

Original Article

Serum leptin and insulin levels during chronic diurnal fasting

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Ramadan fasting is a unique model that is associated with restriction of the timing of food and fluid intake from dawn to sunset and reduction in meal frequency and sleep duration. Leptin levels are thought to play a role in long-term regulation of caloric intake and fat deposition. However, the long-term changes in leptin levels during this pattern of fasting are not known. The study was conducted on lean (N=26, BMI = 22.5 ± 0.4) and obese (N=18, BMI=33.1±1.0) healthy female volunteers. Fasting serum levels of leptin, insulin and glucose were estimated at baseline (day 1), days 14 and 28 of the month of Ramadan and 2 weeks after Ramadan. Baseline serum levels of leptin were significantly higher in obese (13.5 ± 1.96 µg/L, $P < 0.05$) compared with lean subjects (9.60 ± 0.80 µg/L) and correlated positively with body fat ($r = 0.82$, $P = 0.0004$). Serum leptin levels exhibited a significant and comparable increase by 39% and 37% throughout the month in lean and obese subjects, respectively. In addition, a significant correlation ($r = 0.52$, $P = 0.003$) was found between changes in serum leptin and serum insulin levels. We conclude that chronic diurnal fasting is associated with significant elevations in serum leptin. These elevations appear to be mediated by changes in serum levels of insulin. These data support the role of insulin in the long-term regulation of leptin secretion during chronic diurnal fasting followed by nocturnal eating during the month of Ramadan.

Key Words: leptin, insulin, Ramadan fasting, obesity, Bahrain

Introduction

Leptin, the product of the *ob* gene, appears to play a key role in long-term regulation of body weight and energy homeostasis. It conveys information to the brain about the size of energy stores and stimulates the hypothalamic centers responsible for regulation of energy intake and expenditure.^{1,2} Plasma leptin levels are elevated in obese humans and are positively correlated with the indices of body fatness.³⁻⁵ Previous studies indicated that short-term total fasting⁶ or chronic reduction in caloric intake^{7,8} results in reduction of leptin down to 30-66 % of its basal levels. However, the reduction of leptin levels in these studies did not correlate with the changes in body fat mass. On the other hand, chronic overfeeding is associated with significantly elevated levels of leptin than would be expected by the increase in body mass index or percentage of body fat.^{7,8} Although the mechanisms responsible for regulation of leptin are unclear, multiple factors besides body adiposity have been proposed to influence its secretion, including insulin, glucose, cytokines and dietary factors such as the time of administration of the meal⁹ and the food composition.¹⁰

During the month of Ramadan, Muslims abstain from food and fluid intake from dawn to sunset and are permitted to eat during the whole night. These changes in

the timing of food intake are also associated with reduction in meal frequency and sleep duration.¹¹ Energy intakes during Ramadan exhibit regional variations. Previous studies indicate that energy intakes increase,¹² decrease¹³ or remain unchanged¹⁴ compared with pre-Ramadan levels. Food composition has also been reported to shift towards consuming more fat and less carbohydrates despite no change in total energy intake.¹⁴ Despite these inversions in food habits, metabolic changes in diurnal energy expenditure and substrate oxidation seem to keep body weight constant.¹⁴⁻¹⁶ The changes in serum leptin associated with this unique type of fasting and the possible link to these metabolic changes, however, have not been tested.

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We aimed to test the effects of inversion in dietary habits associated with long-term diurnal fasting on the serum levels of leptin in lean and obese individuals. We wanted also to test if there is a correlation between the changes in serum leptin and changes in other factors that have been proposed to play a role in regulating its release. To achieve these goals, we examined the anthropometric variables, food intakes and serum levels of leptin, insulin and glucose during baseline (day1), days 14 and 28 of the month of Ramadan and two weeks after Ramadan fasting in lean and obese individuals.

