Effects of Ramadan on food intake, glucose homeostasis, lipid profiles and body composition

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Abbreviations

Body mass index	BMI
waist-to-hip ratio	WHR
mass of body fat	MBF
Total body water	TBW
percent of body fat	PBF
lean body mass	LBM
soft lean Mass	SLM
Low density lipoprotein	LDL
High density lipoprotein	HDL
Triglyceride	TG
Total cholesterol	TC
Fasting blood glucose	FBS
Homeostatic model assessment of insulin resistance	HOMA-IR
Food frequency questionnaire	FFQ

ABSTRACT

Background: Changes in food consumption patterns during Ramadan may cause metabolic changes, but these have not been well studied.

Objective: We aimed to determine food intake, glucose homeostasis, lipid profiles and body composition before, during and after Ramadan fasting.

Methods: 160 healthy men were enrolled and investigated at three times (before, at the end of, and one month after Ramadan). Body composition was estimated by bio-impedance. Fasting blood samples were obtained for measuring fasting blood sugar (FBS), lipid profiles and insulin level. Insulin resistance was identified by the homeostatic model assessment (HOMA) of peripheral insulin resistance (IR). Food intake was measured using a validated food frequency questionnaire before and during Ramadan. Statistical analysis was performed by SPSS 16 and $P<0.05$ considered the level of significance.

Results: Anthropometric parameters such as body weight, body mass index, and body fat percentage (BFP) as well as FBS and circulating triglycerides were all decreased significantly at the end of Ramadan compared to the same indices measured prior to Ramadan (all $P<0.001$). In contrast, at the end of Ramadan, HOMA-IR was significantly elevated ($P<0.001$). One month after Ramadan, these traits had all started to return to their pre-Ramadan levels, but were still disrupted. Food intake of all food groups except carbohydrates were decreased during Ramadan.

Conclusion: Ramadan fasting may lead to both positive and negative health effects such as a decrease in FBS, weight , BFP, increase in LDL and insulin resistance in healthy adults. However, these effects were all transitory.

Key words Ramadan, Blood glucose, Body composition, Insulin resistance, Calorie restriction.

INTRODUCTION

At least, one billion of the total global population of Muslims (approximately 1.5 billion) (1) abstain from eating or drinking from sunrise to sunset during the holy month of Ramadan (2). Since the Islamic calendar is lunar, the beginning of the Islamic year advances 11 days each year, compared with the Gregorian calendar. Therefore, Ramadan starts at different times of year over a 33-year cycle (3). The fasting period per day may vary depending on the geographical location of the country and the season, and can be as long as 18 hours/day in the summer (4) in some locations.

Adherence to the Ramadan fast causes radical changes in lifestyle, eating patterns, and the quality of ingested nutrients (4,5). The effects of Ramadan on energy balance and weight regulation have been well studied: but with some conflicting outcomes. Hence some studies indicate weight loss (including both fat and fat-free mass)(6-13), but in other studies body weight and body composition remain unchanged (14-20) and may even increase (1). Effects on food intake generally are not significant (8,11,12,18,20) but that may reflect the poor quality the tools to resolve food intake differences (21). Resting metabolic rate and physical activity levels may decline (10, 22) but these seem to be offset by a reduction in sleeping time, and hence there is no impact on total daily energy expenditure as measured by doubly-labeled water (22). All studies agree, however, that there are profound impacts on the circadian patterns of various hormones including leptin, adiponectin, prolactin, insulin and cortisol. These changes may have downstream impacts on insulin resistance (5,23). Moreover there are documented impacts of Ramadan on fasting glucose and serum lipid profiles, but the effects are also inconsistent across studies (24,25,26). In the present study we investigated changes in anthropometric indices, fasting blood sugar, serum lipid profiles, serum insulin and insulin resistance at three times (before, at the end of and one month after Ramadan) and food intake before and during Ramadan in a group of 160 males.

