The effects of Ramadan fasting on activity and energy expenditure

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Short running head: "energy expenditure in Ramadan"

Abbreviations

ANCOVA: Analysis of co-variance

APE: atom percent enrichment

BMI: body mass index

CV: coefficient of variation

DLW: doubly labeled water

GLP1: Glucagon-like peptide-1

k_o: rates at which Oxygen18 is flushed from the body

k_d: rate at which Deuterium is flushed from the body

N_o: isotope dilution space calculated using oxygen18

N_d: isotope dilution space calculated using deuterium

N_d/N_o: dilution space ration

R^2: coefficient of determination

rCO_2: CO_2 production rate

RMR: resting metabolic rate

RQ: respiratory quotient

SD: standard deviation

TEE: total energy expenditure

TEF: thermic effect of food

VO_2: volume of oxygen

VCO2: volume of carbon dioxide

AFM: Absolute Fat Mass

FFM: Fat Free Mass

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ABSTRACT

Background: Fasting during the month of Ramadan entails abstinence from eating and drinking between dawn and sunset and a major shift in meal times and patterns with associated changes in several hormones and circadian rhythms; whether there are accompanying changes in energy metabolism is unclear.

Objective: We have investigated the impact of Ramadan fasting on resting metabolic rate (RMR), activity and total energy expenditure (TEE).

Design: Healthy non-obese volunteers (*n*=29, *n* female= 16) fasting during Ramadan were recruited. RMR was measured using indirect calorimetry (Cosmed Quark RMR). In subgroups of participants, activity (*n*=11, *n* female =5) and TEE (*n*=10, *n* female =5) in free-living conditions were measured, using ActiGraph GT9X accelerometers and the doubly-labeled water technique respectively. Body composition was measured using bio-electrical impedance (Seca 515). Measurements were repeated after a wash-out period of between 1-2 months after Ramadan. Non-parametric tests were used for comparative statistics.

Results: Ramadan fasting did not result in any change in RMR (1365.7 ± 230.2 v 1362.9 ± 273.6 kcal/day for Ramadan and post-Ramadan respectively, *P*=0.713, *n*=29). However, controlling for the effects of age, sex and body weight, RMR was higher in the first week of Ramadan than in subsequent weeks. During Ramadan, total number of steps walked were significantly lower (*n*=11, P=0.001), while overall sleeping time was reduced and different sleeping patterns were seen. TEE did not differ significantly between Ramadan and post Ramadan (2224.1 ± 433.7 v 2121.0 ± 718.5 Kcal/day for Ramadan and post-Ramadan, P=0.7695, n=10).

Conclusions: Ramadan fasting is associated with reduced activity and sleeping time, but no significant change in RMR, or TEE. Reported weight changes with Ramadan in other studies are more likely to be due to differences in food intake.

INTRODUCTION

The practice of fasting during Ramadan is observed by many of the world's over 1.6 billion Muslim population for a full lunar month every year (1). As such, most healthcare professionals including physicians and dieticians are likely to see patients needing advice on medical and nutritional aspects of the Ramadan fast. The fast entails abstinence from eating and drinking between dawn and dusk for a whole lunar month (29-30 days). It represents a major shift from established routines and "normality". In particular, timing and composition of meals change (2): an early breakfast is taken just before dawn (suhoor) and lunch is omitted. The fast is broken at dusk when the main meal (iftar) is taken. Sleeping times and patterns also change (3) to allow the morning meal to be consumed before dawn. Missing lunch, and the long gap between major meals affect appetite (4), hormonal responses to food, and aspects of energy and glucose metabolism. In particular, omitting breakfast has been shown to be associated with a lower exercise-induced thermogenesis (5) and in people with diabetes, causes blunting of insulin and GLP1 response to food (6).

With the longer gaps between the two major meals of the day, reduced physical activity and exercise during the day might be expected. These changes have implications on several biological processes; alterations in several hormonal and metabolic processes including circadian rhythms (7), serum cortisol (8), thyroid function (7), plasma leptin (9), adiponectin (10) and neuropeptide Y (11) in health and disease have been previously reported. Ramadan fasting has been associated with variable weight changes, ranging from modest weight gain (12) to weight neutrality (13) and weight loss (14), with a reported reduction in total calorie intake in some (15), but not all (13) populations. Weight loss observed in some subjects tends to be regained shortly after Ramadan (14).

Investigating energy fluxes in the context of the Ramadan fast could provide a better evidence base for managing patients, particularly those in whom weight management or glycemic control of diabetes is important. The Ramadan fast is also a useful model for investigating the impact of major deviations from meal patterns to physiology and weight changes. While several studies have previously explored dietary changes with Ramadan fasting in various populations, there have been few attempts at quantifying different aspects of energy expenditure during Ramadan. In the current study, we have assessed resting metabolic rate (RMR), physical activity and 'free-living' total energy expenditure (TEE).

