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## Hormonal appetite control is altered by shift work: a preliminary study

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### ABSTRACT

Shift work has been associated with a higher propensity for developing nutritional problems and obesity. However, the possible changes in leptin and ghrelin (2 hormones that contribute importantly to the central regulation of food intake) concentrations in this population are poorly described. The objective of the study was to evaluate the daily concentrations of leptin, nonacylated ghrelin, and acylated ghrelin and the appetite ratings in men working different shift schedules. Daily concentrations of nonacylated ghrelin, acylated ghrelin, and leptin and appetite were measured in 3 groups of subjects: workers on fixed night shifts (n = 9), fixed early morning shifts (n = 6), and fixed day shifts (n = 7). Appetite was evaluated by a validated questionnaire. Blood samples were collected every 4 hours over the course of 24 hours for a total of 6 samples. When comparing the 3 groups, leptin concentrations at 8:00 AM and 4:00 PM for those workers on the day shift were significantly lower than for those on the early morning shift; and concentrations at noon for those workers on the day shift were significantly lower than for those on the night shift. Nonacylated and acylated ghrelin concentrations were significantly lower for those workers on the early morning shift than for those on the day shift. In general, appetite was the lowest in those working the early morning shift. Shift workers on the early morning shift have lower appetites and concentrations of leptin and nonacylated and acylated ghrelin than the workers on other shifts. Further studies are required to better understand the detailed needs of these individuals.

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### 1. Introduction

Shift work is extremely prevalent in many services and industries and requires flexibility on the part of the workforce

[1]. In developed countries, the proportion of shift workers is estimated to be at least 20% of the entire working population [2,3]. No comparative data are available for the total population of Brazil, but research performed in the area around the

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city of Sao Paulo has suggested that 11% of the working population does shift work [4].

Shift-working populations have been associated with a higher propensity to develop long-term nutritional and metabolic problems, such as obesity [5,6] and altered nutritional metabolism [7,8]. Other recent studies have reported that diabetes, insulin resistance, and dyslipidemias also tend to occur more frequently in shift workers [9-11].

It has been known for some time that the eating habits of shift workers are altered [3,12-14], and there is a higher caloric intake [15] and an increased consumption of saturated fat and foods with a high glycemic index [1].

Leptin and ghrelin are peripheral signals that contribute to the central regulation of food intake [16]. Leptin, a hormone released by adipocytes, improves glucose and fat metabolism and provides information about energy status to regulatory centers in the brain [17]. In humans, circulating leptin concentrations are responsive to acute changes in energy balance resulting from increased or decreased caloric intake [18]. Ghrelin is synthesized mainly in stomach endocrine cells but has also been identified in other tissues, including the hypothalamus [19,20]. This hormone stimulates growth hormone secretion and has a potent influence on carbohydrate metabolism via stimulating glucose levels and inhibiting insulin levels. In contrast to the anorexigenic effects of leptin, ghrelin increases food intake and appetite and promotes body weight gain [19]. In addition, evidence has shown that both peptides can be altered by sleep changes [21,22].

In a previous study comparing night, early morning, and day shift workers, we found that the early morning shift was associated with a tendency to have higher homeostasis model assessment (a measure of insulin resistance [10]) values. Considering the potential roles of leptin and ghrelin in the control of metabolism and food intake, there is a need to understand if these hormones change in the shift-working population. However, the concentrations of these peptides in shift workers are unknown.

Because leptin and ghrelin are associated with food intake and the pattern of food intake varies with different shifts, changes in the daily variations through a 24-hour period of these 2 hormones would be predicted. Added to this alteration in their nutritional pattern, shift workers tend to suffer from rhythm misalignment, which has been associated with reduced levels of leptin [23]. Accordingly, we hypothesize that concentrations of leptin will be lower and, consequently, those of ghrelin will be higher in shift workers compared with day workers.

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## 2. Materials and methods

### 2.1. Subjects

The study included 2 all-male groups of blue-collar workers employed on fixed shifts in the steel industry. The factory in question was located in the city of Diadema, São Paulo State, Brazil. A day shift group that had conventional hours for work, meals, and sleep was included as a control. Because no workers worked only day shifts at the factory, day shift

volunteers who had the same types of jobs with the same physical demands were selected from another company to serve as the day shift control group.

The workers in the 3 groups all drove forklift trucks; and no significant differences were observed in their physical activity levels, according to Baecke et al [24]. The questionnaire consisted of 3 sections: work, sport (exercise), and nonsport leisure activity. Most of the questions were scored on a 5-point Likert scale, with the descriptors ranging from “never” to “sometimes” or “very often.” Three additional questions required reporting the type of sport (exercise) activity and both the number of hours per week and the number of months per year during which the respondent participated in the activity. Each section had a maximum score of 5 points, with a maximum of 15 points for the total activity index. Each index was rounded to the nearest 0.1.

The work hours of the 3 groups were as follows: the night shift ( $n = 9$ ) was 10:00 PM to 6:00 AM; the early morning shift ( $n = 6$ ) was 6:00 AM to 2:00 PM; and the day shift ( $n = 7$ ) was 8:00 AM to 5:00 PM. All of the groups worked 6 continuous days and then had 1 day off. All of the subjects had been working on the same fixed shift for at least 2 years, were healthy, were nonobese (body mass index [BMI]  $<30$ ), took no medication, and did not drink alcohol or smoke. The subjects who had had major variations in body mass in the prior 2 years were excluded from the study.

