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Differences in leptin, ghrelin, and glucagon-like peptide-1 levels between religious fasting and normal fasting

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Background/aim: Leptin, ghrelin, and glucagon-like peptide-1 (GLP-1) affect hunger, satiety feelings, and food intake. We hypothesized that during Ramadan, if the brain knows that the body will be hungry until sunset, there may be differences between leptin, ghrelin, and GLP-1 levels in Ramadan and non-Ramadan fasting.

Materials and methods: This study had two phases. In the first phase, the participants were asked to skip the dawn meal of Ramadan (suhur), so that 12 h of fasting could be achieved. Participants ceased food intake at midnight, and at noon blood was drawn. Eight participants were selected as a subgroup. These participants gave blood three times a day to detect hormonal changes during Ramadan. Six months later, in the second phase, blood samples were obtained at noon from participants after 12 h of fasting.

Results: Analysis was conducted on 30 patients [19 males (63.3%) and 11 females (36.7%)]. There was a significant difference in leptin, ghrelin, and GLP-1 levels between Ramadan fasting and non-Ramadan fasting ($P = 0.04$, $P = 0.02$, and $P < 0.001$, respectively). In the subgroup analysis, there was no statistically significant difference in leptin, ghrelin, and GLP-1 levels over time.

Conclusion: The results of this study suggest that the nervous and gastrointestinal systems may behave differently in religious fasting than in nonreligious fasting.

Key words: Leptin, ghrelin, glucagon-like peptide-1, fasting

1. Introduction

It is a known fact that there is a link between the central nervous system and the gastrointestinal system. In recent years, this link has been further elucidated, especially with the discovery of new molecules affecting both brain and gut.

One of these molecules is leptin, also known as the 'satiety' hormone. It is produced mostly by adipose tissue (1) and inhibits hunger in the hypothalamus (2). Another molecule interacting with appetite is ghrelin. Ghrelin is produced in the gastrointestinal tract, and its main purpose is to stimulate hunger in the brain (3). It acts on the hypothalamus and increases hunger and gastric secretions (4). Glucagon-like peptide-1 (GLP-1) is a gut peptide, an incretin that is produced mainly from intestinal L cells in the gastrointestinal system (5) and from the solitary tract nucleus of the brain (6). In modern practice, although its analogs are used for treatment of type 2 diabetes, its functions include lessening the motivational effects of eating and decreasing the quantity and frequency of food consumption, leading to an early 'fullness' feeling (7).

Ramadan is a unique month in the Islamic calendar. During Ramadan, Muslims fast from sunrise to sunset. When they wake up, they know that they will be hungry for the rest of the day. We hypothesized that if the brain knows that the body will be hungry until sunset, the aforementioned appetite-related hormone levels may be different than in normal fasting. In this regard, we aimed to identify the differences between leptin, ghrelin, and GLP-1 levels in Ramadan and non-Ramadan fasting.

2. Materials and methods

2.1. Study design

Ethics committee approval for this study was obtained from the relevant ethics board (Decision No. 2013/0024). The study consisted of two phases: the first phase was in the month of Ramadan in 2013 (July) and the second phase was 6 months later (January 2014). In the first phase,

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healthy volunteers were recruited by hospital staff and blood samples were collected in the last week of Ramadan. In the second phase, blood samples were obtained 6 months later from the same participants.

2.2. Participants

Power analysis was conducted to determine the sample size needed. Thirty participants were recruited according to the power analysis, and five more were included in the study to compensate for possible drop-outs. The study protocol was explained to each screened candidate. Inclusion criteria of the participants consisted of being ≥18 years old, fasting for the whole month, and giving consent. Exclusion criteria consisted of having a medical disorder or using any medical agent, pregnancy, and not fasting for the whole month. To adjust the neurological and gastroenterological system to fasting, we recruited patients who fasted the whole month and we collected the blood samples in the last week of Ramadan.

2.3. Protocol

2.3.1. First phase

Participants were asked to skip the dawn meal of Ramadan (suhur) so that 12 h of fasting could be achieved. They ceased food intake at midnight and blood was drawn at noon. Eight participants were selected as a subgroup. We collected blood samples from the subgroup patients three times (0900, 1200, and 1700 hours) to detect hormonal changes during the day in Ramadan. The remaining participants gave blood only at noon (1200 hours).