METHODS

Participant Screening

Participants were screened clinically, and using standard hematological and biochemical tests and excluded if they had conditions that could influence metabolic rate including thyroid dysfunction, anemia, renal and liver dysfunction and active infection.

Study Design

This study was approved by the Medical Research Ethics Committee of the Imperial College London Diabetes Centre and was conducted in 2015-2016. Healthy non-obese participants intending to fast during the month of Ramadan were recruited (**Supplemental Figure 1**). Sample size calculation was based on our pilot study which indicated a standard deviation for resting metabolic rate of 230 kcal/day in the context of Ramadan fasting. The current study was powered to detect a mean change of 100-150 kcal/day between the Ramadan and post-

Ramadan periods, using a paired crossover design. With α=0.05 and β=0.8, we required 35 participants to detect a change of 100 kcal/day, or 16 to detect a change of 150 kcal/day. All participants had anthropometric and RMR measurements during Ramadan and within 2 months after Ramadan. In addition, in a subgroup of participants, RMR repeat measurements were performed at week 1, week 2, week 3 and week 4 of Ramadan. TEE and activity were measured in subgroups of subjects in both Ramadan and post-Ramadan periods.

Anthropometric Measurements

A trained nurse assessed all participants. Anthropometric measures including weight, height, body composition by bio-electrical impedance (BIA-Seca mBCA 515, Hamburg, Germany), were assessed and recorded before every RMR measurement (during and after Ramadan). Body mass index (BMI) was calculated as weight/height^2.

RMR Measurements

Resting metabolic rate was measured by indirect calorimetry, using the ventilated hood technique (COSMED Quark RMR, Rome, Italy) and following best practice recommendations (16). The device gives values for Respiratory Quotient (RQ) as well as RMR. Measurements were performed (Ramadan and post-Ramadan) after a minimum of 9 hours of fasting from the previous meal including complete abstinence from nicotine and caffeine. In the majority of participants this was between 2 and 3 PM (as suhoor was taken around 4 AM). Some participants had a late-night meal, rather than a traditional suhoor. In such cases, measurements were done at least 9 hours following the last meal which might have been around 10 AM. Participants were also asked to refrain from exercise on the day of

RMR measurements. Calibration of the flowmeter and the gas analyzers were performed regularly and according to manufacturers' instructions. Measurements were performed for a minimum of 20 minutes at room temperature with subjects in light clothing and lying supine, with a ventilated hood over their head and upper body. Data of the first 5 minutes was discarded and only subsequent measurements that had valid steady-state conditions (≤10% coefficient of variation (CV) in RMR, VO_2 and VCO_2) were included.

Activity Levels

ActiGraph GT9X (ActiGraph LLC, Pensacola, FL, USA) tri-axial accelerometers were used to measure physical activity in a subgroup of participants who were encouraged to maintain their normal daily activities. The participants wore the accelerometer on their non-dominant wrist (left). ActiLife 6 software was used for initialization and data upload. A sample rate of 30 Hz was chosen with the accelerometers set to 'blind' mode, to avoid the influence of accelerometer data on activity. Participants wore the device for at least 7 days. Individual activity data was considered valid if the accelerometer was worn for at least 7 days, wearing days included at least 2 weekend days, and the accelerometer was worn for at least 10 hours per day (17). Wearing time validation was performed using the Choi wear time validation tool (18) in the ActiLife software.

Total Energy Expenditure (TEE) Measurement

The doubly labeled water (DLW) method was used for measuring free-living energy expenditure over a period of 4-20 days (19, 20). The technique is based on the exponential disappearance of the stable isotopes ^{2}H and ^{18}O from the body after ingestion of a bolus dose of water labeled with both isotopes. These isotopes mix with the hydrogen and oxygen in body water within a few hours. As energy is expended, ^{2}H is lost as water and ^{18}O as both water and CO_2. After correction for isotopic fractionation, the excess disappearance rate of ^{18}O relative to ^{2}H is a measure of CO_2 production rate, which in turn can be used to estimate total energy expenditure by using a modified Weir's formula (21) based on the CO_2 production rate (rCO_2) and respiratory quotient (RQ- measured by indirect calorimetry). During, and 1-2 months after Ramadan, participants received a dose of DLW based on body size (44-88 g) to match body water enrichment. During Ramadan, the DLW dose could only be administered after iftar and these were taken at patient's own home after careful weighing. After Ramadan, the dose could be taken during the day and under observation. The timings were recorded accurately and taken into account when isotope disappearance calculations were made. The dose enrichment was 10.47APE 18oxygen and 4.57 APE 2hydrogen. A pre-dose urine sample was collected to assess baseline isotope enrichments. The dose was administered between 7-11 pm (after iftar) during Ramadan. Post-Ramadan DLW dose was taken during the day; ingestion times and actual dose taken were recorded. Urine samples were then collected daily from day 1 (day of DLW intake) up to day 14. Urine samples were aliquoted into 2ml cryotubes and stored at -80^{o}C until analysis. The samples were sealed into capillary tubes, which were then vacuum distilled to collect the water, which was then analyzed using an off-axis laser spectroscopy liquid water isotope analyzer (22, 23). Samples were run alongside international laboratory standards of known enrichment (24) for standardization. To derive the isotope dilution spaces (No and Nd for oxygen and hydrogen respectively) and the isotope washout constants (ko and kd respectively for oxygen and hydrogen) we log converted the excess isotope enrichments and then fitted linear relationships to the resultant linearized exponentials. The back extrapolated intercepts were used to evaluate the dilution spaces. Isotope enrichments were converted to daily CO_2 production using the modified two pool model equation A6 from Schoeller et al as recommended for humans (25, 26).