To ensure that the sample size was large enough for meaningful inferences, a required sample size was determined using the Statistica 6.0 software (StatSoft, Tulsa, OK, USA). We specified the following quantities for the calculation: the significance level ( $\alpha = .05$ ), the power (80%), and the expected standard deviation of the variables (leptin and ghrelin), as estimated by measurements previously performed in our laboratory with volunteers and by studies on the daily patterns of leptin and ghrelin in healthy men of similar ages [21,25].

### 2.2. Ethics

This study was approved by the Ethics Committee of the Federal University of Sao Paulo and was conducted according to international ethical standards. An informed written consent was obtained from all volunteers before starting the study.

### 2.3. Preliminary evaluations

#### 2.3.1. Energy intake

Food intake was determined through a self-administered food diary that was kept over the course of 7 successive days. An analysis of the energy intake (EI) and nutrient intake was performed using the Virtual Nutri, version 1.0, software (University of São Paulo, Brazil, 1996).

#### 2.3.2. Body composition measurements

Skinfold thickness measurements were taken on the right side of the body by a trained investigator using a calibrated Lange caliper (Beta Technology Cambridge, MD, USA) according to the recommendations of Jackson and Pollock [26]. Body density was estimated using sex-specific, 3-site equations [27] and converted to percentage fat using the Siri equation [28].

### 2.3.3. Sleep

Sleep variables were recorded by the participants for a week using a previously validated sleep diary [22]. The sleep quality was determined using a version of the Pittsburgh Sleep Quality Index (PSQI) that has been validated for the Portuguese language [29]. The PSQI has 7 items and a total score of 0 to 21; higher scores indicate poorer sleep quality. A PSQI score of at least 5 was considered to indicate poor sleep quality [29].

## 2.4. Experimental protocol

The experimental protocol maintained the normal routine of the shift workers (with regard to EI, work schedule, and sleep patterns) as much as possible during the 24-hour testing period. The participants were also asked not to deviate from their usual eating habits and sleep patterns during the week preceding the workplace data collection. For the workplace data collection, the blood samples were collected and the food intake diaries were completed on the third or fourth day of the shift. The measurements were taken between 6:00 AM and 2:00 PM for the early morning workers, 10:00 PM and 6:00 AM for the night workers, and 8:00 AM and 5:00 PM for day workers. After work, the research team transported the subjects to the Sleep Institute at the Federal University of Sao Paulo. The subjects stayed in private rooms at the Sleep Institute; and they were able to rest and sleep at their habitual times, have their meals, use the telephone and computers, read, and play video games. During the experimental protocol, the usual sleep durations of the subjects were 5.7 hours for the day shift, 6.9 hours for the early morning shift, and 5.8 hours for the night shift. No naps were taken. No vigorous physical activities were allowed. After completing the 24-hour study period and all evaluations, the subjects were driven home or back to their workplace.

### 2.4.1. Blood sampling

Venous blood samples were collected every 4 hours over the course of 24 hours (at 8:00 AM, 12:00 PM, 4:00 PM, 8:00 PM, 12:00 AM, and 4:00 AM), beginning at the start of the shift.

When the blood samples were collected at the workplace, the subjects went to the infirmary; and a nurse from the research team took the sample. For the collections at the Sleep Institute, the subjects had a catheter inserted; and a nurse entered the room and collected a blood sample during sleep without disturbing the subject. To minimize any stress to the volunteers, all the blood sampling procedures were performed by the same experienced phlebotomist. All the samples were taken from an intravenous catheter inserted into a forearm vein. A weak light was used during sample collection. Two blood samples were taken on each occasion.

For the nonacylated ghrelin and leptin measurements, the blood samples were collected into dry glass tubes containing EDTA. The tubes were centrifuged, and the plasma was extracted. For the acylated ghrelin, the blood samples were collected into tubes containing EDTA and aprotinin and centrifuged. The tubes were centrifuged, and the plasma was extracted. The plasma was then acidified to stabilize the *n*-octanoyl modification of the serine residue and prevent rapid deacylation of the ghrelin [30]. The plasma samples were kept frozen at  $-80^{\circ}\text{C}$  until analysis.

A total of 210 mL of blood was collected over the course of 24 hours (6 samples of 35 mL), an amount that did not cause a significant change to the hematocrit [31].

## 2.5. Assays

The leptin concentrations were measured using a commercially available competitive enzyme-linked immunoassay kit (Phoenix Pharmaceuticals, Burlingame, CA, USA). The plasma concentrations of nonacylated and acylated ghrelin were also quantified using an enzyme-linked immunoassay kit (Linco Research, St Charles, MO). The assays were performed according to the instructions provided by the manufacturer.

