Adipokine Levels Are Altered by Shiftwork: A Preliminary Study

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INTRODUCTION

Shiftwork is associated with an increased risk for developing nutritional, cardiovascular, and metabolic disorders (Ardekani et al., 2008; Boivin et al., 2007; Chen et al., 2010; Foster & Wulff, 2005; Padilha et al., 2010; Scheer et al., 2009). There is evidence that both male and female shiftworkers in various occupations are more likely to develop metabolic syndrome over a 4–5-yr period than day-working controls (Lin et al., 2009; Pietrolusti et al., 2010). In addition, we recently reported that a group working early shifts (i.e., 06:00–14:00 h) exhibited altered nutrition metabolism (Crispin et al., 2011) and higher insulin resistance (Padilha et al., 2010).

Although individuals who are subjected to shiftwork schedules appear to be predisposed to develop insulin resistance, little is known about the underlying etiology. However, studies that were conducted over the past few decades clearly indicate that adipokines may play at least a partial role in the development of insulin resistance. Adipokines were initially described as adipose tissue–derived cytokines but are now known to be a large, diverse group of substances secreted by adipose tissues that have endocrine or paracrine functions (Meijer et al., 2011). One such adipokine, adiponectin, is abundant in the human circulation, and its concentration is inversely associated with the risk of developing type 2 diabetes. This peptide can reduce the production of proinflammatory cytokines, and it positively modulates insulin signaling pathways (Luo et al., 2010). In addition, adiponectin has antiatherogenic properties (Nells & Olefsky, 2006). In contrast, adiponectin levels can be significantly reduced by tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Tilg & Moschen, 2006), two proinflammatory cytokines that are secreted primarily by inflammatory cells, and TNF-α has been demonstrated to induce insulin resistance. Moreover, these
cytokines are overexpressed when adipose tissue mass increases, due to increased infiltration of inflammatory cells into tissues. Both IL-6 and TNF-α have been consistently shown to be associated with chronic disorders, in particular, cardiovascular disease, obesity, and type 2 diabetes (Singh & Newman, 2011).

Because adipokines are recognized as playing a principal role in the pathogenesis of metabolic and cardiovascular disorders and because shiftworkers have a higher propensity to develop such disorders, changes in the daily variation of these peptides throughout a 24-h period might be the link between adipokines and disease. Since such daily variations are found in subjects living normally and generally assumed to be part of the integration between individuals and their environment, any circumstance where such an integration is disturbed—as in shiftwork—becomes a source of possible concern. Accordingly, the objective of this study was to measure the daily variation in the concentrations of adiponectin, TNF-α, and IL-6 in a sample of men who were exposed to shiftwork (one group worked a fixed night shift and the other a fixed morning shift) and a group of dayworkers. Those involved in the fixed night and early morning day schedules were considered to be the shiftworkers group given their work times.

MATERIALS AND METHODS

Participants

This study included two all-male groups of blue-collar workers who worked in fixed shifts within the steel industry. The factory was located in the city of Diadema, São Paulo State, Brazil. A day-shift group that had conventional hours of work, meals, and sleep was included as a control. Because there were no workers with corresponding job characteristics in the day shifts at this factory, day-shift volunteers with similar physical and cognitive demands were selected from another company to serve as the day-shift control group. No significant differences were observed in their physical activity levels based on the protocol of Baecke et al. (1982).

The work hours of the three groups were as follows: the night shift (n = 9) 22:00 to 06:00 h; the early morning shift (n = 6) 06:00 to 14:00 h; and day shift (n = 7) 08:00 to 17:00 h. Each group worked for six continuous days and then had one off day. Each subject had been working the same shift schedule for at least 2 yrs and was healthy, nonobese (body mass index [BMI] <30 kg/m²), medication free, and nonconsumers of alcohol or tobacco products. Any subjects who had experienced a variation in body mass (±2 kg) in the previous 2 yrs were excluded from study.

Ethics

This study was approved by the Ethics Committee of the Universidade Federal de São Paulo (protocol number 0591/07) and was conducted according to the principles of international ethical standards (Portaluppi et al., 2010). Informed written consent was obtained from each volunteer before starting the study. The participants had the option to leave the study whenever they wished.

Preliminary Evaluations

Energy Intake

Food intake was determined through a self-administered food diary that was kept over the course of seven successive days. An analysis of the energy intake (EI) and nutrient intake was performed using the Virtual Nutri software (version 1; University of São Paulo, Brazil, 1996). Macronutrient intake was analyzed according to recommendations of the Dietary Reference Intakes (Institute of Medicine [IOM], 2005).

