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Effect of Ramadan fasting on serum concentration of apelin-13 and new obesity indices in healthy adult men

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Background: The aim of this study was to determine how Ramadan fasting (RF) affected the recently described new obesity indices [visceral adiposity index (VAI), waist circumference to height ratio (WHtR), body adiposity index (BAI)], and serum concentration of apelin-13 (RF) in healthy adult men.

Material/Methods: For this purpose, 42 healthy adult men were selected. Anthropometric parameters were measured and a sample of venous blood was obtained for biochemical assays on the first and last days of Ramadan.
When all subjects were evaluated, all anthropometric parameters changed except VAI. Serum apelin-13, triglyceride (TG), HDL-cholesterol (HDL-C), and insulin levels did not change. When patients were divided into 3 groups according to body mass index (BMI), BAI decreased in normal-weight subjects and WHtR decreased in other groups, but VAI and apelin-13 did not change in any groups.

Results: We demonstrate for the first time that while some anthropometric parameters changed, VAI and serum apelin-13 levels did not change with RF. BMI, waist circumference (WC), TG, and HDL-C were evaluated together in calculation of VAI. TG, VAI, and HDL-C remained unchanged by RF. Even if body weight (BW) and BMI decreased, apelin-13 was not affected by RF. The data on serum apelin-13 may have been influenced by the small-percentage decrease in BW, as well as insignificant improvements in metabolic parameters such as lipid profiles, glucose, and insulin.

Conclusions: We found that Ramadan fasting in healthy adult men was associated with significant decreases in BW, BMI, WHtR, and BAI, but we found no significant changes in VAI and serum apelin-13 concentrations.

MeSH Keywords: Body Adiposity Index • Waist Circumference • Intra-Abdominal Fat • Apelin-13 • Fasting

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Background

Ramadan is the holiest month in the Islamic calendar and Muslims fast during this month. They abstain from eating and drinking from Sahur (the meal taken just before dawn during the Ramadan fast) until Iftar (sunset) during Ramadan, which is a lunar month of 29 or 30 days. This is referred to as Ramadan fasting (RF), which lasts from 8 to 18 hours, according to the season and latitude. Ramadan fasting entails major changes in sleep pattern, physical activity, and eating habits, which may cause changes in metabolism. Many aspects of these changes have been studied.

Visceral adiposity index (VAI), waist circumference-to-height ratio (WHtR), and body adiposity index (BAI) are newly described obesity indices [1–5]. The VAI is postulated to be a good indicator of visceral obesity and insulin sensitivity and is significantly correlated with visceral adiposity. Increased VAI has been found to be strongly associated with cardio-metabolic risk [2–6].

Apelin is a bioactive peptide that is synthesized and secreted by adipocytes [7]. Its gene encodes a pre-proprotein that is processed to generate bioactive peptides consisting of 36, 17, or 13 amino acids (apelin-36, apelin-17, and apelin-13, respectively). Apelin-13 acts primarily in the peripheral and central nervous systems, playing important roles in regulating cardiovascular function, fluid homeostasis, and hypertension, as well as regulating eating behavior, gastrointestinal function, and insulin sensitivity [8–10].

We aimed to investigate how new anthropometric obesity parameters (VAI, WHtR and BAI) and serum concentration of apelin-13 are affected by RF in healthy adult men.

Material and Methods

This study was conducted in the month of Ramadan, from 20 July to 20 August, 2012 in Kahramamaras. The length of the fasting was 15-16 hours per day and the mean temperature was 35°C. The study adhered to the guidelines of the Declaration of Helsinki and all procedures were approved by the Sutcu Imam University Institutional Research Ethics Committee (2012/14-4). Written informed consent was obtained from all subjects.

Subjects

The study included 42 healthy males without any disease or drug use, with a mean age of 35.0±8.9 years, who declared that they would fast for the entire month of Ramadan. The subjects were divided into 3 groups according to the World Health Organization (WHO) criteria of BMI as normal (n=15), overweight (n=17) and obese (n=10).

Anthropometric parameters

A 10-mL fasting blood sample of was collected twice from the median cubital vein by using a vacuum sampling method from each subject. Height, body weight (BW), waist circumference (WC), and hip circumference (HC) were measured for twice on the first day (R1) and last day (R2) of Ramadan at 11:00 a.m. (8–9 hours after the last meal [Sahur]). Serum aliquots were preserved at –80°C until biochemical analyses.