### 2.5.1. Food intake

The researchers monitored all meals provided to the subjects during the experiment; and a questionnaire was administered immediately before, during, and after each of the 4 meals. The researchers also recorded the quality and quantity of the food eaten. The questionnaire asked about the factors that influenced the kind of food eaten and the subjective responses to these meals (hunger before and satiety afterward) [12]. To assess hunger, the subjects were asked to mark their response to the question “How hungry do you feel right now?” on a 10-cm scale (with “not at all hungry” on the left and “extremely hungry” on the right). To assess appetite, the subjects were asked to indicate on a 10-cm scale how much they would enjoy eating foods from each of 7 different food categories. The 7 food categories were (1) sweets; (2) starchy food; (3) fruits and fruit juices; (4) vegetables; (5) meat, poultry, fish, and eggs; (6) dairy products; and (7) salty foods. The subjects were asked to provide a response for each food category based only on their appetite at the moment, without concern for calorie or fat intake or for a healthy diet [21]. To assess satiety, the subjects were asked to mark their response to the question “How much would you enjoy eating?” for each food category on a 10-cm scale (with “not at all” on the left and “very much” on the right).

During work and when at the Sleep Institute, the subjects ate 4 standard meals during the 24-hour experimental protocol. The composition of the meals reflected the subjects' habitual food intake as recorded in their food diaries. The meal times also mimicked the usual eating patterns of the subjects, with care being taken that the meals were not consumed near the blood collection times. The meals were consumed at the following times: 6:00 AM, 12:30 PM, 4:30 PM, and 8:15 PM for the day workers; 05:30 AM, 12:30 PM, 4:30 PM, and 8:15 PM for the early morning workers; and 2:15 PM, 8:30 PM, 2:00 AM, and 6:15 AM for the night workers. The food intake analysis was divided into the meals taken before, during, and after the work shift and the times of day when the meals were consumed during the different shifts.

## 2.6. Statistical analysis

All the values are presented as the means and SEM. Hormone levels are presented as the daily variations over a 24-hour period (6 sample points), the 24-hour plasma means, and the first values obtained after sleeping. A 1-way analysis of variance (ANOVA) model was used to compare the

morphological, dietary, sleep, and physical activity variables between the 3 groups. A 2-way (group and time) repeated-measures ANOVA model was used to compare the changes in leptin and nonacylated and acylated ghrelin between the groups and at different times of the day. The repeated measure was time (the 6 sample points), and the group was used as the between-subjects factor (3 levels). This analysis indicates not only if there were time-of-day effects and differences between the groups but also, by investigating the interactions, if there were any differences between the groups in time-of-day effects. Significant differences were investigated using the Tukey test for post hoc analysis. Statistical significance was defined as  $P < .05$ . The data were analyzed using the Statistica 6.0 software (StatSoft).

### 3. Results

A comparison between the 3 groups for age; years of shift work; physical activity level; and morphological, sleep, and dietary variables are shown in Table 1. No significant differences were observed in the morphological variables, ages, years of shift work, physical activity levels, awakenings, and feelings upon waking.

Significant differences between the day shift and early morning shift groups were present in most of the nutritional variables. Subjects on the early morning shift consumed less energy than did the day or night workers, obtained less of their energy from carbohydrate sources than those on the day shift,

and obtained more of their energy from fat than did the day or night workers.

Usual sleep duration was significantly higher in the early morning shift group than in the day shift group (Table 1). In addition, 5 (56%) of the night workers, 4 (57%) of the day workers, and 6 (100%) of the early morning workers were diagnosed as “poor sleepers,” with a PSQI total score greater than 5.

Table 2 shows the mean 24-hour concentrations and values immediately after sleep for plasma leptin and non-acylated and acylated ghrelin in the 3 groups. The concentrations of leptin after sleep were higher in those working the early morning and night shifts than in the day workers. The concentrations after sleep and 24-hour average concentrations of acylated ghrelin were significantly lower in those working the early morning shift than in the other 2 groups. The concentrations of nonacylated ghrelin immediately after sleep were significantly lower in both of the shift workers groups than in the day shift group, whereas the 24-hour average was significantly lower in the early morning workers than in the other 2 groups.

The daily profiles of the appetite-regulating hormones (leptin, nonacylated and acylated ghrelin) are shown in Fig. 1. In all of the groups, the time of day did not have a significant effect on leptin ( $F_5 = 1.509$  and  $P = .479$  for the day shift group,  $F_5 = 1.846$  and  $P = .126$  for the night shift group, and  $F_5 = 0.479$  and  $P = .788$  for the early morning shift group), acylated ghrelin ( $F_5 = 0.758$  and  $P = .587$  for the day shift group,  $F_5 = 0.369$  and  $P = .867$  for the night shift group and  $F_5 = 0.304$ , and  $P = .906$  for

**Table 1 – Morphological, sleep, dietary, and physical activity patterns in day, early morning, and night shift workers**