Body Composition Measurements

Body composition was assessed by measurement of triceps, subscapular, and abdominal skinfold thickness. Skinfold thickness was measured on the right side of the body (Jackson & Pollock, 1985) by a trained investigator using a calibrated Lange calliper (Beta Technology, Santa Cruz, CA, USA) with .1-mm precision. Measurements were taken three times and the median of the three measurements was used in the analysis. Body density was estimated using sex-specific three-site equations (Jackson & Pollock, 1978) and was converted to a percentage of fat using the Siri equation (Siri, 1961). Waist circumference was measured to the nearest .1 cm with a flexible steel measuring tape at the midpoint between the lowest rib and the iliac crest. The measurement was made at the end of a normal expiration while the subject stood upright, with feet together and arms placed freely by the side of the body.

Sleep

Sleep variables were recorded by the participants for 1 wk using a previously validated sleep diary (Taheri et al., 2004). Sleep quality was determined using a version of the Pittsburgh Sleep Quality Index (PSQI) that was translated into Portuguese and validated (Bertolazi et al., 2008). The PSQI has seven items and a total possible score of 0 to 21; a higher score indicates poorer sleep quality. A PSQI score ≥5 was considered as an indicator of poor sleep quality (Bertolazi et al., 2008).

Chronotype

Chronotype was assessed using a Brazilian adaptation of the Morningness/Eveningness Questionnaire (Benedito-Silva et al., 1990).

Experimental Protocol

The experimental protocol maintained the normal routine of the shiftworkers with regard to energy intake, work schedule, and sleep patterns as much as possible during the 24-h testing period. The experimental protocol was conducted during September (winter). The participants were also asked not to deviate from their usual...
eating habits, sleep patterns, and work schedules during the week prior to the testing period, so that the data would duplicate those collected in preliminary assessments. For data collection in the workplace, blood samples were collected on the third or fourth day of the shift. The samples were obtained between 06:00 and 14:00 h for the early morning workers, between 22:00 and 06:00 h for the night workers, and between 08:00 and 17:00 h for the dayworkers. After work, the research team transported the subjects to the Sleep Institute at the Universidade Federal de São Paulo for collection of samples at the other times of the day, as explained in the next section. The subjects stayed in private rooms and were allowed to rest and sleep and to eat their normal type of meal at their usual time. In the workplace, volunteers remained inside the factory in warehouses with small windows. In the Sleep Institute, volunteers slept in rooms with windows fitted with blackout curtains. During wakefulness, subjects were allowed to use the telephone and a computer at the times they normally did so. No naps were taken, and no caffeine intake or vigorous physical activities were allowed throughout the experimental protocol. The volunteers were monitored by the research team throughout this time. After completing the 24-h study period and all evaluations, the subjects were driven either to their home or to workplace.

**Blood Sampling**

Venous blood samples were collected every 4 h over the course of 24 h (at 08:00, 12:00, 16:00, 20:00, 24:00, and 04:00 h), beginning at the start of the shift. Blood samples at work were collected by a nurse from the research team. At the Sleep Institute, blood samples were obtained using an indwelling catheter. During sleep, a nurse entered the room and collected the blood sample in dim light (≈6 lux) without disturbing the subject. To minimize stress to the volunteers, blood samples were collected by the same experienced phlebotomist. A total of 210 mL of blood was collected over the course of the 24-h (the total volume of the two samples at each time point being 35 mL). These amounts did not cause significant change in hematocrit level (Simon et al., 1994).

**Assays**

The concentrations of adiponectin, TNF-α, and IL-6 were measured using the respective commercially available competitive enzyme-linked immunoassay (ELISA) kits (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). The assays were performed according to the instructions provided by the manufacturer in the AFIP-Medicina Laboratorial Validation and Research Department. Sensitivity was .106 pg/mL, .039 pg/mL, and 1.5 ng/mL for TNF-α, IL-6, and adiponectin, respectively. The amount of peptide in each sample was determined from absorbance values using standard curves. The ranges of the standard curves were .550–2.816 pg/mL, .447–9.96 pg/mL, and 1.5–100 ng/mL for TNF-α, IL-6, and adiponectin, respectively.

