m$^{-2}$ is not surprising, except that this is an early indication of increased risk for cardiovascular disease events (CRP >1.0 mg·L$^{-1}$) in younger men (29). However, we did not detect basal differences between normal and overweight young men for sTNFR1, IL-6, sgp130, IL-10, or sICAM-1. Thus, although CRP was theassociated with obesity is present in young, overweight, otherwise low disease risk men. This finding is consistent with previous research with elevations in inflammation in young, overweight youths 10–14 yr old (36).

Eccentric exercise involves forcing muscles to lengthen against resistance and has been used as a research model to induce inflammation (12,23), and this exercise was used as a model to study inflammation differences between normal weight and overweight men. Inflammation is the general response of the body to tissue injury, with the overall goal being healing. The inflammatory response is a sequential process involving cytokine and chemokine signals, leuko-cytes, oxidative stress, and acute phase proteins (38). Strength loss, serum CK activity, and muscle soreness are indicators of muscle damage (5). The changes that we mea-sured in this study indicate that muscle damage was induced by the eccentric exercise, but there were no differences between groups. As a result, we infer that differences be-tween groups in the level of inflammation induced by the eccentric exercise resulted from differences related to BMI, the grouping variable, and not from differences in the degree of muscle damage. Previous research comparing responses of high-force eccentric exercise of the knee extensor muscles in women measured greater changes in indices of muscle damage, peak torque, soreness, and serum CK activity, in overweight compared with normal weight women (28). Our findings may differ because of difference in sex of the par-ticipants or because of our use of the arm versus the previous researchers' use of the weight-bearing leg muscles.
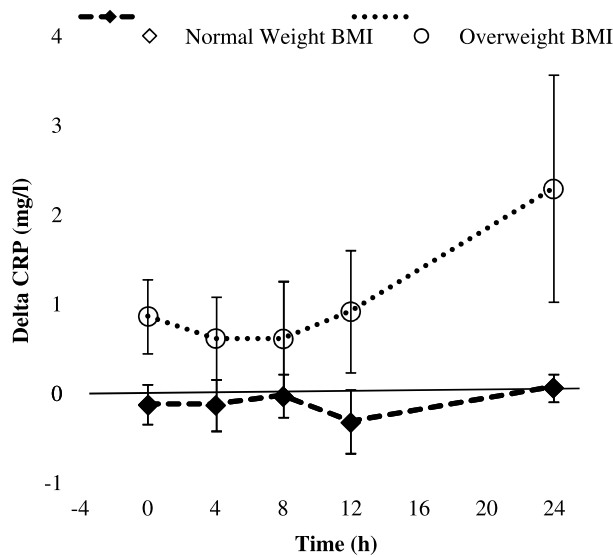
**FIGURE 3—Delta (EX value − CON value) scores across time for the normal weight and overweight BMI groups for CRP. Values = mean ± SEM.**

TABLE 3. Indirect markers of muscle damage in eccentric exercise (EX) condition for normal and overweight BMI groups.

| | Normal Weight Group | | Overweight Group | | P Time, Group, T x G |
|---|---|---|---|---|---|
| Strength loss (%) | | | | | |
| 0 h postexercise | −33.6 ± 4.6 | † | −25.8 ± 6.6 | | <0.001(T) |
| 24 h | −29.0 ± 5.4 | † | −22.4 ± 7.6 | | 0.431(G) |
| 48 h | −25.7 ± 5.6 | † | −21.5 ± 8.0 | | 0.888(T x G) |
| 96 h | −19.8 ± 5.3 | † | −9.9 ± 7.5 | | |
| 120 h | −12.3 ± 5.7 | † | −6.6 ± 8.1 | | |
| Soreness (mm) | | | | | |
| Preexercise | 2.5 ± 1.7 | | 2.3 ± 2.3 | | <0.001(T) |
| 4 h | 14.5 ± 3.2 | † | 19.1 ± 4.6 | | 0.743(G) |
| 8 h | 13.2 ± 3.8 | † | 19.6 ± 5.4 | | 0.773(T x G) |
| 12 h | 18.5 ± 4.0 | † | 21.0 ± 5.7 | | |
| 24 h | 36.5 ± 4.8 | † | 37.5 ± 6.8 | | |
| 48 h | 43.9 ± 4.4 | † | 48.3 ± 6.3 | | |
| 96 h | 27.3 ± 5.7 | † | 21.4 ± 8.0 | | |
| 120 h | 12.4 ± 3.8 | † | 13.6 ± 5.4 | | |
| CK (IU·L$^{-1}$) | | | | | |
| Preexercise | 155.2 ± 16.3 | | 179.6 ± 22.5 | | 0.002(T) |
| 24 h | 316.1 ± 60.5 | † | 368.4 ± 83.4 | | 0.809(G) |
| 48 h | 826.9 ± 307.4 | † | 768.0 ± 423.7 | | 0.348(T x G) |
| 96 h | 4184.9 ± 1474.9 | † | 1802.4 ± 2033.0 | | |
| 120 h | 4044.7 ± 1304.4 | † | 1649.0 ± 1798.0 | | |

Values are presented as mean ± SEM unless stated otherwise.
† $P < 0.05$ compared with preexercise.
T indicates main effect for time; G, main effect for group; and T x G, time by group interaction.
CK = serum CK enzyme activity.