BMI, waist circumference-to-hip circumference ratio (WHR), WHtR, VAI, and BAI were calculated. The BW, height, WC (measured at a level midway between the lowest rib margin and the iliac crest), and HC (widest diameter over the greater trochanters) were measured using standardized procedures to calculate obesity indices as follows [5]:

WHtR = Waist circumference (cm)/height (cm)

VAI = [WC/(39.68+1.88×BMI)]×[TG (mmol/L)/1.03]×[1.31/HDL-C (mmol/L)] (for males)

BA I= (HC/height^{1,5})–18

Biochemical Assays

Serum Triglyceride (TG), Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were analyzed using standard methods.

Serum apelin-13 levels were determined with ELISA (enzyme-linked immunosorbent assay) method by using a commercial kit (USCN Life Science Inc.), an automatic ELISA microplate reader (Thermo Scientific, Finland), and a computer program (Skanlt for Multiscan FC 2.5.1). Sensitivity was 37.2 pg/mL and the detection range was 98.8–8000 pg/mL. Intra-assay CV was <10% and inter-assay CV was <12%. Results were observed as ng/mL.

Statistical analysis

Results are expressed as mean ±SD, number, and percent. Data were analyzed in SPSS Program 15.0 (SPSS Inc., Chicago, IL, USA). Paired t test (in all subjects) and Wilcoxon t test (in groups according to BMI) were used to match variables between R1 and R2. Statistical significance was assumed when the p-value was less than 0.05.

Results

Demographic, anthropometric and biochemical data obtained from the subjects at R1 and R2 with p values are listed in Table 1. All anthropometric measurements except VAI were

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Table 1. Biochemical and anthropometric parameters of all subjects in R1 and in R2 (n=42).

parameters	R1	R2	p value
Age, years	35.0±8.9		
Smoking, n	3		
BW, kg	80.4±14.3	78.8±13.7	<0.001
WC, cm	95.4±11.9	94.3±11.4	<0.001
HC, cm	104.6±7.8	104.0±7.5	0.001
WHR	0.91±0.05	0.90±0.05	0.005
WHtR	0.55±0.07	0.54±0.07	<0.001
BMI, kg/m^2	26.64±4.40	26.11±4.18	<0.001
BAI	27.78±3.51	27.51±3.58	0.001
VAI	1.82±1.21	1.71±1.01	0.364
Glucose, mg/dL	74.2±12.67	84.2±10.71	<0.001
Insulin, µU/mL	6.51±3.21	7.05±3.52	0.406
TC, md/dL	170.4±40.9	182.4±36.7	<0.001
TG, mmol/L	1.38±0.79	1.31±0.72	0.447
HDL-C, mmol/L	1.09±0.19	1.1±0.19	0.744
LDL-C, mg/dL	100.1±30.8	110.6±27.9	<0.001
Apelin 13, ng/mL	4.64±0.92	4.19±1.26	0.069

Table 1 was obtained from anthropometric and biochemical data of all subjects by using paired t test.
BAI – body adiposity index; BMI – body mass index; HDL-C – high-density lipoprotein cholesterol; HC – hip circumferences; LDL-C – low-density lipoprotein cholesterol; TC – total cholesterol; TG – triglyceride; VAI – visceral adiposity index; WC – waist circumference; WHR – waist circumferences-to-hip circumferences ratio; WHtR – waist circumference-to-height ratio.

decreased at R2 (p<0.05). Glucose, TC, and LDL-C increased and TG, HDL-C, insulin, and apelin-13 remained unchanged.

The demographic, anthropometric, and biochemical data of groups according to BMI score with p values are listed in Table 2. BAI decreased in normal-weight subjects and WHtR decreased in overweight and obese subjects at R2.

BW was decreased in 35 subjects and increased in 7 subjects (2 normal-weight and 5 overweight). Mean decreases of BW were 2.61% in all subjects and 2.7%. 2.17%, and 2.61% in normal-weight, overweight, and obese subjects, respectively. VAI, WHtR, and BAI were decreased in subjects who lost weight, but there was no significant difference in subjects who gained weight. When subjects who gained weight were excluded, apelin-13 was not significantly different.