Data Analysis

All quantitative data were checked by at least two investigators and entered into a spreadsheet. SPSS version 20.0 was used for statistical analyses. Non-parametric tests were used to compare Ramadan and post-Ramadan values for RMR, Activity parameters and TEE.

RESULTS

Resting Metabolic Rate and Ramadan Fasting

A total of 29 individuals, 13 males and 16 females, aged 19 to 52 years completed the study and had full paired (Ramadan and post-Ramadan) RMR data. All participants were non-obese (15 with $20<BMI\leq 25$ and 14 with $25<BMI<30$, with a mean BMI of 25.8 ± 2.2 and 23.6 ± 3.1 kg/m^2 in males and females, respectively, **Table 1**). Hours of fasting from last meal to RMR measurement during Ramadan and post-Ramadan periods were similar (Median 10.3 and 10.5 hours respectively). Mean RMR was 1365.7 ± 230.2 kcal/day for Ramadan and 1362.9 ± 273.6 kcal/day for post-Ramadan; the difference was not statistically significant (*P*=0.713). Furthermore, no significant change in RMR/FFM was found between Ramadan and post-Ramadan periods (30.1 ± 9.2 kcal/day/kg during Ramadan v 30.1 ± 10.0 kcal/day/kg after Ramadan; n=29; P-value=0.779). However, RQ during and after Ramadan were significantly different (n=29; P<0.0001) with a lower value of 0.80 ± 0.06 during Ramadan compared to 0.88 ± 0.05 after Ramadan.

Resting Metabolic Rate in Early and Late Ramadan

The effect of the duration of Ramadan fasting on resting metabolic rate was analyzed by including the week of measurement as categorical variable in multiple linear regression in all individuals in whom a measurement of RMR was performed at least in the first week (n=19) of Ramadan (**Table 2**). The number of subjects who had measurements performed in week 2, 3, 4 and after Ramadan is shown in Table 2. Overall the model fit was good with an adjusted $R^2 = 0.70$. Controlling for the effects of sex, age, weight, and number of hours since suhoor, RMR was significantly lower in the second, third and fourth weeks of Ramadan than in the first week of Ramadan [β= -138.62 (-255.45 , -21.8); p<0.05; β= -155.55 (-274.83 , -36.27); p<0.05; β= -223.84 (-373.33 , -74.35); p<0.01, respectively].

Activity

Paired measurements of activity levels, measured in number of steps, were obtained from 11 individuals, 6 males and 5 females, during Ramadan and 2 months after Ramadan. The total number of steps per day (converted from activity counts using ActiGraph proprietary software) during Ramadan (9950 ± 1152) was significantly lower (P=0.001) than the total number of steps after Ramadan (11353 ± 2054; **Table 1**). Activity levels had a unique pattern through timings at other times of the day in Ramadan compared to after Ramadan (**Figure 1**). At night (00:00 – 06:00), activity levels were higher in Ramadan compared to post-Ramadan

(P=0.001). In contrast, in the morning (06:00 – 12:00) and afternoon (12:00 – 18:00), activity levels were lower in Ramadan compared to post-Ramadan (P=0.001 and P=0.002 respectively). Unlike other timings of the day, differences in activity levels in the evening (18:00 – 00:00) in Ramadan compared to post-Ramadan were not observed (P=0.70). During Ramadan, three main patterns of activity could be identified; some subjects stayed awake after iftar and retired to bed after suhoor, waking up later in the morning (**Supplemental Figure 2A**). Some others broke their sleep; retiring to bed before midnight and wake up for eating suhoor, retiring for a second time around 4.30 in the morning for a brief period of sleep, and waking up for a second time for going to work (**Supplemental Figure 2B**). We also found some subjects who did not sleep (**Supplemental Figure 2C**) at all during the night; some participants made up for this in an afternoon nap (not shown). **Figure 2** shows the cumulative median activity curve for all participants.