	Day shift (n = 7)		Early morning shift (n = 6)		Night shift (n = 9)		P <sup>1</sup>
	Mean	SEM	Mean	SEM	Mean	SEM	
Age (y)	26.7	2.6	31.8	1.5	30.1	1.4	.20
Duration of shift work (y)	2.9	1.1	3.7	0.58	4.2	0.8	.45
Morphological variables							
Height (m)	1.7	0.02	1.7	0.03	1.8	0.02	.54
Weight (kg)	82.6	3.9	79.8	3.9	80.4	4.0	.07
BMI (kg/m <sup>2</sup> )	27.2	1.0	27.6	1.16	26.1	1.4	.20
Fat mass (%)	22.4	1.9	21.7	2.2	21.3	2.6	.21
WC (cm)	91.4	2.9	92.7	3.2	89.5	3.2	.07
WHR	99.3	3.0	100.5	2.1	98.1	2.9	.21
Sleep variables							
Usual sleep duration (min)	341.8 <sup>b</sup>	17.6	413.2 <sup>a</sup>	16.4	350.9 <sup>b</sup>	20.5	.05
Awakenings	0.3	0.1	0.4	0.2	0.8	0.2	.17
Feeling up waking (%)	63.7	4.0	80.9	5.2	72.3	6.3	.18
Dietary variables							
Grams of food	3313.6	342.9	2447.9	204.5	3075.5	144.8	.06
EI (kJ/d)	14.867 <sup>b</sup>	1.534	11.089 <sup>a</sup>	873	14.491 <sup>b</sup>	477	.03
Protein intake (%EI)	16.2 <sup>c</sup>	0.6	20.2 <sup>a</sup>	1.1	23.1 <sup>b</sup>	0.5	<.0005
Carbohydrate intake (%EI)	59.6 <sup>b</sup>	1.4	45.3 <sup>a</sup>	1.6	48.7 <sup>a</sup>	1.6	<.0005
Total fat intake (%EI)	24.2 <sup>b</sup>	1.3	34.4 <sup>a</sup>	1.9	28.2 <sup>a</sup>	1.7	.003
Cholesterol	270.4 <sup>a</sup>	29.7	263.8 <sup>a</sup>	22.2	468.9 <sup>b</sup>	32.0	<.0005
Other variables							
Physical activity level	2.7	0.4	2.8	0.4	2.8	0.3	.86

The values are means and SEM. Results are shown for early morning, night, and day workers. The values in a row with different superscripts are significantly different;  $P < .05$  (ANOVA and Tukey tests). WC indicates waist circumference; WHR, waist to hip ratio; P<sup>1</sup>, P values calculated by ANOVA.



**Table 2 – The postsleep and 24-hour average concentrations of plasma leptin, nonacylated ghrelin, and acylated ghrelin**

	Subjects						P <sup>1</sup>
	Day shift (n = 7)		Early morning shift (n = 6)		Night shift (n = 9)		
	Mean	SEM	Mean	SEM	Mean	SEM	
<i>Plasma leptin</i>							
Concentration after sleep, ng/mL	2.3 <sup>b</sup>	0.84	4.6 <sup>a</sup>	1.7	4.7 <sup>a</sup>	1.4	.04
Mean 24-h concentrations, ng/mL	2.9	1.4	4.4	1.1	4.5	1.2	.39
<i>Plasma nonacylated ghrelin</i>							
Concentration after sleep, pg/mL	482.9 <sup>b</sup>	112.5	169.2 <sup>a</sup>	69.9	394.3 <sup>a</sup>	150.1	.05
Mean 24-h concentrations, pg/mL	513.3 <sup>b</sup>	115.6	227.0 <sup>a</sup>	26.2	368.3 <sup>b</sup>	104.5	.02
<i>Plasma acylated ghrelin</i>							
Concentration after sleep, pg/mL	163.2 <sup>b</sup>	48.7	26.8 <sup>a</sup>	18.8	114.3 <sup>b</sup>	41.3	.04
Mean 24-h concentrations, pg/mL	174.7 <sup>b</sup>	38.1	45.1 <sup>a</sup>	15.8	99.1 <sup>b</sup>	40.7	.003

Values with different superscripts in a row are significantly different;  $P < .05$  (ANOVA and Tukey tests). P<sup>1</sup> indicates P values calculated by ANOVA.

the early morning shift group), or nonacylated ghrelin ( $F_5 = 1.438$  and  $P = .239$  for the day shift group,  $F_5 = 0.587$  and  $P = .710$  for the night shift group, and  $F_5 = 1.373$  and  $P = .268$  for the early morning shift group).

When comparing groups, it was found that the leptin concentrations at 8:00 AM and 4:00 PM were significantly lower for the day shift workers than for the early morning shift workers and that the leptin concentration at noon was significantly lower for the day shift workers than for the night shift workers. The nonacylated ghrelin and acylated ghrelin were both significantly lower at all 6 measurement times in those on the early morning shift than in those on the day shift.

The appetite ratings were significantly lower in those on the early morning shift than in those on the day or night shifts; this finding was particularly true for the prework meal; for the intake of sweets, starchy foods, vegetables, meat, poultry, fish, eggs, and dairy produce; and for overall appetite and eating enjoyment (Table 3).