Discussion

In this study, we demonstrate for the first time that apelin-13 was not affected by RF in all subjects and in groups according to BMI. Some studies reported that decreasing of serum apelin levels was associated with reduction in BMI by diet, exercise program, and bariatric surgery [11,12]. Krist et al. and Castan et al. established that decreases in serum apelin levels and improved insulin sensitivity, fasting blood glucose, and other metabolic indices were simultaneous. In our study, while some anthropometric parameters were improved, TC, LDL, glucose and insulin levels increased at the end of RF. These results may be due to insignificant differences of serum apelin-13 levels between R1 and R2.

In contrast, Heinonen et al. reported that no significant change in plasma apelin was observed in men and women with metabolic syndrome despite substantial and sustained improvements in body weight, BMI, body adiposity, and glucose metabolism, and a small improvement in blood pressure in response to a very low-calorie diet and 6-month weight maintenance period [13]. In our study, the weight loss rates were lower and the period was shorter than in the study by Heinonen et al. In addition, none of the subjects in our study had metabolic syndrome. Our findings are similar to those of Heinonen et al. When we evaluated the subjects who lost weight (mean weight loss was 2.61%), no significant difference was obtained between R1 and R2 in serum apelin-13 levels.

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Table 2. Biochemical and anthropometric parameters of normal, overweight and obese subjects in R1 and in R2.

Parameters	In normal (n=15)		In overweight (n=17)		In obese (n=10)		p values		
	R1	R2	R1	R2	R1	R2	p1	p2	p3
Age, years	29.5±7.7		39.4±7.0		36.0±10.5				
Smoking, %	0		11.8		14.3				
BW, kg	69.1±7.3	67.8±6.9	81.8±6.8	80.5±7.0	101.1±15.1	98.3±13.9	0.006	0.003	0.018
WC, cm	85.5±6.6	84.7±6.1	97.2±4.5	95.9±4.5	112.3±12.4	110.7±11.8	0.061	0.018	0.041
HC, cm	98.4±4.8	98.1±4.5	106±4.1	105.3±3.9	114.6±8.7	113.7±8.2	0.202	0.02	0.063
WHR	0.87±0.04	0.86±0.04	0.92±0.03	0.91±0.03	0.98±0.05	0.97±0.04	0.13	0.083	0.102
WHtR	0.49±0.04	0.49±0.03	0.56±0.03	0.55±0.03	0.65±0.07	0.64±0.06	0.075	0.02	0.042
BMI, kg/m^2	22.87±1.63	22.45±1.57	27.17±1.55	26.70±1.45	33.45±4.44	32.55±4.06	0.006	0.004	0.042
BAI	26.96±2.13	26.69±2.06	26.85±3.45	26.59±3.24	31.79±3.61	31.49±3.46	0.028	0.093	0.068
VAI	1.16±0.72	1.18±0.6	2.03±1.41	1.98±1.21	2.73±0.71	2.2±0.74	0.82	0.723	0.128
Glucose, mg/dL	68.8±8.4	80.6±7.2	76.7±13.5	86.8±13.3	79.4±15.7	85.6±9.2	0.001	<0.001	0.176
Insulin µU/mL	5.37±2.79	5.17±1.61	6.37±3.32	7.42±4.36	9.30±2.25	10.24±1.28	0.887	0.196	0.612
TC, mg/dL	148.9±24.4	173.4±30.4	174.9±43.5	185.1±43.7	205.3±39.5	195.0±26.9	<0.001	0.007	0.204
TG, mmo/L	81.9±27.4	84.6±29.2	135.0±85.4	135.8±83.9	175.6±46.3	136.4±28.9	0.798	0.756	0.043
HDL-C, mmol/L	44.5±8.1	44.8±8.9	41.2±7.5	41.6±6.1	39.1±3.7	38.9±5.4	0.777	0.856	0.916
LDL-C, mg/dL	83.1±19.1	102.2±25.7	103.0±30.5	111.9±31.6	129.7±29.7	125.1±17.0	0.001	0.004	0.735
Apelin 13, ng/mL	4.41±0.96	3.91±1.14	4.59±0.87	4.33±1.48	5.21±1.04	4.76±0.93	0.14	0.469	0.345

p1: between R1 and R2 in normal; p2: between R1 and R2 in overweight; p3: between R1 and R2 in obese.
Table 2 was obtained from anthropometric and biochemical data of normal, overweight and obese subjects by using Wilcoxon test.
BAI – body adiposity index; BMI – body mass index; HDL-C – high density lipoprotein cholesterol; HC – hip circumferences;
LDL-C – low density lipoprotein cholesterol; TC – total cholesterol; TG – triglyceride; VAI – visceral adiposity index;
WC – waist circumference; WHR – waist circumferences-to-hip circumferences ratio; WHtR – waist circumference-to-height ratio.