2.3.2. Second phase

To ensure that participants were free of the influence of Ramadan, the study protocol was repeated 6 months later. If the participant had a different BMI or waist circumference value from the first phase's results, they were excluded from the analysis. Participants were asked to cease food intake again at midnight, and after 12 h of fasting, blood samples were obtained at noon. No subgroup was formed in the second phase.

2.4. Biochemical measurements

2.4.1. Blood samples

Venous blood samples were collected into two different blood tubes: a plain blood tube to obtain a serum sample for measuring glucose, insulin, and leptin, and a prechilled BD P800 blood collection tube (Becton, Dickinson and Company, NJ, USA), which contained spray-dried K_2EDTA anticoagulant and proprietary additives to collect and preserve plasma for measuring metabolic markers, including GLP-1 and ghrelin. After the collection of blood, serum and plasma samples were immediately separated by cold centrifugation and stored at –80 °C until further analysis.

2.4.2. Analytical methods

All the samples were measured together centrally to prevent interassay variation. Serum glucose was measured by hexokinase method, using an Architect c8000 biochemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA). Serum insulin levels were measured using a chemiluminescent immunoassay method with a UniCelDxI 800 immunoassay system (Beckman Coulter Inc., Carlsbad, CA, USA). A commercial ELISA kit was used for serum leptin determinations (Catalog No. EIA-2395; DRG Instruments GmbH, Marburg, Germany). The lowest limit of this assay was 1.0 ng/mL. The intraassay coefficients of variation (CVs) at 3.15 and 24.62 ng/mL for leptin were 5.95% and 6.91%, respectively. The interassay CVs of the test were 11.55% and 6.66 % at 2.71 and 26.15 ng/mL, respectively.

Plasma total ghrelin was measured with a total ghrelin (both intact and des-octanoyl forms) ELISA kit (Catalog No. EZGRT-89K; Millipore, Watford, UK). The analytical sensitivity of the test was 50 pg/mL. The intraassay CVs at 384.6, 904.5, and 1522.4 pg/mL for ghrelin were 1.26%, 0.9%, and 0.99%, respectively. The interassay CVs of the test were 7.81%, 6.28%, and 6.18% at the previous concentrations, respectively. Plasma GLP-1 levels were measured by a GLP-1 active ELISA kit (Catalog No. EGLP-35K; Millipore), in which active GLP-1 (7-36 amide) can be measured without cross-reacting with the inactive forms of GLP-1. The lowest level that could be detected by the assay was 2 pM. The intraassay CVs at 4, 8, 12, 28, and 76 pM for active GLP-1 were 8%, 7%, 6%, 7%, and 9%, respectively. The interassay CVs of the test were 13%, 12%, 7%, 7%, and <1% at the previous concentrations, respectively.

Manufacturers' protocols were followed for all commercial kits. Insulin resistance was assessed based on the fasting insulin and plasma glucose values, using the homeostasis model assessment (HOMA)-IR model.

2.5. Statistical analysis

Statistical analyses were conducted with SPSS 21.0 (IBM Corp., Armonk, NY, USA). The normality of distribution of variables was assessed with the Shapiro–Wilk and Kolmogorov–Smirnov tests. Subjects were compared for differences in two measurements using the paired samples t-test or the Wilcoxon test. The correlations between variables were determined by Pearson correlation test or Spearman's rho. Bonferroni correction was used when necessary. Data were expressed as means ± SD. $P < 0.05$ (two-tailed) was considered statistically significant. For subgroup analysis, we first executed a Friedman test and then used Bonferroni correction. Wilcoxon signed-rank tests were used to assess the parameters that showed significant differences with time.

3. Results

A total of 35 participants were recruited. Three were lost in follow-up, one became pregnant during the second phase, and one had different BMI and waist circumference values from the first phase. Analysis was conducted on the remaining 30 patients [19 males (63.3%) and 11 females (36.7%)]. The demographic characteristics of the patients are shown in the Table.

There was no significant difference in glucose levels between Ramadan and non-Ramadan fasting, whereas the mean insulin level was significantly higher in the Ramadan group (P = 0.001).

Mean leptin levels were 7.1 ± 6 ng/mL in Ramadan fasting and 5.8 ± 4 ng/mL in non-Ramadan fasting (P = 0.04). Ghrelin levels were higher in non-Ramadan fasting compared to Ramadan fasting, i.e. 589 ± 335 pg/mL vs. 535 ± 355 pg/mL (P = 0.02). Mean GLP-1 levels were 5.6 ± 1 pg/mL in Ramadan fasting and 4.6 ± 0.7 pg/mL in non-Ramadan fasting (P < 0.001) (Figures 1A–1C).