The ANOVA model for appetite level indicated that there were significant ( $P < .004$ ) group effects (indicating that the 3 groups of shift workers were different when all 3 meals were considered) and significant ( $P < .0005$ ) time effects (indicating that, on average, the 3 groups of workers presented different appetite levels before, during, and after their shifts). However, the major interest of this study was in the interaction between these 2 factors. Significant interactions between time and group were found for appetite in all 3 meals. These significant interactions indicated that the meal content and appetite varied not only with the timing of the meal relative to the time of work (pre-shift, during-shift, and post-shift meal) but also with the type of shift. That is, the enjoyment of food and the type of food eaten were markedly changed according to the shift being worked. These changes are shown in Table 3. They were particularly marked for the following intakes: meat, poultry, fish, eggs, and dairy products (for the day shift); fruit and fruit juices (for the early morning shift); and intake of vegetables and meat, poultry, fish, and eggs (for the early morning and night shifts) (Table 3). Thus, appetite was significantly higher at the meal before work compared with the meal during work for meat, poultry, fish, and eggs (for the day and early morning shifts)

and for dairy products (for day shift). The night shift showed significant differences in appetite between the pre- and postwork meals for vegetables and meat, poultry, fish, and eggs (Table 3).

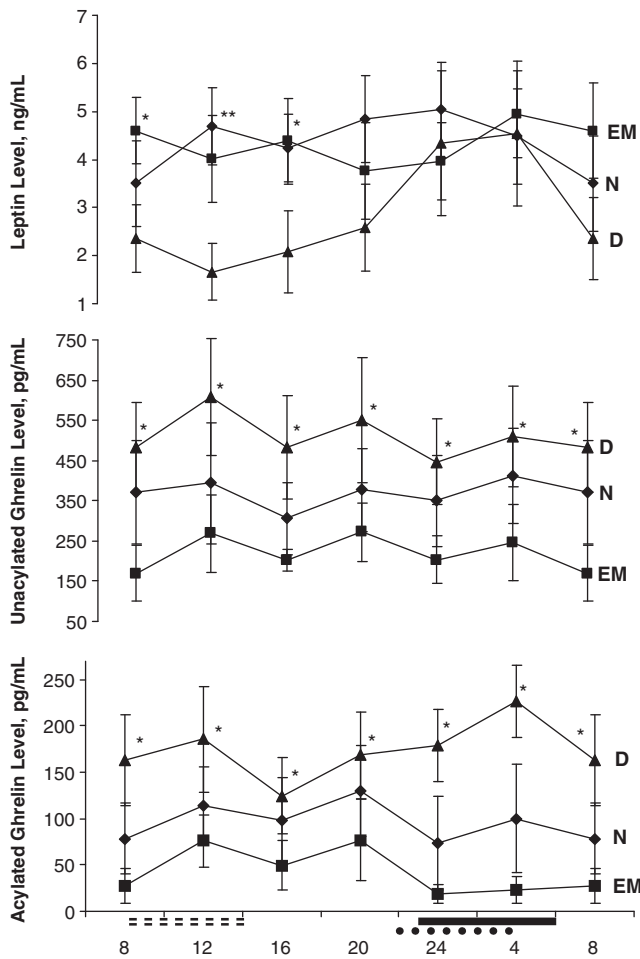
## 4. Discussion

This study evaluated leptin and nonacylated and acylated ghrelin levels in shift workers and showed significant differences from the day shift subjects. We found that leptin concentrations were significantly lower for the day shift than for the early morning shift at 2 time points (8:00 AM and 4:00 PM) and lower than the night shift at noon (Fig. 1). In addition, there were lower levels of ghrelin (both acylated and nonacylated) throughout the day in the early morning shift workers. That is, the changes from the day shift workers were at least as significant in the early morning shift workers as in the night shift workers. These findings are important because a modified secretion of these peptides is associated with altered food intake [16,32] and because, in general, early morning shift work has received little attention in the literature.

### 4.1. Leptin concentrations

Although the 3 groups showed leptin concentrations that were within the reference range [33], the leptin concentrations after sleep and the 24-hour mean values were similar to those of age-matched healthy men [21,34–36] only in the day shift group. The leptin concentrations found in both the early morning and night shift groups were comparable to those observed in healthy subjects around 50 years old [37,38].

The prevalence of obesity is higher in shift workers [15,39,40], and other evidence highlights a positive relationship between BMI and the duration of shift work exposure [6]. We evaluated only nonobese workers in our study, but the differences in leptin levels between the 3 shifts could still be important for understanding the metabolic changes that lead to a predisposition to obesity. Although the leptin levels in the 2 shift worker groups were higher than those in the day shift



**Fig. 1 – Mean ( $\pm$ SEM) concentrations of leptin and total and active ghrelin at 6 time points in early morning (EM, squares), night (N, diamonds), and day shift (D, triangles) workers. Intergroup differences and intragroup circadian variations were evaluated by using a 2-factor repeated-measures ANOVA model, followed by Tukey tests when a factor showed a significant effect ( $P < .05$ ). \*Differences between early morning and day subjects; \*\*differences between day and night subjects ( $P < .05$ ). = =, Mean sleep time of night shift workers; ••, mean sleep time of early morning shift workers.**

group (Table 2 and Fig. 1), it remains to be established if such “normal” values are appropriate for shift workers and others with altered daily rhythms.