In regulating cardiovascular function, apelin inhibits water intake and vasopressin production [10,14]. Water deprivation, while increasing the activity of vasopressin neurons, reduces plasma apelin concentrations and induces an intra-neuronal accumulation of the peptide, thereby decreasing the inhibitory effect of apelin on vasopressin release and preventing additional water loss at the kidney level. Apelin counteracts the effects of vasopressin in the maintenance of body fluid homeostasis [15]. Apelin expression in fat cells is strongly inhibited by fasting and recovered after re-feeding [5]. Intracerebroventricularly injected apelin-13 inhibits gastric emptying and gastrointestinal transit [16]. Apelin has been shown to be involved in stimulating gastric cell proliferation, cholecystokinin (CCK) secretion, histamine release, gastric acid and bicarbonate secretion, and regulation of gastrointestinal motility [17,18]. Fasting for 30 days did not significantly affect circulating apelin-13 levels. Subjects in the present study did not have any disorders or diseases. We hypothesize that if we had taken subjects having conditions such as higher BMI, hypertension, and metabolic syndrome, we might have found significant differences in serum concentrations of apelin-13 during RF.

Other findings of our study involved anthropometric parameters. BAI decreased in normal-weight subjects and WHtR decreased in overweight and obese subjects. These findings mean the abdominal fat reduction was more marked in overweight and obese subjects. WHO reported that BMI value should be between 18.5 and 24.9 kg/m^2 and median value should be between 21.0 and 23.0 to achieve and maintain optimal health conditions [19]. In our study, median BMI value was 26.2 on the first day of Ramadan and 25.5 on the last day, which is closer to the WHO target. These findings may be seen as a positive effect of RF on health. According to WHO, at least 2.8 million people die each year due to overweight or obesity. Being overweight or obese drastically increases risk of noncommunicable diseases such as cardiovascular disease, cancer, and DM.

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There is increased co-morbidity for overweight individuals and severe co-morbidity for obese individuals. Both general and abdominal adiposity are related to the risk of death [20–22].

The majority of health-specific findings related to RF are equivocal. A previous study measured the effects of RF on BMI, WHR, and abdominal and visceral fat distribution measured by CT, finding that BMI and BW were not changed, but the visceral fat compartment was reduced in women and young subjects because they were more physically active [23]. Another study reported that restriction in feeding, reduction in activity, and increased sleeping time may be the causes of increased BW. In contrast, decreased total amount of food consumed per day and non-decreased physical activity together during RF may be the causes of reduction in BW [24].

In our study, the results revealed that in addition to VAI, the levels of TG and HDL did not change. VAI is measured by using BMI, WC, TG, and HDL [5]. Some studies reported that HDL-C significantly increased and there was a non-significant rise in the TG value together with weight loss at the end of Ramadan fasting [24–27]. Unalacak et al. reported that no significant difference was found in HDL-C, LDL-C, and TG in normal/-weight and obese subjects following RF [28]. On the contrary, Ziaee et al. showed reductions in concentrations of blood glucose and HDL, and increases in LDL, in healthy individuals during RF [29].

Changes in anthropometric and biochemical parameters are variable and depend on the quality and quantity of food intake, physical activity and exercise, changes in body weight, and biochemical response to starvation [24–30]. Differences in the results of glucose, TC, LDL-C, TG, and HDL-C detected in our study may be related to these factors. Hence, the changes that occurred following 30 days of fasting were not sufficient to affect VAI.

Limitations

We did not investigate the effects of calorie intake and energy expenditure, sleeping harmony, and diurnal rhythm-related hormones, and we did not follow up the effects of RF. Because all subjects were healthy, insulin resistance and sensitivity and their relation with apelin-13 were not examined.

Conclusions

There were significant decreases in BW, BMI, WHtR, and BAI, but no significant changes in VAI and serum apelin-13 concentrations during Ramadan fasting in healthy adult men.

Further studies considering the calorie intake and energy expenditure, physical activity, nutrition, and sleeping harmony, and recording the follow-up effects of RF may provide more detailed findings related to the effects of RF on health.

Conflict of interest

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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