Total Energy Expenditure and Ramadan Fasting

Ten participants (5 males and 5 females, **Table 1**) completed doubly-labelled water (DLW) experiments. Typical washout curves for an individual during and post-Ramadan are presented in **Supplemental Figure 3**. Linearity in the log converted curves was excellent, with R^2 averaging 0.995 (SD = 0.007) for the ^{18}oxygen curves and 0.994 (SD = 0.006) for the ^{2}hydrogen curves. Individual estimates of N_o, Nd, ko and kd are presented in supplementary materials **Supplemental Table 1**. The dilution space ratio Nd/No averaged 1.036 (SD = 0.011) during Ramadan, and 1.027 (SD = 0.008) post-Ramadan. The isotopic washout ratio was 1.226 (SD = 0.060) during Ramadan and was 1.213 (SD = 0.060) post-Ramadan. There was no significant difference in TEE during and post-Ramadan (2224.1 ± 433.7 v 2121.0 ± 718.5 kcal/day; P=0.7695, **Table 2**). Analysis of co-variance (ANCOVA) showed no significant difference between Ramadan and post-Ramadan regression lines-**Figure 3** (ANCOVA; t = 0.35, P = 0.727); the main factor influencing TEE was body weight (ANCOVA; t = 2.72, P = 0.015).

DISCUSSION

Ramadan is unique and differs from both prolonged and short term starvation. The former decreases RMR (27-29), whereas short-term starvation (up to 4 days) may increase RMR, and this has been attributed to a rise in norepinephrine levels (30). We have investigated Ramadan as a separate entity that may be of relevance to the growing trend to prescribe 'intermittent fasting' for therapeutic weight loss. There are very few studies of energy expenditure in this context (31, 32). El Ati and colleagues (33) investigated the effect of Ramadan fasting on anthropometric and metabolic variables in healthy Tunisian Muslim women; despite marked changes in food intake during Ramadan, there were no significant changes in body weight, body composition or resting energy expenditure. More recently, McNeil and colleagues (34) conducted a study to examine the effect of Ramadan fasting on variations in eating behavior, appetite ratings, satiety efficiency, and energy expenditure in 20 Muslim participants. They reported no significant differences in anthropometric measures, before Ramadan compared to during Ramadan, and after Ramadan, and no significant difference in RMR, thermic effect of food (TEF) and TEE before and after Ramadan. Notably however, in this study, RMR, TEF and TEE were not measured during Ramadan. Similarly, Harder-Lauridsen and colleagues found only minor differences in body mass index, and no significant change in body composition in fasting men before and Ramadan (35). In spite of the lack of significant change in RMR with Ramadan fasting, our study has shown a lower RQ during Ramadan compared to post-Ramadan period, a finding similar to that reported by El Ati et al (33) and suggestive of a shift towards fat rather than carbohydrate as source of fuel during Ramadan.

We used the DLW technique to measure free-living 24-hour energy expenditure in the context of Ramadan fasting. DLW is a well-validated technique to measure free living 24-hour energy expenditure (36-39). We have also explored the other important aspects of energy expenditure, namely RMR and physical activity. Resting metabolic rate was significantly lower in mid and late Ramadan of fasting compared to the first week of Ramadan. It has previously been demonstrated that acute starvation causes an increase in resting metabolic rate in association with increasing serum norepinephrine (39), raising the possibility that the same phenomenon may also occur in the context of intermittent fasting. Our results suggest however, that this may not be the case and that after a few days of intermittent fasting there is some metabolic adaptation with a reduction in RMR. This may be of importance in some diets that promote skipping and spacing meals and may be an explanation for difficulties in weight loss and weight loss maintenance after the first few days of such dietary practices. Ramadan is often seen as a period of relative inactivity and as such we had hypothesized that activity energy expenditure and thus 24-hour energy expenditure should also be reduced with Ramadan fasting. We used accelerometers to measure activity in a smaller group of individuals and showed a trend to support the hypothesis that people are less physically active during Ramadan fasting. Our study did not show a significant difference in TEE in Ramadan and post-Ramadan periods (2224 v 2121 kcal/day). Our data has also indicated inter-individual variability in activity patterns and how they change during Ramadan. In most participants, we have shown a shift in the timing of activity from mainly day time before Ramadan to mainly night-time during Ramadan. Many of our participants seemed to have much reduced sleeping times during Ramadan. In our group of subjects, we were also able to demonstrate different patterns of activity during Ramadan, confirming a reduction in sleeping time and broken sleep in majority of subjects. This reduction in sleep time may offset any reductions in expenditure produced by lower activity and lower RMR leading to the non-significant effects on TEE.

In common with other Ramadan studies, we faced reluctance from potential subjects in participating in the study who did not wish to deviate from their normal Ramadan routines. Many of our initial potential volunteers found wearing accelerometer wristbands and/or multiple urine specimens during Ramadan unacceptable. Other limitations of our study included inherent methodological issues. We have used ActiGraph wristbands to monitor activity. These accelerometers are very useful in monitoring steps, but other activities such as cycling or prayers may not be captured so may underestimate activity. We observed considerable variability in minute to minute RMR records during indirect calorimetry; as a result and to maximize accuracy, we had to repeat the procedure in some subjects.