Leptin levels show daily variation, with leptin rising during nocturnal sleep [25,41]. However, we found a (nonsignificant) daily change only in the day shift workers (in whom the levels were lower during the day and higher during sleep); neither the early morning nor the night shift workers showed this variation. These responses may have reflected the influence of sleep patterns. It has been shown that both short-term, experimental sleep restriction [21] and chronic, self-reported decreased sleep duration [22] are associated with decreased leptin. This information is partially consistent with our results. Although the 24-hour average concentrations of leptin were not significantly different between the groups,

we found a higher level of this peptide in the early morning shift group at 2 evaluation points (8:00 AM and 4:00 PM; Fig. 1); and this group also had a significantly longer sleep duration. We also found that the early morning workers had the highest percentage (100%) of poor sleep quality (a PSQI score  $\geq 5$ ). Although the relationship between sleep duration and leptin has been well documented [21,42], the influence of sleep quality on leptin levels has not been thoroughly explored. It is also important to consider the effects of rhythm misalignment, which is common in shift workers [43], on leptin levels. Sheer et al [23] evaluated 10 adults over the course of 10 days who ate and slept about 12 hours out of phase from their habitual times. Development of rhythm misalignment was found, together with a significant fall ( $-17\%$ ,  $P < .001$ ) in leptin levels. The authors separated out statistically the effects of changed sleep efficiency and rhythm misalignment and concluded that rhythm misalignment, independent of its effects on sleep, was mainly responsible for the changes in leptin levels.

It is well documented in the literature that leptin concentrations are influenced by different factors, such as sleep time [21,22,42], changes in energy expenditure [44], caloric intake and the timing of meals [45], and concentrations of other hormones, such as insulin [46]. In particular, insulin levels may explain the leptin results obtained. Other studies have found that circulating insulin concentrations are an important independent predictor of leptin [47,48], with insulin acting to regulate leptin production in synergy with cortisol [33]. We recently showed [10] that a group working in the early morning had higher insulin resistance (homeostasis model assessment of insulin resistance) than nocturnal and day workers ( $P < .05$ ). Other evidence has shown that shift work is associated with disordered insulin metabolism [39]. Thus, the increased insulin concentrations may have been associated with the higher leptin concentrations observed in our shift workers.

Another possible explanation for the current results relates to food intake, given that shift workers tend to show differences compared with those working during the day [5,12,13]. The present study found that the early morning group had a food EI that was significantly lower than that of the other groups (Table 1), and leptin and ghrelin release is influenced by food intake [45].

In summary, there are marked changes in leptin levels and profiles in individuals on different shifts. These differences may reflect changes in sleeping patterns, but the effects of altered eating patterns (see below and Table 3) are an obvious alternative or an additional cause. Such alterations may have important metabolic consequences, and it is important to evaluate the prognostic value of these changes when giving advice on sleeping and eating habits.

#### 4.2. Ghrelin concentrations

Changes to ghrelin and leptin levels in opposing directions have been found in many studies [25,38,49], and they were confirmed in the present study. The early morning workers showed the lowest nonacylated and acylated ghrelin levels at baseline (Table 2) and over most of the 24 hours (Fig. 1). This profile may have led to the low food intake observed in these subjects (Table 3).

**Table 3 – The average appetite ratings and associated variables in those working the day, early morning, and night shifts**