Although there was a correlation between BMI and ghrelin, GLP-1, and leptin levels in Ramadan fasting (P = 0.009, r = –0.45; P = 0.006, r = –0.52; and P = 0.001, r = 0.44, respectively), a correlation with BMI was observed only with ghrelin levels in non-Ramadan fasting (P = 0.009, r = –0.55). The only significant correlation with waist circumference was observed with leptin levels only in Ramadan fasting (P = 0.03, r = 0.57).

In the subgroup analysis, there was no statistically significant difference in leptin, ghrelin, and GLP-1 levels over time (0900, 1200, and 1700 hours); however, there was significant difference in glucose levels (P = 0.01 for 1200 and 1700 hours, and P = 0.01 for 0900 and 1700 hours).

4. Discussion

The results of this study indicate that there is difference in metabolic parameters between religious fasting and nonreligious fasting. To the best of our knowledge, this is the first study to show the differences of ghrelin and GLP-1 levels in religious fasting.

This difference can be attributed to neurohumoral mechanisms. In Ramadan fasting, one wakes without the intention to eat; however, in non-Ramadan fasting, one intends to eat after the prohibition is lifted (for health purposes, after 8 h of fasting when blood is drawn, etc.). This intention to eat or not to eat may be the reason for the difference we observed.

In our study, leptin levels were high during Ramadan fasting and lower in non-Ramadan fasting, indicating that the brain had adjusted itself to satiety during Ramadan. Similarly, GLP-1 levels were higher in Ramadan fasting, whereas 'hunger' hormone ghrelin levels were lower compared to non-Ramadan fasting, thus supporting our hypothesis.

There are other studies that have tested this hypothesis. In a report by Alzoghaibi et al., ghrelin levels did not show any significant difference between months prior to Ramadan and Ramadan (8). Furthermore, although leptin levels were reduced, there was no significant difference between Ramadan and non-Ramadan months. Their study had a relatively smaller cohort than our study with eight participants, and their methodology differed regarding the timeline.

In another study by Ajabnoor et al., there was a statistical difference between leptin levels prior to Ramadan with 10 h of fasting and during Ramadan with 6–7 h of fasting (9). The number of participants in this study was similar to our study. In this study, the mean leptin levels of the participants in Ramadan fasting was more than twice as high as our cohort's results. This discrepancy is probably due to methodological differences. In our study, because the participants had skipped suhur, 12-h fasting was possible. However, in Ajabnoor et al.'s study, the patients stopped eating at 0430 hours and samples were drawn at 0900 hours. Therefore, the subjects in Ajabnoor et al.'s study were not as hungry as our participants.

In a report by Hadi et al., there was no difference in leptin levels before, during, and after Ramadan fasting

Table. Demographic characteristics of the participants.

Variables	Mean (SD)	
Age, years	36.1 ± 8	
Body mass index (kg/cm^2)	26 ± 3.8	
Waist circumference (cm)	Males	Females
	91.5 ± 9	81.7 ± 11
Weight (kg)	75 ± 13	
Height (cm)	170 ± 9	