We made no attempts at investigating two specific aspects of energy balance in the context of Ramadan: food intake and thermic effect of food. The former has been the subject of several previous studies (15, 40, 41), with most, but not all finding an overall reduction in food intake; a change in composition of food with an increase in carbohydrate intake has also been shown, although these are in part determined by local culture and food preference.

Our findings may be of relevance to weight and weight loss with Ramadan. Several studies have investigated the impact of Ramadan on body weight and metabolic health. Results have been inconsistent, largely due to differences in dietary habits, gender, age, and ethnicity. A recent systematic meta-analysis of 30 self-controlled cohort studies (42) have reported the beneficial effects of Ramadan on metabolic status, including lower blood glucose concentrations, improved lipid profiles, and reduced body weight. Our results indicate that the reduction in activity in Ramadan is not universal and that some people were actually more physically active during this period. It is therefore possible to be as active in Ramadan as other months of the year, and this should be emphasized and encouraged. Ramadan has been associated with increased insulin resistance and this would make the continued physical activity even more important.

In conclusion our results suggest that Ramadan is associated with reduced physical activity, and reduced RMR after the first week of fasting. These reductions in components of the energy expenditure however did not translate into an overall reduction in TEE. This was possibly because there was also a large reduction in time spent sleeping which might offset the energy savings from reduced activity and RMR. Ramadan results in profound disruptions of daily activity patterns but overall energy balance does not appear greatly affected. Our results are of relevance to millions of Muslims who observe Ramadan fasting and may have implications to dietary restriction programs that promote skipping and spacing meals.

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Authors Contributions:

NL designed the research, analyzed data, wrote the manuscript and had primary responsibility for final content

IS conducted the study, and analyzed data, contributed to manuscript.

BA contributed to manuscript

CH conducted DLW measurements and contributed to data analysis and the manuscript

AB contributed to data analysis and writing of the manuscript

NF edited and contributed to the manuscript

JS edited and contributed to manuscript

MTB edited the manuscript

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Table 1:Patient characteristics at baseline and energy metabolism parameter during and after Ramadan fasting in the study population.

			N	Age [years]	BMI [kg/m2]	Weight [kg]	AFM [kg]	FFM [kg]	RMR [kcal/day]	Total Steps per day	TEE [kcal/day]
All	Ramadan (Baseline)	All	29	33.3 ± 8.7	24.6 ± 2.9	68.5 ± 12.3			1365.7 ± 230.2	NA	NA
		Males	13	35.8 ± 7.1	25.8 ± 2.2	78.1 ± 7.3	19.7 ± 3.9	58.9 ± 6.8	1538.8 ± 215.2	NA	NA
		Females	16	31.4 ± 10.1	23.6 ± 3.1	60.7 ± 9.8	21.8 ± 7.4	39.5 ± 3.2	1225.0 ± 121.5	NA	NA
	Post-Ramadan	All	29		24.6 ± 2.9	68.3 ± 12.5			1362.9 ± 273.6	NA	NA
		Males	13		26.0 ± 2.1	78.1 ± 7.4	19.5 ± 6.4	59.3 ± 17.5	1572.1 ± 267.1	NA	NA
		Female	16		23.5 ± 3.1	60.3 ± 9.9	21.1 ± 7.5	39.7 ± 3.3	1192.9 ± 117.9	NA	NA
Activity experiment	Ramadan (Baseline)	All	11	34.1 ± 7.3	24.4 ± 3.1	69.6 ± 13.7			NA	9950 ± 1152	
		Males	6	36.3 ± 7.5	25.4 ± 1.4	77.9 ± 7.1	21.0 ± 4.4	57.4 ± 3.8	NA	9710 ± 1135	
		Females	5	31.4 ± 6.2	23.3 ± 4.2	59.6 ± 13.6	20.6± 11.3	39.5 ± 3.1	NA	10239 ± 1192	
	Post-Ramadan	All	11		24.9 ± 3.1	71.1 ± 14.4			NA	11353 ± 2054	
		Males	6		26.1 ± 1.4	80.4 ± 7.2	22.4 ± 3.6	58.5 ± 4.7	NA	10987 ± 2156	
		Females	5		23.4 ± 3.9	60.1 ± 13.3	20.2 ± 11.5	40.4 ± 2.6	NA	11792 ± 1864	
DLW experiment	Ramadan (Baseline)	All	10	32.6 ± 11.3	25.0 ± 3.2	70.5 ± 13.2			1387.0 ± 249.9	NA	2224.1 ± 433.7
		Males	5	37.6 ± 14.0	26.3 ± 1.8	79.6 ± 5.6	21.0 ± 3.7	59.1 ± 3.9	1597.0 ± 147.3	NA	2538.8 ± 257.7
		Females	5	27.6 ± 5.6	23.8 ± 4.0	61.4 ± 12.3	22.3 ± 9.7	39.6 ± 3.6	1177.0 ± 92.3	NA	1909.4 ± 330.5
	Post-Ramadan	All	10		24.8 ± 3.2	69.6 ± 13.1			1355.2 ± 255.7	NA	2121.0 ± 718.5
		Males	5		25.9 ± 1.9	78.4 ± 6.0	20.3 ± 3.6	58.6 ± 3.4	1539.2 ± 214.6	NA	2534.9 ± 716.2
		Females	5		23.6 ± 4.0	60.9 ± 12.5	21.4 ± 10.1	40.0 ± 3.4	1171.2 ± 127.9	NA	1707.1 ± 469.6