Subjects and methods

Healthy female volunteers aged 18–45 years were sought for participation in the study. All subjects signed a written consent form before being involved and the experimental procedures included in the study were explained in the form. Subjects were specifically excluded if fasting blood glucose was greater than 6.3 mmol/L, if blood pressure was above 140/90 mmHg or if they were smokers. All Muslim subjects included in the study abstained from food and drinks during the day from dawn to sunset and food was allowed only during the night. The study included 26 non-obese and 18 obese females and obesity was defined as BMI>27.3 according to the National Institute of Health (NIH) criteria for defining obesity.¹⁷ All variables were measured during baseline (day1), days 14 and 28 of the month of Ramadan and two weeks after Ramadan fasting in lean and obese individuals. The Research and Ethics Committee at the College of Medicine and Medical Sciences (CMMS), Arabian Gulf University approved all the experimental protocols.

Anthropometric variables

Subjects were weighed bare-footed, wearing light clothes and standing on a standard pre-calibrated balance and measurements were approximated to the nearest 1kg and height was simultaneously measured. Waist circumference was measured with a standard tape at a level midway between the xiphisternum and the symphysis pubis. Hip circumference was measured at the widest point in the hip region. The measurements of the height and waist and hip were approximated to the nearest 0.1cm. Body fat was assessed by using bio-impedance technique.

Blood pressure measurements

Blood pressure was measured according to Joint National Committee (JNC-VI) guidelines for Detection, Diagnosis and Treatment of Hypertension.¹⁸ Subjects were seated and the suitable cuff size was used as necessary. Two pressure recordings 2 minutes apart were taken during each setting. If the difference between the two readings was more than 5 mmHg, another measurement was taken and all the readings were averaged.

Energy intake

During each of the experimental days, subjects were given a form where they reported the type and exact quantities of all the food they had eaten during the night. Nutrient intakes were estimated utilizing a computer

program (Diet Expert for windows, version 2.1) and from food composition tables published by the Nutrition Unit at the Ministry of Health in Bahrain.¹⁹

Biochemical assays

In addition to the anthropometric and blood pressure measurements, fasting venous samples were taken at 1:00-2:00 PM for measurement of serum levels of leptin, insulin, glucose and lipids. Serum leptin levels were measured by radioimmunoassay (*Linco, St. Charles, MO*) as previously described.²⁰ The human leptin sensitive radioimmunoassay detects leptin with a sensitivity of 0.05µg/L. The intra and inter-assay coefficients were 7.5% and 8.9%, respectively. The human insulin specific radioimmunoassay detects insulin with a sensitivity of 2µU/L. The intra and inter assay coefficients were less than 4.4% and 6.0%, respectively. The blood glucose was measured by the hexokinase method (*Roche Diagnostics, Mannheim, Germany*) in plasma from fluoride oxalate tube. Serum cholesterol was measured by cholesterol oxidase method (*Roche Diagnostics, Mannheim, Germany*) and serum triacylglycerol was measured by enzymatic method utilizing glycerol kinase and peroxidase (*Roche Diagnostics, Mannheim, Germany*). All the samples of collected sera were kept at -20°C until analysis and all the hormonal assays were run in the same assay in duplicate.

Statistical analysis

All the data in the study are expressed as mean ± SEM. Data were analyzed using *SSPS program (version 11)* by using analysis of variance (*ANOVA*) for repeated measures. When the interaction was found with repeated measures among groups, differences between groups were determined by using factorial *ANOVA* followed by Bonferroni adjustment. A *P* value < 0.05 was considered statistically significant. To determine any possible linear correlation between the different variables, Pearson's correlation coefficient was used.

Table 1 shows the baseline measurements of the anthropometric variables in lean and obese subjects during Ramadan fasting. The anthropometric variables (body weight, BMI, body fat and waist circumference) included were significantly higher ($P<0.5$ to $P<0.01$) in obese compared with lean subjects. However, no changes in these variables were observed across the study. Energy intake was significantly increased ($P<0.05$) compared with baseline levels in lean and obese individuals. It seems that most of the increase in energy intake was due to higher intake of fats ($P<0.05$). No changes in the intake of carbohydrates or protein were observed among lean or obese individuals over the month.