METHODS

This observational study was performed in Kermanshah, Iran in the summer of 2014. The study protocol was approved by the Ethics Committee of the Kermanshah University of Medical Sciences (approval no: 91058) and was registered as a clinical trial with the Iranian clinical trials registry (registration number IRCT201702269856N5). The study population consisted of people who fasted during the entire month of Ramadan. One hundred and sixty healthy men with no diagnosed disease who volunteered to participate in the study were selected from five mosques in different districts of the city. A list of eligible subjects was developed before the commencement of Ramadan. The selected individuals were matched for age and the socioeconomic status in different districts. Data were collected at three different times (1-7 days before, 1-4 days before the end and one month after Ramadan had ended). A flow chart for the study is presented in figure 1.

Demographic data, including age, educational level and socioeconomic status were collected by a researcher-developed questionnaire. Dietary intake was assessed by a semi-quantitative food frequency questionnaire (FFQ). The FFQ's validity and reliability have been confirmed previously (27). This questionnaire consists of 168 foods with a standard portion size. The mentioned value for each food is based on recommended portion size. The questionnaire was only administered prior to and at the end of Ramadan. The amounts of macronutrients and energy intake were estimated at each of the time point.

Body composition was measured by bio-impedance body analyzer model Avis Plus 333. This device is a standard instrument for assessing the composition of the body based on a multi-frequency bioimpedance signal allowing estimation of the mass of body fat (BF), body fat percentage (BF%), lean body mass (LBM), soft lean mass and total body water (TBW). To assess changes in blood biochemical indices, 5-ml venous blood samples were taken from the participants at each time point. Blood samples were obtained after 12-14 h

fasting, at 8-9 am 2-5 days prior to Ramadan, at 6-8 pm on the 25-28th days of Ramadan, and at 8-9 am 28-30 days after Ramadan. After separation of blood cells by centrifugation at 3000 rpm for 15 min at 4°C, serum was stored with 0.1% Na_2-EDTA in the freezer at −40°C. Serum triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined quantitatively by enzymatic colorimetric kits (Pars Co., Iran) with an auto analyzer device (Technic on RA-XT, Ireland). FBS was measured according to a standard photometric method by an auto analyzer (RA1000-RAXT, Pars Co., Iran). Homeostatic model assessment-insulin resistance (HOMA-IR) and beta cell function (β) indices were calculated by the formula below: (FPI: Fasting plasma insulin)(FPG: Fasting plasma glucose)

$$\text{HOMA-IR} = [\text{FPI (mIU/L)} \times \text{FPG (mmol/L)}]/22.5$$

$$\text{HOMA-\%}\beta = [20 \times \text{FPI (mU/L)})/(\text{FPG (mmol/L)} - 3.5]$$

For the conversion of fasting glucose units from mg/dl to mmol/l, the number was multiplied by 18 (28).

Statistics

The data were analyzed by SPSS 16. We analysed the data across all three time points using repeated measures analyses of variance, followed up where appropriate by paired t-tests to locate differences of interest. We used correlation to explore the relationship between weight loss during Ramadan and weight regain afterwards. Correlation was used to investigate the association between food groups and anthropometric and biochemical parameters. $P<0.05$ was considered the level of significance.

RESULTS

The mean age of the participants was 39.35±10.7 (range: 21-63) years. At baseline, 160 participants were enrolled in the study, of whom eight people were excluded because of incomplete information and the data of 152 people (compliance rate 95%) was analyzed. The

mean weight of the participants was 76.33±11.5 kg before Ramadan and 74.4±11.3 kg at the end of Ramadan (paired t-test: t = 19.95, *P*<0.001), and had returned to the baseline level (76.31 kg) one month after Ramadan (paired t-test compared to pre-Ramadan levels, t =.13, P =.89). BMI also decreased significantly between baseline and the end of Ramadan (paired t-test, t = 20.38, *P*<0.001) but returned to the baseline level one month later (table 1). There was a significant positive correlation between weight loss during Ramadan and weight regain over the month after Ramadan (R=0.359, P: 0.01). The mean of TBW and SLM were significantly decreased at end of Ramadan (P<0.001).The mean BF was 18.2±6.2 kg before Ramadan, decreased by about 0.7 kg ~~and reached 17.6 ±6.1kg~~ at the end of Ramadan (paired t-test, t = 7.21, *P*<0.001) and had increased to 19.25±6.38 kg one month later (paired t-test compared to pre-Ramadan, t = -8.47, *P*<0.001). The mean weight loss and glucose levels in people who were overweight or obese were slightly higher than those who had normal weight. However, these differences were not significant. BF% decreased by about 0.3% at the end of Ramadan compared with before Ramadan (paired t-test, t =2.91, *P*<0.001). TBW was 41.9±5.14% before Ramadan, decreased to 41.0±4.58% at the end of Ramadan and increased to 41.1±4.93% one month later (paired t-test, t = 9.24, *P*<0.001). LBM decreased from 58.2±7.14 kg to 56.9±6.47 kg at the end of Ramadan and increased to 57.1±6.85 kg one month later (*P*<0.001).