Subgroup analysis on participants of total energy expenditure and activity monitoring study are shown. RMR measurements were conducted on all subjects. Activity (using accelerometers; n=11) as steps/day and total energy expenditure (TEE-using doubly labelled water technique; n=10) were measured on two separate subgroups. Apart from activity ($P<0.001$), no significant difference between Ramadan and post-Ramadan values were seen. AFM= absolute fat mass, FFM= fat free mass, RMR=resting metabolic rate, TEE=total energy expenditure, DLW=Doubly Labeled Water.

Table 2: Resting metabolic rate in early and late Ramadan

Covariate	N	Estimate (95% CI)	Std. Error	Sig.
Intercept		741.91 (372.05 , 1111.78)	183.40	<0.001
Week 2	9	-138.62 (-255.45 , -21.8)	57.93	0.021
Week 3	9	-155.55 (-274.83 , -36.27)	59.15	0.012
Week 4	5	-223.84 (-373.33 , -74.35)	74.13	0.004
Post-Ramadan	16	-46.30 (-140.6 , 47.99)	46.76	0.328
Time since Suhoor (h)		-4.85 (-24.46 , 14.77)	9.72	0.621
Male sex		203.89 (95.02 , 312.77)	53.99	<0.001
Age (years)		-0.73 (-5.7 , 4.24)	2.46	0.768
Weight (kg)		10.10 (6.22 , 13.97)	1.92	<0.001

The effect of the duration of Ramadan fasting on resting metabolic rate was analyzed by including the week of measurement as categorical variable in multiple linear regression. Resting metabolic rate is significantly lower in the second, third and fourth weeks of Ramadan than during the first week, when controlling for time since suhoor, sex, age and weight.

Figure 1

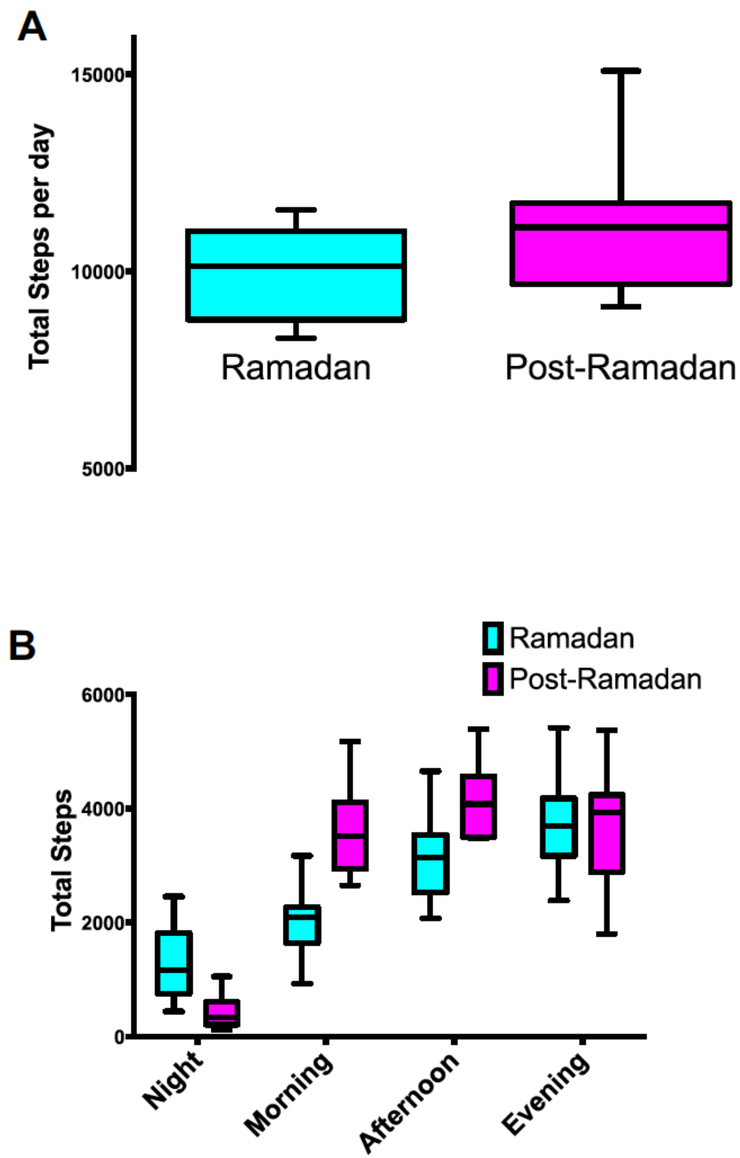

A
15000
10000
5000
Total Steps per day
Ramadan
Post-Ramadan
B
Ramadan
Post-Ramadan
6000
4000
2000
0
Total Steps
Night
Morning
Afternoon
Evening