Table 2 shows the biochemical changes in lean and obese subjects. Obese subjects had significantly ($P<0.05$) higher serum levels of insulin, triglyceride and leptin. In addition, serum leptin levels were significantly increased ($P=0.03$) in both lean and obese subjects. This increase in serum leptin was associated with comparable and significant ($P=0.04$) increases in serum insulin. Serum triglycerides were marginally higher ($P=0.05$) in obese women during the month of Ramadan. No changes in serum glucose were observed during the month in either

lean or obese groups. The correlation between serum leptin and indices of obesity as well as serum insulin were analyzed. Significant correlations were found between serum leptin and percent fat ($r = 0.70$, $P = 0.001$) and also between leptin and the amount of fat in kg ($r = 0.82$, $P = 0.0004$) in each group. In addition, serum leptin concentrations correlated significantly ($r = 0.52$, $P = 0.003$) with serum insulin in lean and obese groups.

Discussion

The main finding in this study is that exclusive voluntary abstention from food and drinks from dawn to sunset for one month (Ramadan) is associated with progressive elevations in serum leptin in both lean and obese women. In addition, the changes in serum leptin correlate with the changes in serum insulin that occurred during the study. Increased plasma leptin in obese compared with lean women confirm the results of previous studies indicating that hyperleptinaemia is an essential feature in the majority of obese humans.²¹ The cause of increased leptin levels in obesity has been explained by selective leptin resistance, which could be mediated by abnormal leptin synthesis, reduced leptin catabolism or decreased leptin transport across the blood brain barrier.¹ It is conceivable that increased leptin levels in obese women and its positive correlation with body fat in our study is supporting the speculation that leptin could be used as a marker of body fat stores.^{22,23} However, the lack of changes in body adiposity in our study indicate that body fat is unlikely to be an important factor in mediating the increased leptin levels during Ramadan.

The pattern of fasting during Ramadan (for approximately 13 hours a day) followed by re-feeding was associated with changes in serum insulin that were correlated with the changes in serum leptin. A recent study has also indicated that in weight-reduced obese women, fasting insulin and leptin levels were closely correlated suggesting that insulin could contribute to the long-term regulation of plasma leptin.²⁷ These observations are supported by others^{6,28} that found that insulin and glucose might regulate leptin secretion, especially in response to alterations in the energy status.¹⁰

Energy restriction studies have indicated that serum insulin and leptin levels are decreased with fasting, and the decrease in leptin levels correlates closely with the reduction in plasma glucose.^{7,8} Furthermore, leptin levels remain unchanged when serum insulin was prevented from decline by intravenous infusion of small amounts of glucose.²⁴ Therefore, our data confirm and support a role of insulin in long-term regulation of leptin secretion under conditions of chronic diurnal fasting followed by nocturnal feeding.

Potential non-adiposity factors that could be responsible for elevated serum leptin and insulin are those related to diet such as the amount of energy intake or food composition. Leptin secretion decreases in response to fasting^{7,24} and increases in response to positive energy balance induced by overfeeding.⁶ Food composition also alters the secretion of leptin, although the data regarding this issue has been controversial.^{10,25,26} Since energy intake were increased during Ramadan, it is possible that elevated levels of leptin and insulin may reflect a state of

Table 1. Anthropometrical variables and energy intake in lean and obese women during the study