LDL cholesterol (~~95.4±20.4 vs 98.7±20.8,~~ paired t-test, t = -3.32, p<0.001) and TC (~~185.94±52.6 vs 192.7±39.7,~~ paired t-test, t =-2.21, p<0.03) increased during Ramadan, and then decreased, but had not returned to their baseline levels one month after Ramadan (~~95.4±20.4 vs 96.9±22.8,~~ paired t-test, NS). HDL cholesterol was not significantly different across the three different time points (ANOVA). TG decreased significantly during Ramadan (~~151.3±83.6 vs 140.9±74.2~~, paired t-test, t =2.01, p<0.04) but increased dramatically and even reached higher level than the baseline level one month after Ramadan(~~151.3±83.6 vs 161.2±87.6,~~ paired t-test, NS) (Table 1).

FBS decreased at the end of Ramadan (paired t-test, t = 6.7 p<0.001) but increased dramatically and also reached higher level than the baseline level one month later (paired t-test, t = -4.1, p<0.001) The insulin level increased during Ramadan (paired t-test, t = -8.16, *P*=0.001) and decreased (paired t-test, t = 2.34, *P*=0.02) but did not return to baseline level one month after Ramadan. The HOMA-IR index increased at the end of Ramadan compared with before Ramadan (paired t-test, t = -6.44 P=0.001). Although it subsequently decreased slightly but it had not returned to the baseline level one month later (paired t-test, t = -6.47,p=0.001). HOMA-β significantly increased at the end of Ramadan compared with before Ramadan, and decreased one month later but did not return to the baseline level (Table 1).

The consumption of all food groups (except fruits) decreased significantly during Ramadan compared with the pre- Ramadan level (Table 2). Among the macronutrients, carbohydrate intake increased significantly during Ramadan compared with before Ramadan (paired t-test, t = -4.46, P<0.001) both protein (paired t-test, t = 17.01, P<0.001) and fat (paired t-test, t = 13.82, P<0.001) intake decreased significantly during Ramadan compared with baseline. Estimated energy intake decreased significantly during Ramadan compared with before Ramadan (paired t-test, t = 5.78, P<0.001) (Table 3). There was no significant correlation between the change in body weight and the change in food intake from before to during Ramadan (p < .05). There were significant associations between HDL and bread and cereals intake (R=-0.185, P: 0.01) as well as between body weight and fruit intake (R= 0.214, P: 0.006) before Ramadan, and between insulin and dairy products intakes (R=0.156, P: 0.045), LDL and vegetables (R=0.194, P=0.012) and TC and vegetables (R=0.213, P: 0.006) at the end of Ramadan.

DISCUSSION

Although Ramadan is generally called ‘Ramadan fasting’ there is little evidence that food intake levels are actually decreased during the period of Ramadan (8,11,12,18,20). Nevertheless many studies have suggested that body weight and fat mass decline during Ramadan (6-13) suggesting individuals are in a state of negative energy balance. Consistent with these previous studies we also observed that individuals during Ramadan lost body weight, BMI and body fat. However, using a validated food intake measurement tool we were also able to detect a decrease in caloric intake during the Ramadan period, supporting the idea that individuals are in negative energy balance and lose weight because of lowered intake. However, weight loss was not correlated to the change in intake. This could be because the instrument we used to monitor food intake was not accurate enough to reflect changes at the individual level. Contrasting our lack of a significant association, Shariatpanahi et al observed that participants who had greater weight loss ingested fewer calories (29).
In this study, the weight of 96% of participants was reduced during Ramadan. This reduction may be the primary cause of metabolic improvements. People during Ramadan usually eat in two main meals: suhoor, which is served before dawn, and iftar, which is served after sunset. In suhoor, fasting people usually have a tendency to consume little food, and this can lead to an increase in lipolysis and gluconeonogenesis so it can potential be the cause of some of the observed adverse metabolic effects. In this study, the mean of TBW and SLM were significantly decreased at the end of Ramadan.