Figure 2

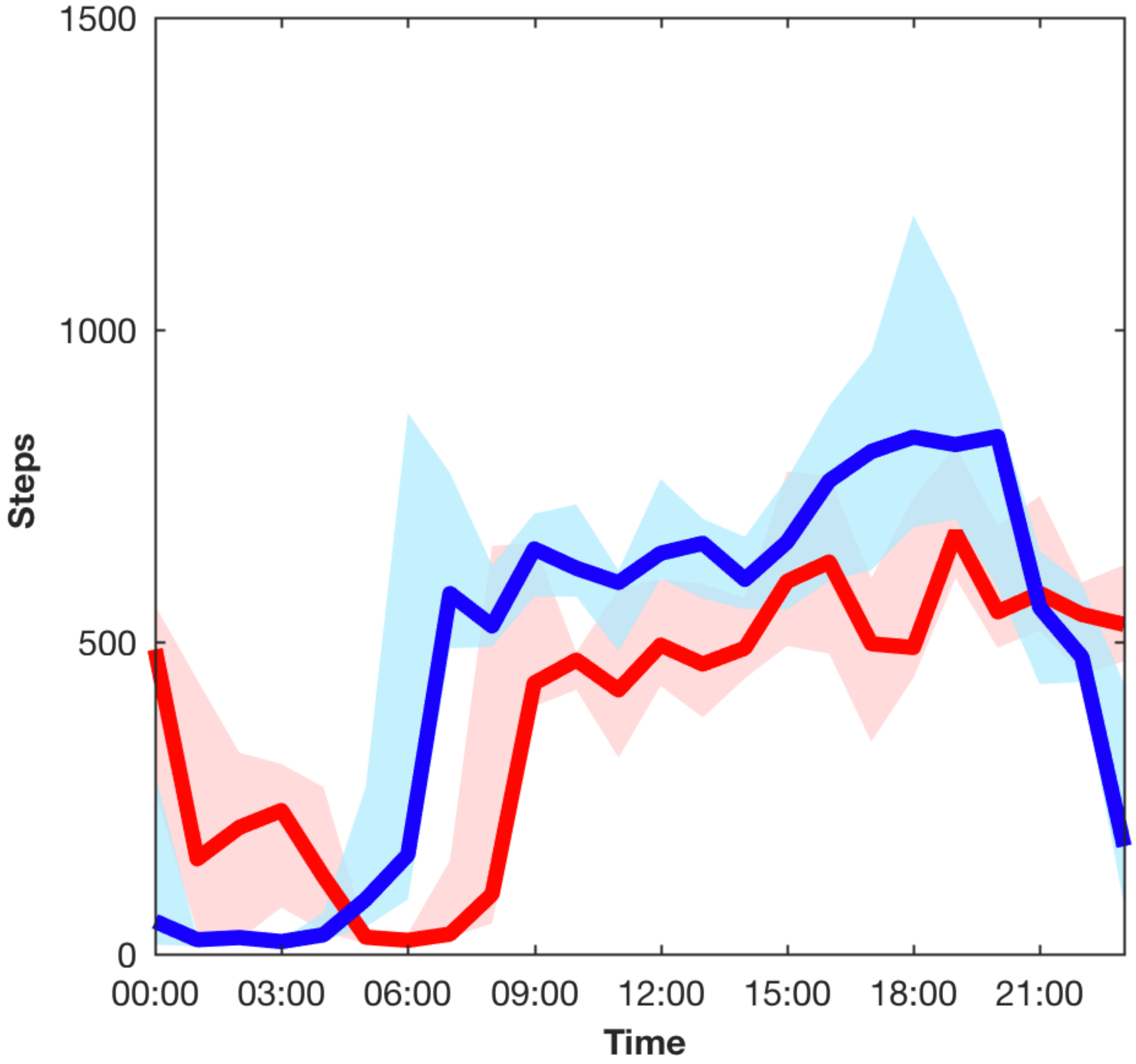
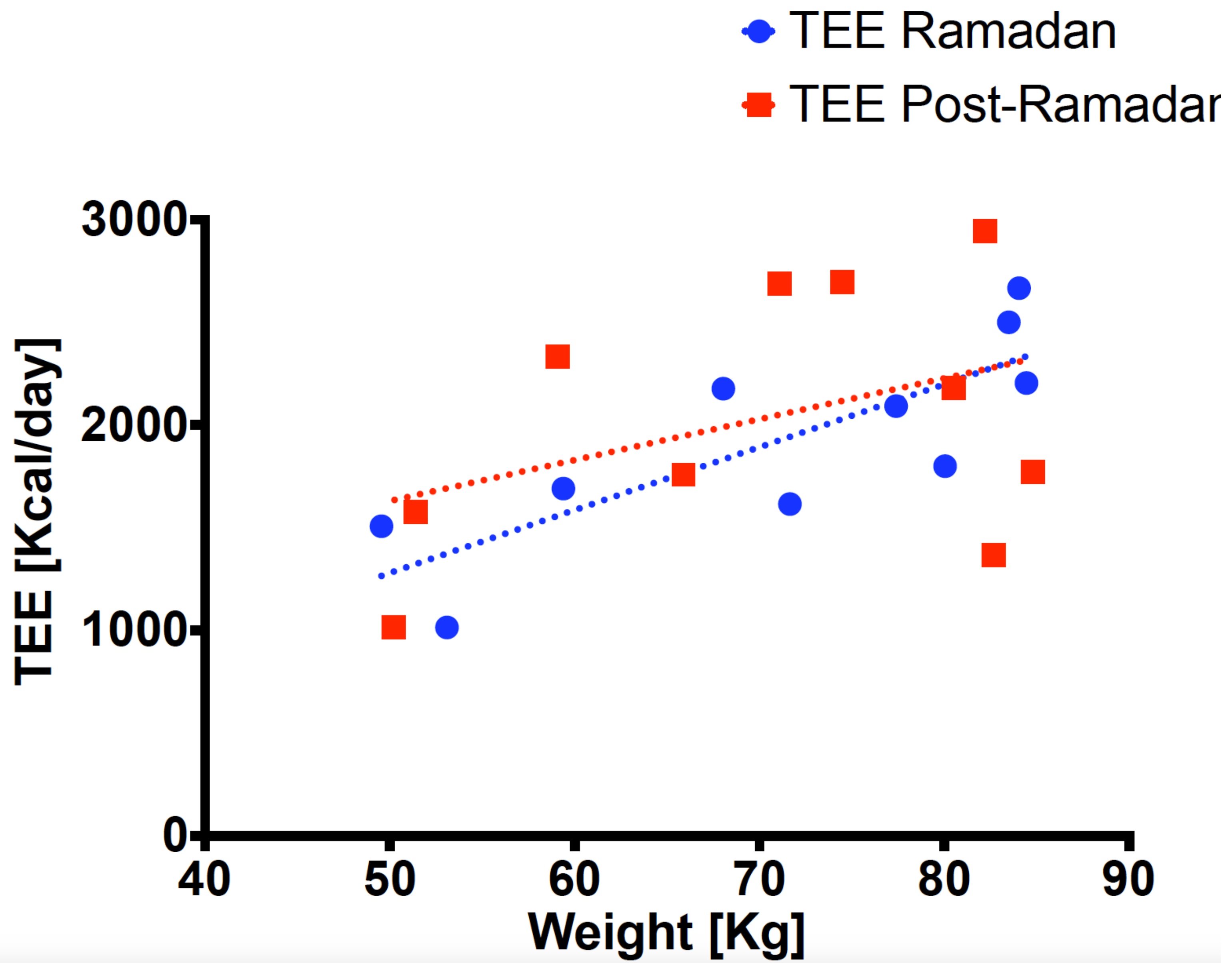