Food category	Day shift (n = 7)		Early morning shift (n = 6)		Night shift (n = 9)		P <sup>1</sup>
	Mean	SEM	Mean	SEM	Mean	SEM	
<b>Prework meal</b>							
Sweets (cake, candy, cookies, ice cream, and pastry)	2.4 <sup>a</sup>	1.1	0.3 <sup>b</sup>	0.2	4.1 <sup>a</sup>	0.9	.01
Starchy food (rice, bread, pasta, cereal, and potatoes)	8.1 <sup>a</sup>	0.8	4.1 <sup>b</sup>	0.9	4.3 <sup>b</sup>	0.7	.003
Fruits and fruit juices	5.4	1.2	3.3 <sup>*,†</sup>	1.3	6.1	0.5	.16
Vegetables	0.7 <sup>a</sup>	0.7	0.3 <sup>a,*</sup>	0.2	5.9 <sup>b,†</sup>	0.3	.0001
Meat, poultry, fish, and eggs	1.1 <sup>a,*</sup>	0.8	0.3 <sup>a,*</sup>	0.2	6.9 <sup>b,†</sup>	0.6	.0001
Dairy (milk, cheese, and yogurt)	7.1 <sup>a,*</sup>	1.1	6.0 <sup>a</sup>	1.4	2.3 <sup>b</sup>	0.5	.003
Salty food (chips, salted nuts, pickles, and olives)	2.6	1.3	0.3	0.2	3.3	0.8	.06
Overall appetite	7.9 <sup>a</sup>	0.7	3.7 <sup>b</sup>	0.7	6.8 <sup>a</sup>	0.5	.0006
Enjoyment of eating foods	8.6 <sup>a</sup>	0.7	5.1 <sup>b</sup>	1.1	7.1 <sup>c</sup>	0.5	.02
Hunger before meal	7.9 <sup>a,†</sup>	0.7	3.6 <sup>b,*</sup>	0.7	6.8 <sup>a,†</sup>	0.5	.0006
Satiety after meal	6.6	0.8	6.5	0.7	5.4	0.4	.34
<b>During-work meal</b>							
Sweets (cake, candy, cookies, ice cream, and pastry)	3.0	1.3	1.5	1.1	3.1	0.9	.57
Starchy food (bread, pasta, cereal, and potatoes)	6.3	1.4	6.3	0.5	4.6	0.8	.36
Fruits and fruit juices	4.7	0.9	5.3	1.0	5.8	0.9	.68
Vegetables	4.3	1.6	6.0 <sup>†</sup>	0.1	5.1	1.1	.67
Meat, poultry, fish, and eggs	7.9 <sup>†</sup>	0.7	6.7 <sup>†</sup>	0.6	6.3 <sup>†</sup>	1.1	.48
Dairy (milk, cheese, and yogurt)	0.3	0.2	0.3	0.2	2.2	0.9	.05
Salty food (chips, salted nuts, pickles, and olives)	2.0	1.0	0.3	0.2	2.2	1.0	.36
Overall appetite	9.1 <sup>a</sup>	0.5	5.9 <sup>b</sup>	0.7	4.8 <sup>b</sup>	0.7	.0003
Enjoyment of eating foods	8.9	0.5	7.3	0.5	7.8	0.6	.19
Hunger before meal	9.2 <sup>a,†</sup>	0.5	5.9 <sup>b</sup>	0.7	4.7 <sup>b</sup>	0.7	.0003
Satiety after meal	7.7 <sup>a</sup>	0.8	6.2 <sup>b</sup>	0.5	5.1 <sup>b</sup>	0.3	.01
<b>Postwork meal</b>							
Sweets (cake, candy, cookies, ice cream, and pastry)	3.4	1.3	0.5	0.5	2.7	0.8	.13
Starchy food (bread, pasta, cereal, and potatoes)	5.9	1.2	6.0	0.6	5.7	0.9	.97
Fruits and fruit juices	6.3	1.0	5.2	1.1	6.2	0.8	.69
Vegetables	0.4	0.3	1.3	1.3	1.5	0.5	.52
Meat, poultry, fish, and eggs	0.4	0.3	1.0	1.0	1.4	0.5	.51
Dairy (milk, cheese, and yogurt)	3.1	1.0	4.0	0.9	4.0	1.2	.86
Salty food (chips, salted nuts, pickles, and olives)	2.6	1.0	0.7	0.6	2.4	0.9	.33
Overall appetite	4.7	0.4	5.0	0.8	6.5	0.5	.07
Enjoyment of eating foods	6.8	0.8	7.2	0.5	8.7	0.5	.09
Hunger before meal	6.5	0.5	5.0	0.8	4.7	0.4	.07
Satiety after meal	7.2 <sup>a</sup>	0.6	6.8 <sup>b</sup>	0.6	5.5 <sup>c</sup>	0.2	.04

Each category was rated on a 0- to 10-cm visual analogue scale. Values with different superscripts in a row are significantly different;  $P < .05$  (ANOVA and Tukey tests) for significant time  $\times$  group interactions. P<sup>1</sup> indicates P values calculated by ANOVA.

Differences in the food categories: \* Pre- vs during-work meal; † During vs postwork meal; ‡ Pre- vs postwork meal.

Some evidence indicates that ghrelin levels are increased during nocturnal sleep and decreased in the hours before waking [50]. This time course suggests a mechanism for regulating ghrelin production that is independent of food intake. Numerous endocrine circadian rhythms may be involved (cortisol or leptin, for example [51]), and they in turn are related to insulin and blood glucose levels [52]. However, we did not find significant increases in either form of ghrelin during sleep in the present study (Fig. 1). It is possible that 4-hour sampling intervals may have been too infrequent to precisely identify the peaks and nadirs in the 24-hour ghrelin profile or the differences between the waking and sleeping values.

#### 4.3. Food intake

Weight gain in shift workers has been explained by several mechanisms, including changes in caloric intake [14], dietary habits (such as eating fewer meals and more snacks), the

daily distribution of food intake [5,15], physical exercise [15,53], and sleeping habits [15]. Di Lorenzo et al [1] evaluated 718 Italian shift workers, aged 35 to 60 years, and showed that the meals commonly consumed during the night shift were poor in fiber and rich in animal proteins and saturated fat and in foods with a high glycemic index; these kinds of foods are known to produce an increase in body fat in working individuals. In our study, the early morning workers consumed significantly less energy than those in the other 2 groups (Table 1). This finding is in contrast to that of de Assis et al [13], who found that the 24-hour EI in garbage collectors did not differ between work shifts. In the present study, the early morning group also ate more protein and fat and fewer carbohydrates (when expressed as the percentage of total EI) than the day shift group (Table 1). These results confirm the negative effects of shift work upon food intake [1,5]. This pattern of food intake is associated with obesity and an increased risk of cardiovascular disease [54]. The percentage

of total EI contributed by fat, despite significant differences between the early morning and day shift groups, did not exceed current recommendations [55]; by contrast, the percentage of total energy obtained from carbohydrates in the early morning group (45.3%) was right at the lower limit of the recommended range (45%–65%).