In these men both fat mass and lean mass decreased during Ramadan. One month after Ramadan, fat mass had increased again to a level higher than the pre-Ramadan baseline. Besides that, muscle mass increased but did not reach its baseline level. Since the participants in this study abstained from eating and drinking on average for 17 hours each day, in the final hours of fasting, the body activates the gluconeogenesis and lipolysis processes to meet energy and glucose needs. If exacerbation of lipolysis is associated with reduction in access

to carbohydrate, it leads to a marked increase in supply of acetyl coenzyme A. In this situation, acetyl coenzyme A is not able to enter the Krebs cycle so that it is converted into other metabolites such as ketones and cholesterol.

A previous study showed that during Ramadan, the levels of TC and TG decreased significantly. The amount of HDL cholesterol increased and remained stable and LDL decreased one month after Ramadan (30). While another study showed that TG and HDL significantly decreased, but LDL levels increased and TC did not change (24,30). A study in Bojnourd, east Iran showed that the levels of cholesterol, TG, LDL cholesterol, and HDL cholesterol had all decreased by the 28th day of Ramadan compared with the first day (31). In contrast in another study, blood cholesterol and TG increased significantly during Ramadan compared with before Ramadan; however, both parameters decreased after this month (32). Different blood lipid profiles among people in Ramadan might be attributed to the different diets of Muslims during this month.

This study showed that FBS levels changed significantly over time. Two previous studies demonstrated that FBS decreased among healthy people during Ramadan fasting (33-34). In contrast, a different study reported an increase in fasting glucose (35) and a third study showed variations in glucose levels(36). The increased levels of HOMA-IR, seen in the present study, represent increase in insulin resistance during Ramadan, which can be due to metabolic outcomes of fasting rather than pathological conditions. Ramadan fasting can be considered semi-starvation. In early starvation, the adaptive response of energy homeostasis involves several endocrine changes. Insulin secretion is reduced and glucagon and epinephrine release elevated in response to reduction in the concentrations of plasma glucose and free amino acid. These changes lead to decrease in muscle protein synthesis, lipogenesis and growth and increases in lipolysis and glycogenolysis. Peripheral insulin resistance increases probably due to the increase in plasma free fatty acids (37). A previous study in Turkey that examined the

effects of fasting on biochemical and hematological parameters in healthy and overweight people, observed significant reductions in HOMA-IR and FBS in overweight individuals during fasting. Moreover, in the healthy group, HOMA-IR increased significantly but no significant change was observed in FBS (25). In contrast, study in Tehran, demonstrated that the mean FBS in fasting men aged 34-61 years decreased significantly after Ramadan while HOMA-IR did not change significantly (38). The current evidence therefore shows considerable inconsistency in the findings on HOMA-IR variation during Ramadan. This inconsistency may be due to differences in sample size, season of fasting, number of fasting days, and nutritional knowledge of different populations. Taken together, most studies have shown that fasting in Ramadan leads to decrease in blood glucose and increase in insulin sensitivity (39). The beta cell function (HOMA-β) significantly increased during Ramadan compared to before Ramadan. This index also exhibited a significant decrease in the third phase of the study but did not return to its level before Ramadan. A previous study in the USA showed that increased levels of FBS, insulin and HOMA-IR were significantly associated with increased risk of diabetes while increased HOMA-β significantly reduced the risk of type 2 diabetes (40). Insulin sensitivity and insulin secretion follow a circadian pattern. In this study insulin levels had been measured at different time points for blood sampling so this may be a reason for the difference seen in the resultsfor both insulin and HOMA-IR.

A strength of the current study was the large sample of individuals that participated, relative to similar studies conducted previously. However, there were also several weaknesses and limitations. The study only included males and hence the impacts of Ramadan fasting on females could not be evaluated. Moreover, in common with most studies of Ramadan conducted in strict Muslim countries, we did not have a control group who did not partake in the fasting. Abstaining from the fasting is permitted under certain conditions – for example for pregnant or lactating women, senile individuals or for individuals that are ill.