**Food Intake**

The researchers monitored all of the meals that were provided to the subjects during the experiment. During work and when at the Sleep Institute, the subjects ate four standard meals during the 24-h experimental period (breakfast, lunch, afternoon snack, and dinner). The composition of the meals reflected the subjects’ habitual food intake as recorded in their food diary. The meal times also mimicked the usual eating patterns of the subjects, and care was taken to ensure the meals were not consumed close to the times when blood was collected. The meals were consumed at the following times: 06:00, 12:30, 16:30, and 20:15 h for the dayworkers; 05:30, 12:30, 16:30, and 20:15 h for the early morning workers; and 14:15, 20:30, 02:00, and 06:15 h for the night workers.

**Statistical Analysis**

All of the values are presented as mean ± SEM. Hormone levels are presented as the daily variation over the 24-h period (from six sample points), the 24-h plasma means, and the first values that were obtained after sleeping. Variables were normally distributed, and a one-way analysis of variance (ANOVA) was used to compare the morphological, dietary, sleep, and physical activity variables between the three groups. Two-way mixed design ANOVAs were conducted to compare changes in adiponectin, TNF-α, and IL-6 over the course of 24 h in the three groups that were working at different times of the day. The between-subject factor was shift (three levels), and the within-subject factor was time of day (six levels). Differences were tested for significance using the Tukey’s test for post-hoc analysis. The minimal statistical significance was set at p < .05, and the analyses were performed using SPSS 12.0 for Windows (SPSS, Chicago, IL, USA).

The single cosinor method (Nelson et al., 1979) using a 24-h period was used for analyzing circadian rhythms of each individual for adiponectin, TNF-α, and IL-6 levels. The cosinor parameters—mesor (with the equal sampling protocol, the 24-h mean), amplitude (one-half the peak-to-trough variation due to rhythmicity), and acrophase (peak time relative to local midnight)—were then combined to perform a group cosinor analysis for each of the three variables for the day-, early-morning-, and night-shift workers. Acrophases with both single and group cosinor analyses were assessed with reference to local midnight rather than some other parameter such as mid-sleep, for example.

**RESULTS**

**Subject Characteristics**

No significant differences were found in the age, years of shiftwork, morphological variables, and physical activity levels between the three groups of workers (Table 1).

The dietary pattern was significantly different between groups. The early-morning-shift group had significantly lower total energy intake than the other two groups. The day-shift group ingested a significantly lower
percentage of protein and fat, and a higher percentage of carbohydrate, than the other two groups. Despite the statistically significant differences, the macronutrient intakes were within the recommended values of the Dietary Reference Intakes (IOM, 2005) in all three groups.

Sleep duration was significantly longer in the early-morning-shift group than in the other groups (Table 1). PSQI scores did not differ statistically between the three groups. However, five (56%) of the night workers, four (57%) of the dayworkers, and six (100%) of the early morning workers had a PSQI total score that was >5, suggesting they were “poor sleeper.”

Chronotype analysis showed similarities between the study groups, and most individuals were classified as intermediate (88% of night workers, 86% of early morning workers, and 83% of dayworkers).

The Effects of Work Shift on Hormone Profiles
Table 2 shows the mean 24-h plasma concentrations of adiponectin, TNF-α, and IL-6 for the workers of each of the three shifts. An ANOVA revealed that adiponectin and TNF-α, but not IL-6, exhibited a significant main effect of shift. The early morning group had lower mean levels of adiponectin than the other two groups (p = .016), and the day group had lower mean levels of TNF-α than the other two shift groups (p < .0005). The 24-h mean IL-6 levels did not differ significantly between groups (p = .147).

The daily profiles of adiponectin, TNF-α, and IL-6 are shown in Figure 1. Time-of-day did not have a significant effect on adiponectin (p = .829), TNF-α (p = .779), or IL-6 (p = .979) levels in any group.

Cosinor analysis revealed only one significant rhythm when levels of adiponectin, TNF-α, and IL-6 were assessed on an individual worker basis. Group cosinor analysis was then performed for each of the three variables per each of the three groups of workers (Table 3). No significant group rhythm was found. That is, there was no evidence for significant 24-h cosine rhythms in any variable for the vast majority of individuals or any group of workers.

DISCUSSION
In this study, we found that the early morning group had lower levels of adiponectin than the other two groups and that both shiftwork groups (night and early morning...
groups) had higher levels of TNF-α than dayworkers. These findings should be emphasized, as they might contribute to our understanding of the higher prevalence of metabolic disorders and insulin resistance observed in shiftworkers. This higher prevalence is independent of excess fat mass (Crispim et al., 2011; Padilha et al., 2010), and consistent with this, our sample did not contain significant differences between BMI or body fat percentage.