In addition to higher leptin levels (at 8:00 AM and 4:00 PM) and lower ghrelin levels, the early morning group also had lower appetite ratings for 4 of the 7 food categories (sweets, fruits, vegetables, and salty food). In addition, there was decreased interest in and decreased appreciation of food, especially for the before-work meal. More generally, both of the shift workers groups showed less hunger before the at-work meal than did the day shift workers (Table 3). It has been proposed that eating when on atypical work schedules is more motivated by habit and time pressure and less motivated by hunger and social opportunity [5,12]. In the present study, the appetite measurements were performed reliably. In addition, we compared workers who had the same food availability, as defined by similar meals made at home or at work (the company had no vending machines) and similar cultural and socioeconomic feeding patterns.

#### 4.4. Sleep, food intake, and its relationship with daily rhythm adjustment

Fixed night shifts offer a potential benefit over rotating shift systems in that they may serve to maximize the adjustment of the circadian clock and hence minimize the various health and safety problems associated with night work [56]. However, all shift workers need to alter their food intake to fit their work schedule [3,12]. In our study, we found that the early morning shift had more impact on food intake than did the night shift, suggesting that starting work early (6:00 AM) may lead not only to alterations in the pattern of food intake but also to the possibility of a decrease in health. The timing of food intake should be considered when assessing the impact of shifts on nutrition and health, as should facilities for eating at work and the availability of food of various types [12]. In addition, disturbed circadian rhythms, sleep problems, lifestyle problems, and increased stress [1,8,39–41] have all been implicated as possible risk factors for disease in the shift-working population. The sleep pattern can be particularly disturbed in these individuals (from waking up for work at around 4:00 AM), and they may also have impaired sleep architecture [57]. Surprisingly, the day shift workers normally had less sleep duration than the early morning or night shift workers (Table 1). This finding was unexpected, given that shift workers normally have shorter sleep duration than day workers [57]. However, it is possible that the busy and dynamic lifestyle imposed by a large commercial center in Brazil (the region where the study was conducted) may have affected the sleep profile of the workers, especially the day shift workers. The region where the research was conducted had heavy traffic, and the workers needed to awaken early because of the time required to travel long distances to the workplace during the peak-traffic hours. This consideration may explain why the frequent finding of decreased sleep duration in shift workers due to their work hours [57] was not observed in the current study. Even so, this unexpected result

requires further study, including an assessment of the reasons for staying in bed and arising in shift workers.

An unadjusted body clock may contribute to the inappropriately timed rhythms of gastrointestinal tract activity and hypothalamic feeding centers responsible for eating behavior during shift work [12]. Melatonin, which was not measured in this study, is generally considered to be the best indicator of the phase of the endogenous circadian body clock. Its circadian rhythm appears to be largely unaffected by the timing of sleep, activity, and food ingestion, although its secretion is suppressed by exposure to light [56]. In a recent review, Folkard [56] found that only a small minority (3%) of workers show evidence of a “complete” adjustment in their endogenous melatonin rhythm to night work and that less than a quarter (21.1%) of night workers show evidence of the sufficiently substantial adjustment that would be required to derive any real benefit.

#### 4.5. Further work and summary

Our experimental protocol kept the EI and activity levels of the shift workers as normal as possible over a 24-hour period; furthermore, we demonstrated that the shift work seemed to alter the control of food intake of young individuals who differed in working hours. These data, when consolidated and extended by further research, can then form the rationale for a nutritional approach—based upon work schedules and taking into account the peculiarities of each shift with regard to the control of food intake—for shift workers. In addition, further studies in nutrition to elucidate the social issues that drive food intake in shift workers are needed. The potential value of these is that the current results indicate that conflict might exist between the endogenous and social factors that influence food intake, and this conflict might predispose the individual to nutritional disorders.

The protocol of this preliminary study had some limitations. We evaluated only a small number of young, male participants over a single 24-hour period. Although no time-of-day effect was found for any of the hormones in the 3 shifts studied, the reasons for this might have been the small sample size or alterations to food intake and sleep schedules. More studies using larger samples, including individuals with a wider age range and of both sexes, and separating out effects due to alterations in patterns of sleep and food intake are needed. For example, evidence from older and female shift workers would be valuable and apposite, given that age and sex may affect the neuroendocrine regulation of appetite. In summary, the present study showed that shift workers, especially those on the early morning shift, had different levels of appetite, leptin, and nonacylated and acylated ghrelin than day shift workers.

Similar concerns and suggestions to institute multidisciplinary programs that aim to produce healthier lifestyles have been proposed recently by others (eg, [58,59]). Kim et al [58] evaluated the effects of a 10-month lifestyle intervention program (including dietary counseling, advice on increasing physical activity, and recommendations to stop or limit smoking and alcohol intake) upon the components of the metabolic syndrome. Favorable changes in metabolic parameters and adiponectin were reported. Furthermore, Di Marzo



et al [59], studying the effects of a similar program upon viscerally obese men, found that most risk factors were significantly improved by the intervention.

These intervention studies, coupled with the results of the present study and the further studies outlined above, will all provide information that can be used to underpin better advice for improving the health and well-being of all individuals, including those working on shift-work schedules.

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## Conflict of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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