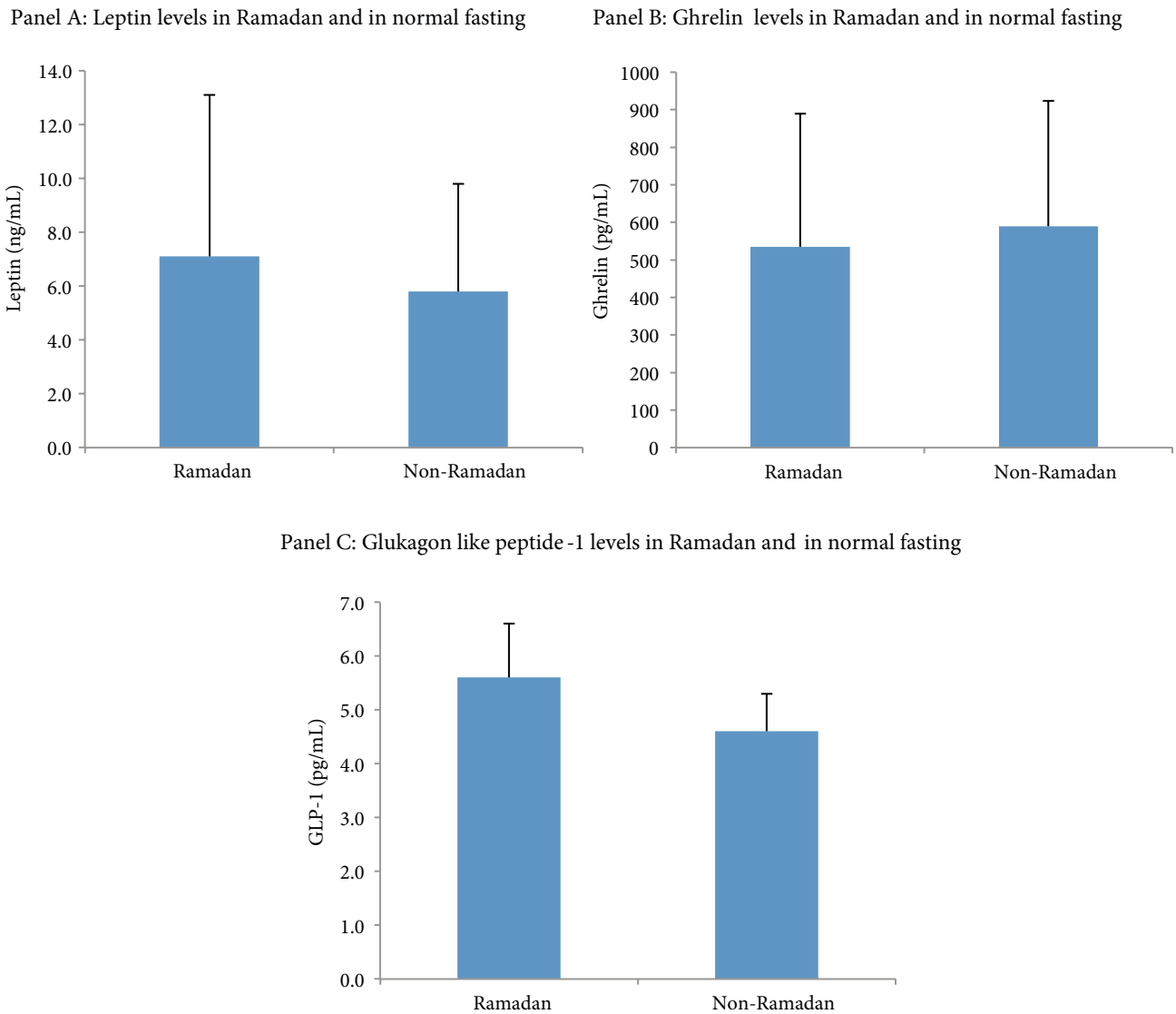

Figure 1. Differences in leptin (A), ghrelin (B), and glucagon-like peptide (C) levels.

(10). In this study, 56 patients with stable cardiac disease were recruited. Approximately 70% of the patients recruited in this study had a BMI of ≥25; in our study, this rate was 60%. Leptin resistance in these patients may have caused higher leptin levels for the participants of this study compared to our results (12.7 ng/mL vs. 7.1 ng/mL).

Another study by Mesci et al. tested this hypothesis with ghrelin, leptin, and adiponectin levels. Although they observed lower leptin and higher ghrelin levels in nonreligious fasting, their results were not statistically significant (11).

Another interesting result of this study was the findings in our subgroup. In our subgroup analysis, there was no decrease or increase in leptin, ghrelin, and GLP-1 levels during the day in Ramadan fasting, although glucose significantly decreased. This supports the fact that in intentional fasting due to religious beliefs, the brain is set up to be hungry for the rest of the day. Consequently, no metabolic change is detected.

There are several limitations to this study. First, our subgroup was relatively small with eight participants. Second, the Ramadan month of the study was in July according to the Gregorian calendar and hence fasting time was almost 17 h. To investigate any possible effect of daytime duration on fasting and hormones, the same protocol should be applied to a Ramadan month in winter. Finally, blood samples were drawn during the last week of Ramadan. For comparison, another set of blood samplings

could have been drawn during the first week. Any possible differences between the results of these weeks would strengthen the study.

In conclusion, we found significant differences in leptin, ghrelin, and GLP-1 levels between Ramadan fasting and non-Ramadan fasting. We also noted that these molecular parameters do not change during the fast. These results constitute progress towards elucidating how the brain adapts to hunger.

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