Adiponectin levels are high in human serum, and adiponectin is secreted by adipose tissue in inverse proportion to the fat content in the tissue (Buechler et al., 2011). Thus, the fact that early-morning-shift workers had lower levels of adiponectin compared with the other two groups is noteworthy, because epidemiological studies have found that low adiponectin levels are associated with insulin resistance (Hui et al., 2004; Neumeier et al., 2006; Targher et al., 2004). In agreement with these findings, Wang et al. (2009) observed that shiftwork is associated with impaired glucose metabolism, and recently we reported that early morning workers had a significantly higher incidence of insulin resistance than night and dayworkers (Padilha et al., 2010). This difference in adiponectin levels between the early morning shift and the other two groups should be better investigated in future studies, particularly because early morning shiftwork has received little attention in the literature.

Despite showing that the mean adiponectin levels over the course of the 24 h differed between the groups of subjects, there was no evidence for a significant daily variation (Figure 1) or 24-h rhythm in an individual or group (Table 3). Sheer et al. (2010) found that adiponectin followed significant day/night rhythms in healthy, lean, nonsmoking men who lived conventional lifestyles, with troughs in the night that were independent of the feeding/fasting cycle. However, daily variations in adiponectin levels in shiftworkers have not been investigated, precluding a comparison of the results of Sheer et al. (2010) with those of the present study. To better understand this topic, the effects of sleep deprivation and rhythm misalignment among shiftworking populations (Rajaratnam & Arendt, 2001) requires investigation.

### FIGURE 1
Mean (± SEM) concentrations of adiponectin, TNF-α, and IL-6 at 6 time points in workers in the early morning (EM - ▪), night (N - ◆), and day (D - ▲) shifts. = Mean sleep duration of the night-shift workers. • Mean sleep duration of the early-morning shift workers. — Mean sleep duration of the day workers.

### TABLE 3. Group cosinor parameters for the adiponectin, TNF-α, and IL-6 levels in day-, early-morning-, and night-shift workers

<table>
<thead>
<tr>
<th></th>
<th>Mesor</th>
<th>Amplitude</th>
<th>Acrophase T&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Acrophase D&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p value&lt;sup&gt;*&lt;/sup&gt;</th>
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<td>Day shift</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Adiponectin</td>
<td>10.24</td>
<td>.41</td>
<td>18.10</td>
<td>271</td>
<td>.34</td>
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<tr>
<td>TNF-α</td>
<td>7.54</td>
<td>.60</td>
<td>5.10</td>
<td>77</td>
<td>.23</td>
</tr>
<tr>
<td>IL-6</td>
<td>3.09</td>
<td>.38</td>
<td>15.40</td>
<td>231</td>
<td>.86</td>
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<td>Early morning shift</td>
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<td></td>
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<tr>
<td>Adiponectin</td>
<td>6.86</td>
<td>.94</td>
<td>15.90</td>
<td>239</td>
<td>.08</td>
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<tr>
<td>TNF-α</td>
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<td>9.30</td>
<td>140</td>
<td>.10</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.32</td>
<td>.16</td>
<td>6.30</td>
<td>94</td>
<td>.26</td>
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<tr>
<td>Night shift</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin</td>
<td>9.76</td>
<td>2.75</td>
<td>2.40</td>
<td>36</td>
<td>.44</td>
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<tr>
<td>TNF-α</td>
<td>9.22</td>
<td>.26</td>
<td>9.00</td>
<td>135</td>
<td>.66</td>
</tr>
<tr>
<td>IL-6</td>
<td>2.77</td>
<td>.21</td>
<td>11.90</td>
<td>179</td>
<td>.32</td>
</tr>
</tbody>
</table>

<sup>a</sup>Statistical significance was set at p < .05.
<sup>b</sup>Acrophases presented as clock hours and fraction of clock hours (where midnight = 24:00 h).
<sup>b</sup>Acrophases presented as degrees (where 24 h = 360° and 0° = midnight).
The infiltration of macrophages into adipose tissue, which in turn is responsible for the local production of TNF-α and IL-6, may be a primary cause of the low-grade inflammation and insulin resistance that are often found in obese subjects (Weisberg et al., 2003; Wellen et al., 2005). However, in the present study, the levels of IL-6 did not show a significant 24-h rhythm in an individual or group (Table 3), and mean 24-h plasma concentrations did not differ significantly between the groups (Table 2). The production of IL-6 by adipose tissue is variable and ranges between 10% and 35% of systemic IL-6 levels (Fried et al., 1998; Mohamed-Ali et al., 1997). Consistent with our study, van Mark et al. (2010) showed recently that day- and shiftworkers had similar serum levels of IL-6.