Higher levels of sTNFR1 and IL-6 in the eccentric exer-cise compared with control condition indicate that early (sTNFR1) and middle (IL-6) phases of inflammation oc-curred eight or more hours after the exercise bout. Although there was a trend (P = 0.09) for the IL-6 response to be greater in the overweight group of men, the sTNFR1 re-sponse was significantly greater in the overweight group. Thus, we infer from this finding that physiological mecha-nisms related to higher BMI are linked to the enhancement of the acute inflammation response. McMurray et al. (22) found that cytokine responses (TNF->, IL-6, and IL-1 re-ceptor antagonist) to high-intensity, intermittent exercise in adolescent boys and girls did not differ between normal and overweight groups, but this exercise may not have produced muscle damage and measurements were only made 0 and 2 h postexercise. The peak of IL-6 occurred 8 h postexercise in our study. Our findings concur with those of Gonzalez et al.(8) who found that inflammation responses were greater in obese compared with normal weight women in response to hyperglycemia. Our findings are evidence that exercise-induced muscle damage is a stimulus to which overweight individuals have a greater inflammation response and that the influence of increased body mass begins before obese body mass levels are reached.

Our interest in the difference in acute inflammation be-tween normal and overweight men stems not only from the interaction between adipose tissue and inflammation me-diators but also from recent evidence that inflammation mediators influence thermogenesis and the accumulation of fat mass (26). In particular, sTNFR1, as demonstrated using knock-out mice lacking this receptor, has a protec-tive role against diet-induced obesity and insulin resistance (27,35,40). As a crucial element in the initiation and am-plification of the inflammation response, TNF-$\alpha$ induces cellular effects primarily through signal transduction in-volving either TNFR1 or TNFR2 (11). Only TNFR1 results in activation of nuclear-factor-$\kappa$B to induce production of pro-inflammatory pathways, and only TNFR1 has been im-plicated in the interference of insulin signaling and insulin resistance (11,40). Cell-associated TNFRs are shed when TNF-$\alpha$ increases, resulting in an increase in soluble recep-tors in the circulation, sTNFR1 and sTNFR2 (1). One ra-tionale for the shedding of the soluble receptors is that they can sequester the TNF-$\alpha$ to increase its half-life in the cir-culation from 6 min to greater than 2.5 h (2). However, independent roles of sTNFRs with respect to influencing obesity-associated inflammation, thermogenesis, and fat mass need to be evaluated. BMI is a rough proxy for adi-posity used in this investigation to differentiate normal and overweight without a specific measure of fat mass. Our finding that sTNFR1 levels were higher during exercise-induced inflammation in overweight but not normal weight men is a unique finding that should be further investigated so that it may be determined whether this difference is linked specifically to body fat (not measured in the present in-vestigation). This may be an indication that overweight men had greater increases in TNF-$\alpha$ to induce shedding of TNFR1. Alternatively, there may be an up-regulation of TNFR1 that leads to greater increases in overweight compared with normal weight men.

The interrelationships of the neuroendocrine and immune systems are complex; thus, the linkage between circadian patterns in endocrine and immune parameters and the development of obesity and related consequences also is complex. This issue is important because some studies have measured associations between short sleep duration (a disruptor

of circadian rhythms) and obesity, diabetes mellitus, and hypertension incidence (6). It also has been demonstrated in animal models that inducing obesity with a high-fat diet disrupts circadian patterns for insulin resistance andmediators of inflammation including TNF-$\alpha$ and IL-6 (3). Thus, there is evidence not only that disruption of circadian patterns, for example, by altering sleep patterns, in-creases the likelihood of becoming obese or acquiring some diseases, but also that becoming obese disrupts circadian variation and influences development of some diseases. In humans, a flattening of the circadian variation in cortisol occurred in the highest (>31 kg·m$^{-2}$) and lowest( <21 kg·m$^{-2}$) BMI groups, a U-shaped association, and for men ($n = 2915$) and women ($n = 1041$) in the Whitehall II study (16). Other researchers found flattening of cortisol var-iation with increasing abdominal obesity in women but not in men (18). Our finding that circadian patterns in cortisol, sTNFR1, and IL-6 were similar for normal and overweight men may be an indication that men are not sensitive enough to body mass–related flattening of circadian patterns for this to be measured in nonobese, over-weight men. Alternatively, it may be that overweight men fall within the same region of the U-shaped association as normal weight men and do not have circadian disruptions. Regardless, we found no evidence that normal and over-weight men differed in circadian patterns for the endocrine and immune variables measured.

We conclude that the elements of the pro-inflammatory state characteristic of obesity that can be detected in over-weight men are an increase basal concentrations of CRP and a modest enhancement of the acute inflammation response. This enhancement was most evident for sTNFR1 than other mediators of inflammation. Whether the enhanced response is a result of a greater TNF-$\alpha$ response or the result of the sTNFR1-specific role in obesity and insulin resistance requires further investigation. Circadian patterns of cortisol and mediators of inflammation are unchanged in young, healthy, overweight men. This finding adds to the body of evidence suggesting that being overweight, not just obese, influences the risk of diseases associated with inflammation and can do so at a relatively early age. Furthermore, our findings suggest that body mass–associated increases in in-flammation include both basal levels of mediators of in-flammation and the magnitude of the acute inflammation response. It is possible that additional differences between groups are present but too small to detect with our experi-mental methods and samples size; thus, it may be most reasonable to conclude that the differences we measured are the most pronounced and additional differences were not of sufficient magnitude for reliable detection. Accordingly, the trends for circadian differences in sTNFR1 and greater IL-6 and CRP during acute inflammation may be emerging differences that will become more pronounced as BMI in-creases further. The primary limitations of this study are that cardiovascular fitness, chronic physical activity levels, and body composition were not measured. These variables in-fluence inflammation, and adjustment for their influence would allow for greater discrimination between BMI groups. Future research that includes these measurements to aid in interpretation of inflammation differences is recommended.

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