None of these groups however provide suitable controls. Formally therefore, without such controls we cannot separate the trends in time from changes due to season. Nevertheless despite this caveat it seems likely that the patterns were caused by Ramadan and were not seasonal effects independent of the fasting behaviour.

The reduction in weight, BMI and body fat and some biochemical parameters such as TG and FBS represents the positive health benefits of fasting during Ramadan. However LDL cholesterol and fasting insulin increased as did HOMA-IR. These positive and negative effects were all transient as they had generally returned to baseline levels one month after Ramadan had finished.

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Table 1: Anthropometric and biochemical indices before, at the end and after Ramadan*

Anthropometric &biochemical Indices	Pre-Ramadan (Mean± SD)	Ramadan (Mean± SD)	Post-Ramadan (Mean± SD)	P_1	P_2	P_3	P_4
Weight(kg)	76.33±11.4	74.22±11.2	76.31±11.5	<0.001	<0.001	0.890	<0.001
BMI(kg/m^2)	26.10±3.79	25.37±3.74	26.08±3.81	<0.001	<0.001	0.699	<0.001
WHR	0.9±0.081	0.89±0.082	0.9±0.078	<0.001	<0.001	<0.001	<0.001
MBF(kg)	18.34±6.1	17.60±6.2	19.36±6.2	<0.001	<0.001	<0.001	<0.001
TBW (%)	41.88±5.1	40.90±4.8	41.14±4.8	<0.001	<0.001	<0.001	0.002
PBF (%)	23.50± 5.6	23.13±5.8	24.8±5.4	<0.001	0.003	<0.001	<0.001
LBM (kg)	58.18±7.09	56.81±6.7	57.14±6.8	<0.001	<0.001	<0.001	0.001
SLM (kg)	53.77±6.5	52.52±6.1	52.74±6.2	<0.001	<0.001	<0.001	0.028
LDL(mg/dl)	95.8±20.6	99.40±21.3	96.95±22.8	0.009	0.001	0.352	0.052
HDL(mg/dl)	44.70±7.9	45.59±9	46.25±9.3	0.033	0.168	0.010	0.195
TG(mg/dl)	151.44±85.2	140.44±75.2	161.25±87.6	0.001	0.044	0.103	<0.001
TC(mg/dl)	187.76±52.4	193.62±40.3	190.72±41.3	0.155	0.071	0.373	0.217
FBS(mg/dl)	80.17±19.3	72.06±18.4	81.3±21.5	<0.001	<0.001	0.630	<0.001
Insulin(mg/dl)	4.63±5.33	11.32 ±9	9.02 ±7.54	<0.001	<0.001	<0.001	0.020
HOMA-IR	0.93±1.09	2.01±1.66	1.84±1.62	<0.001	<0.001	<0.001	0.372
HOMA-β	17.62±24.1	55.57±49.3	37.79±36.2	<0.001	<0.001	<0.001	0.001

Body mass index (BMI), waist-to-hip ratio (WHR), mass of body fat (MBF), Total body water (TBW), percent of body fat (PBF), lean body mass (LBM), soft lean Mass (SLM), Low density lipoprotein (LDL), High density lipoprotein (HDL), Triglyceride (TG), Total cholesterol (TC), Fasting blood glucose (FBS).

*ANOVA test was used to investigate anthropometric and biochemical indices before, at the end and a month after Ramadan.

P_1: comparison of means before, at the end and a month after Ramadan

P_2: comparison of means before and the end of Ramadan

P_3: comparison of means before, and a month after Ramadan

P_4: : comparison of means at end and a month after Ramadan

Table 2: Changes in the consumption of food groups before and the end of Ramadan

Food Groups (serving/day)			**p-value**
	Pre-Ramadan (Mean± SD)	**Ramadan (Mean± SD)**	
Breads and Cereals	6.79±2.90	5.71±1.21	< 0.003
Dairy Products	3.45±1.75	1.85±0.69	< 0.001
Meats	3.97±2.16	2.24±0.85	< 0.001
Fruits	5.97±3.56	8.50±2.67	< 0.001
Vegetables	0.92±0.47	0.90±0.63	< 0.03
Fat and Oils	2.29±0.89	2.33±0.87	< 0.001
Others	2.24±1.64	2.42±2.17	< 0.001