	Lean				Obese			
	Early	Mid	End	Post	Early	Mid	End	Post
Weight (kg)	72.5 ± 2.6	71.7 ± 3.0	71.7 ± 3.0	72.3 ± 2.5	89.0 ± 5.2**	88.1 ± 5.3 **	88.6 ± 5.3 **	88.6 ± 5.3 **
Body mass index (kg/m ²)	22.5 ± 0.4	22.5 ± 0.4	21.8 ± 0.8	22.8 ± 0.7	33.1 ± 1.0 **	33.0 ± 1.0 **	31.7 ± 0.9 **	33.4 ± 1.1 **
Fat (kg)	17.7 ± 1.2	17.8 ± 1.3	17.5 ± 1.3	17.6 ± 1.4	34.9 ± 2.4 **	34.7 ± 2.3 **	31.3 ± 2.0 **	34.8 ± 2.5 **
Fat (%)	29.1 ± 1.5	29.3 ± 1.6	29.5 ± 1.6	29.0 ± 1.7	38.8 ± 1.1**	38.7 ± 1.0**	37.4 ± 1.0**	38.9 ± 1.1**
Waist circumference (cm)	72.2 ± 1.0	72.4 ± 1.1	71.5 ± 1.0	72.2 ± 1.9	93.1 ± 2.7**	92.4 ± 2.6**	89.2 ± 2.5**	93.2 ± 3.1*
Waist/hip ratio	0.70 ± 0.03	0.80 ± 0.02	0.72 ± 0.02	0.81 ± 0.02	0.79 ± 0.02*	0.79 ± 0.02*	0.78 ± 0.02*	0.81 ± 0.02
Energy intake (Kcal/day)	1666 ± 281	1747 ± 327†	1661 ± 349	—	1654 ± 441	1756 ± 412†	1681 ± 289	—

Values are mean ± SEM. * indicates that $P < 0.05$ and ** $P < 0.01$ in obese compared with lean subjects; † indicates $P < 0.05$ compared with beginning of Ramadan.

Table 2. Chemical variables in lean and obese women during the study

	Early	Mid	End	Post	Early	Mid	End	Post
Glucose (mmol/L)	5.27±0.08	4.96±0.14	5.06±0.15	5.71±0.12	5.81±0.47	5.61±0.51	5.84±0.61	6.06±0.42
Triglycerides (mmol/L)	0.72±0.05	0.70±0.03	0.77±0.03	0.81±0.12	0.95±0.16*	0.94±0.16*	1.04±0.21*	0.90±0.21
Cholesterol (mmol/L)	4.36±0.11	4.36±0.12	4.54±0.12	4.37±0.35	4.55±0.18	4.66±0.17	4.68±0.18	4.33±0.40
Insulin (μU/L)	5.3±0.3	6.7±0.4	7.4 ±0.4	7.7±1.6	7.4±1.2*	8.4±1.2*	8.8±1.4*	12.2±3.2*
Leptin (μg/L)	9.6±0.8	12.7±1.0†	14.0±1.1†	11.6±1.3	13.5±1.96*	17.1±2.5*†	20.0±0.01*†	16.6±1.0*

Values are mean ± SEM. * indicates that $P < 0.05$ and ** $P < 0.01$ in obese compared with lean subjects; † indicates $P < 0.05$ compared with beginning of Ramadan.

positive energy balance due to compensatory increase in food intake during the night. These observations agree with previous studies indicating that energy intake is increased,¹² but contradict those that indicate that energy intake is reduced¹³ during Ramadan. The discrepancy between these studies and ours could be due to regional variations in the culture or the economical status of the region.

Plasma leptin is secreted in a pulsatile fashion with peak nocturnal levels and the nadir at noon.⁴ Although the underlying mechanisms responsible for the circadian leptin rhythm is unclear, there is evidence that the diurnal rhythm of leptin secretion is entrained to the meal pattern and shifting the meal timing causes a comparable shift in plasma leptin rhythm.⁹ Since Ramadan fasting is associated with forward shifting of lunchtime by approximately 6 hours, another possible cause of increased levels of leptin in our study could be attributed to shift in the circadian pattern of leptin with progressive proximity towards the peak nocturnal levels. Additional studies that consider taking frequent samples over the 24 hours will be required to examine the circadian variations in serum leptin during Ramadan fasting.

In conclusion, we found that the circulating leptin levels progressively increase throughout the month of Ramadan. These elevations in serum leptin correlate with comparable increases in serum insulin. Increased levels of insulin and leptin could be due to increased energy intake during this month. These data support an important role of insulin in long-term regulation of leptin secretion during peculiar conditions of chronic fasting and re-feeding during Ramadan.

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