Studies have clearly documented that adiponectin expression is down-regulated by TNF-α. This relationship is consistent with our data, in which both shiftworker groups exhibited higher levels of TNF-α than dayworkers. These results differ from those of van Mark et al. (2010), who reported that day- and shiftworkers had similar serum TNF-α levels; however, their findings were based on a single fasting baseline sample, whereas ours were based on six equally timed samples obtained over a 24-h period. Although TNF-α is usually increased in obese subjects (Nells & Olefsky, 2006; Trayhurn & Woods, 2004), our sample was composed of healthy nonobese workers, and neither BMI nor body fat percentage differed between the groups. Therefore, some other factor (s) might be responsible for the low-grade inflammation and for understanding the metabolic changes that predispose workers to glucose metabolism disorders.

TNF-α has been proposed as a link between obesity and insulin resistance; this is because TNF-α is overexpressed in adipose tissues of obese animals and humans, and obese mice lacking either TNF-α or its receptor show protection against developing insulin resistance (Borst, 2004). As TNF-α was higher in night- and early-morning-shift workers when compared with the dayworkers, we postulate that this cytokine might serve as an important marker for recognizing metabolic disease in these workers.

Shiftwork schedules induce chronic sleep debt, and shiftworkers develop significantly more sleep disorders than do dayworkers (Åkerstedt et al., 2010a, 2010b; Ohayon et al., 2010; van Mark et al., 2010). In the present study, habitual sleep duration was significantly longer in the early-morning-shift group than in the other groups (Table 1). Nonetheless, all of the early morning workers showed poor sleep (PSQI >5), perhaps associated with longer time in bed because of nonrefreshing sleep. It is of interest that Puttonen et al. (2011) found that two- and three-shift work schedules were associated with increased level of C-reactive protein, a marker of low-grade systemic inflammation and that Prather et al. (2009) showed that self-reported higher sleep-debt scores predict elevated levels of the cytokine IL-6. However, the literature is unclear with regard to any possible links between cytokine production and sleep disorders. For example, Patel et al. (2009) described a direct correlation between duration of sleep and increase in IL-6 or decline in TNF-α levels, but Okun et al. (2009) found in young healthy women that IL-6 and TNF-α levels were not related with PSQI score or sleep duration, and this was consistent with the report of van Mark et al. (2010). These inconsistencies could be indicative of the characteristics of the study samples as well as the study methods and protocols, i.e., single sampling vs. multiple sampling throughout the 24 h, and demonstrate that the need for further study of the link between sleep and the immune system in shiftworkers.

In our study, we found that the early morning shift had more of an impact on adipokine levels than did the night shift, which suggests that starting work early (e.g., at 06:00 h) can lead both to alterations in the pattern of adipokines and to the predisposition of a decrease in health. Daily physiological rhythms can be particularly disturbed in these individuals who need to wake for work ~04:00 h, and they can also exhibit impaired sleep architecture (Paim et al., 2008), metabolic changes (Padilha et al., 2010), and an altered control of food intake (Crispim et al., 2011), all of which may be involved in both changes in cytokine production and the onset of metabolic problems. Surprisingly, we found no differences in adiponectin levels in night compared with dayworkers. These differences between the two groups of shiftworkers could be attributed to the fact that the early morning workers might try to follow a more typical diurnal lifestyle relative to the fixed night workers, who follow a night activity and a daytime sleep schedule. This could lead to greater circadian disruption and changes in sleep quality of early-morning-shift workers. Anyway, more studies are needed in order to analyze the concentrations of this peptide in larger samples of night workers, given that they are more often affected by metabolic disorders (Pietroiusti et al., 2010), and adiponectin seems to be closely involved in such issues (Neumeier et al., 2006; Targher et al., 2004).

The protocol of this study had some limitations. We evaluated a relatively small number of young male participants over a single 24-h period. Also, the lack of control of sleep can be considered an important limitation of the study. There is a need for additional studies with larger samples that include both male and females (and individuals of a wider age range) and that isolate effects that are due to alterations in sleep patterns.

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