Table 3: Changes in the consumption of Macronutrients (g/day) and Energy intake (MJ/day) before and at the end of Ramadan

Macronutrients	(Mean± SD)		P-Value
	Pre-Ramadan	Ramadan	
Carbohydrate	271.24±93.70	276.33±68.78	< 0.001
Protein	77.72±25.30	49.50±11.68	< 0.001
Fat	48.66±17	32.17±10.20	< 0.001
Energy intake	7.6±2.3	6.6±1.6	< 0.001

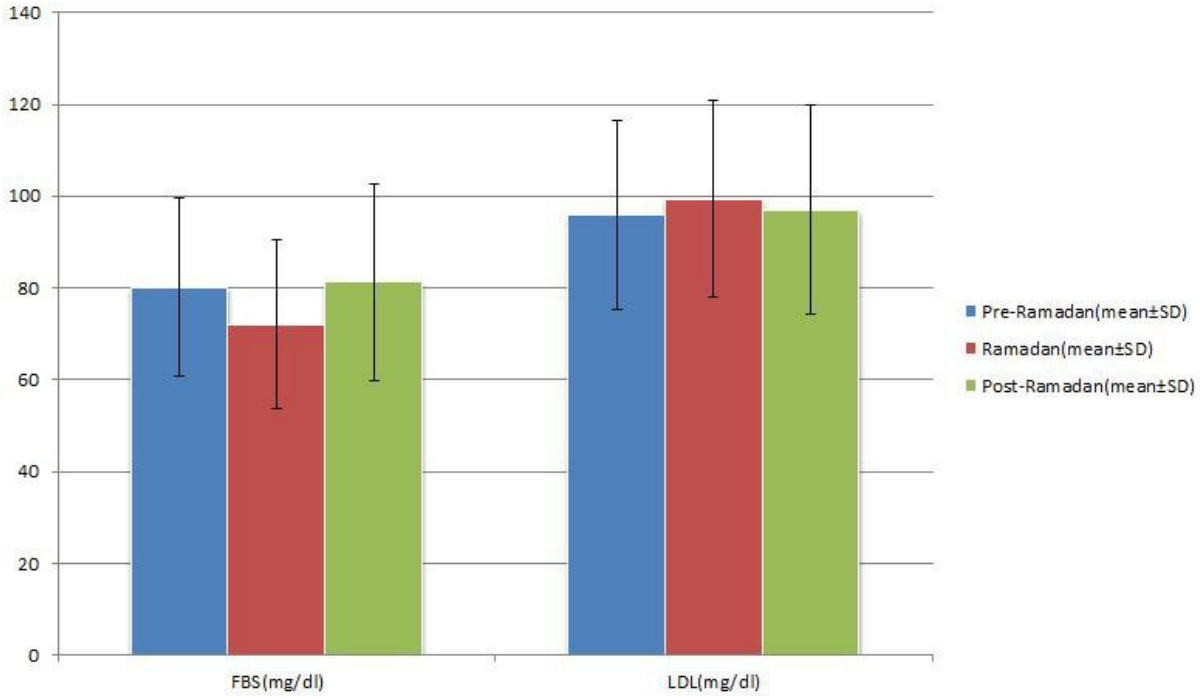

Figure 1. Fasting blood sugar (FBS) and low density lipoprotein (LDL) in people before, at the end, and one month after Ramadan. Error bars are standard deviation.

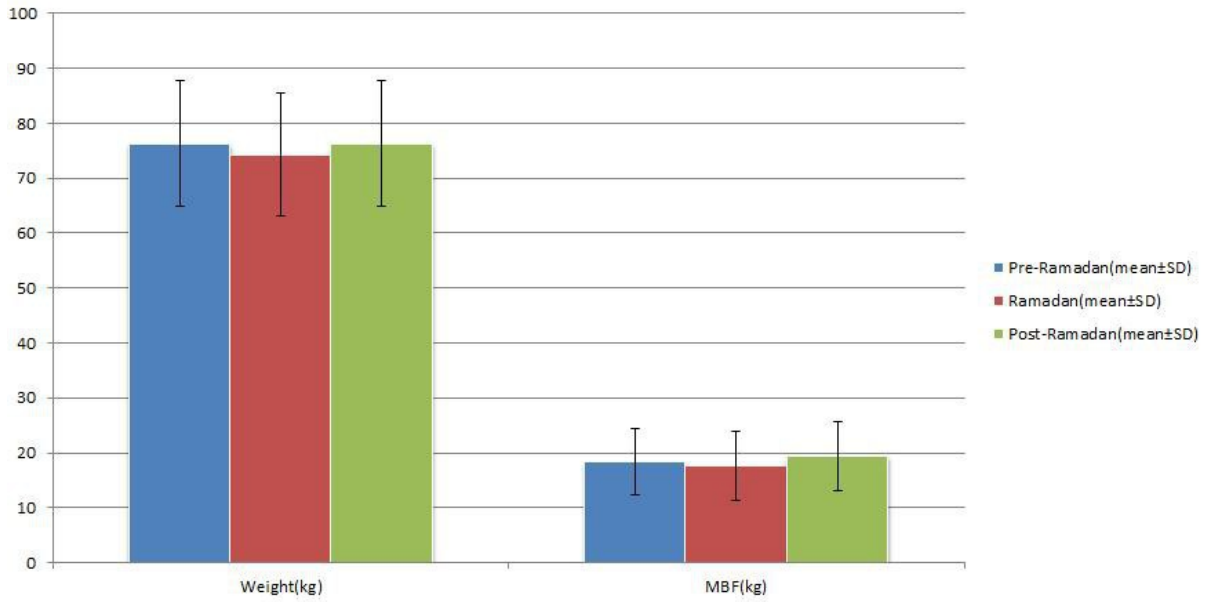

Figure 2. Body weight (FBS) and mass of body fat (MBF) (both in kg) in fasted people before, during and one month after Ramadan. Error bars are standard deviation.

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540 Supplementary materials 1:

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542 STROBE Statement—checklist of items that should be included in reports of observational
543 studies

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	Item No	Recommendation
Title and abstract	1	Effects of Ramadan on food intake, glucose homeostasis, lipid profiles and body composition (lines 1 and 2)
		Food intake, glucose homeostasis, lipid profiles and body composition before, during and after Ramadan fasting among 160 healthy men were investigated. Ramadan fasting may lead to some beneficial effects such as a decrease in FBS, weight and BFP in healthy adults. However, it also led to increased insulin resistance. (lines 53-74)
Introduction		
Background/rationale	2	At least, one billion of the total global population of Muslims abstain from eating or drinking from sunrise to sunset during the holy month of Ramadan. Adherence to the Ramadan fast causes radical changes in eating patterns, and the quality of ingested nutrients. The effects of Ramadan on anthropometric and metabolic indices are inconsistent across studies so the aim of the present study was investigating effect of Ramadan on anthropometric and metabolic indices (lines 79-99)
Objectives	3	In the present study we investigated changes in anthropometric indices, fasting blood sugar, serum lipid profiles, serum insulin and insulin resistance at three times (before, at the end of and one month after Ramadan) and food intake before and during Ramadan (lines 99-102.
Methods		
Study design	4	This study was an observational study (line 104)
Setting	5	Participants in the study were selected from five mosques in different districts of Kermanshah a province in west of Iran. Data were collected at three different times (1-7 days before, 1-4 days before the end and one month after Ramadan. (lines 107-113)
Participants	6	The study population consisted of people who fasted during the entire month of Ramadan. One hundred and sixty healthy men with no diagnosed disease who volunteered to participate in the study were selected from five mosques in different districts of the city. A list of eligible subjects was developed before the commencement of Ramadan. (lines 107-112)
Variables	7	Demographic data, including age, educational level and socioeconomic status were collected by a researcher-developed questionnaire. Dietary intake was assessed by a semi-quantitative food frequency questionnaire (FFQ). Body composition was measured by bio-impedance body analyser. 5-ml venous blood samples were taken from the participants at each time point. After separation of blood cells by centrifugation at 3000 rpm for 15

		min at 4°C, serum was stored with 0.1% Na_2-EDTA in the freezer at −40°C. Serum triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined quantitatively by enzymatic colorimetric kits (Pars Co., Iran) with an auto analyzer device (Technic on RA-XT, Ireland). FBS was measured according to a standard photometric method by an auto analyzer (RA1000-RAXT, Pars Co., Iran). Homeostatic model assessment-insulin resistance (HOMA-IR) and beta cell function (β) indices were calculated (lines 115-141)
Data sources/ measurement	8*	The FFQ's validity and reliability have been confirmed. This questionnaire consists of 168 foods with a standard portion size. The mentioned value for each food is based on recommended portion size. The questionnaire was only administered prior to and at the end of Ramadan. The amounts of macronutrients and energy intake were estimated at each of the time point. Weight, body mass index (BMI), waist-to-hip ratio, mass of body fat (BF), body fat percentage (BF%), lean body mass (LBM), soft lean mass and total body water (TBW) were measured by bio-impedance body analyser. Blood samples were obtained after 12-14 h fasting, at 8-9 am 2-5 days prior to Ramadan, at 6-8 pm on the 25-28th days of Ramadan, and at 8-9 am 28-30 days after Ramadan. After separation of blood cells by centrifugation at 3000 rpm for 15 min at 4°C, serum was stored with 0.1% Na2-EDTA in the freezer at −40°C. (lines 115-126)
Bias	9	It was not possible to blind people to the fact they were engaged in Ramadan fasting. Hence there could be a bias in their reporting as they were aware of the stage in the protocol they were at.
Study size	10	The study population consisted of people who fasted during the entire month of Ramadan. One hundred and sixty healthy men with no diagnosed disease who volunteered to participate in the study were selected from five mosques in different districts of the city (lines 108-110)
Quantitative variables	11	Dietary intake was assessed by a semi-quantitative food frequency questionnaire (FFQ). Body composition was measured by bio-impedance body analyser. 5-ml venous blood samples were taken from the participants at each time point. After separation of blood cells by centrifugation at 3000 rpm for 15 min at 4°C, serum was stored with 0.1% Na_2-EDTA in the freezer at −40°C. Serum triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL) were determined quantitatively by enzymatic colorimetric kits (Pars Co., Iran) with an auto analyzer device (Technic on RA-XT, Ireland). FBS was measured according to a standard photometric method by an auto analyzer (RA1000-RAXT, Pars Co., Iran). Homeostatic model assessment-insulin resistance (HOMA-IR) and beta cell function (β) indices were calculated (lines 115-141)
Statistical methods	12	For comparison of the quantitative variables, repeated measurement test was conducted for three parameters and Post Hoc (Tukey test) was used for two of these. $P<0.05$ was considered the level of significance. Correlation was

used to investigate the association between food groups and anthropometric and biochemical parameters.
(lines 142-147)

Continued on next page

Results		
Participants	13*	One hundred and sixty healthy men with no diagnosed disease who volunteered to participate in the study were selected from five mosques in different districts of the city. A list of eligible subjects was developed before the commencement of Ramadan. The selected individuals were matched for age and the socioeconomic status in different districts. Data were collected at three different times (1-7 days before, 1-4 days before the end and one month after Ramadan had ended). (lines 108-110)
Descriptive data	14*	See table 1
Outcome data	15*	See table 1 and 2
Main results	16	During Ramadan individuals ate less food (in all food categories) and lost weight. There was an improvement in most health indicies but insulin resistance increased. These changes were transient and returned towards baseline after Ramadan was over. (lines 149-194)
Other analyses	17	There was no subgroups in this study
Discussion		
Key results	18	Ramadan fasting generates some improvement in health markers but these changes are transitory (lines 197-255).
Limitations	19	The study only included males and hence the impacts of Ramadan fasting on females could not be evaluated. Moreover, like many studies performed in strict muslim countries it was not possible to have a control group that did not adhere to Ramadan restrictions (lines 256-266)
Interpretation	20	Ramadan fasting may lead to some beneficial effects such as a decrease in FBS, weight and BFP in healthy adults. However, it also led to increased insulin resistance. The effects were all transitory (lines 268-272)
Generalisability	21	There is no reason to believe these results are not widely applicable.
Other information		
Funding	22	The current study was sponsored by the Vice Chancellery of Research & Technology Affairs at Kermanshah University of Medical Sciences, Kermanshah – Iran (Grant no. 91058). (lines 276-277)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.