1500
1000
500
0
Steps
00:00
03:00
06:00
09:00
12:00
15:00
18:00
21:00
Time

Figure 3



TEE Ramadan
TEE Post-Ramadar
TEE [Kcal/day]
3000
2000
1000
0
40
50
60
70
80
90
Weight [Kg]

LEGENDS FOR FIGURES

Figure 1: The effect of Ramadan fasting on activity; (A) box plot of total number of steps/day in 11 participants during and after Ramadan; (B) box plot of total number of steps per night, morning, afternoon and evening in Ramadan and after Ramadan in 11 participants. Comparisons have been made using Wilcoxon signed-rank test. Total number of steps per day (9950 ± 1152 vs. 11353 ± 2053, P=0.001), activity in the morning (1974 ± 583 vs. 3606 ± 715, P=0.001) and afternoon (3193 ± 783 vs. 4164 ± 670, P=0.002 were significantly lower during Ramadan compared to after Ramadan, whereas nocturnal activity was higher during Ramadan (1261 ± 629 vs. 416 ± 279, P=0.001). There was no significant difference in activity level in the evenings between Ramadan and post-Ramadan periods.

Figure 2: 24-hour activity profiles (number of steps per hour) during (red) and after Ramadan (blue) represented as overall median 24-hour profile for all study participants (n=11). Solid lines represent median; highlighted areas represent the 25 and 75 percentiles of all measurement days. Cumulative pattern for all participants indicating the dominant tendency to stay awake during the night and sleep after suhoor, retiring to bed around midnight and waking up very early in the morning. There is more activity at night during Ramadan; after Ramadan, there is more activity during the day.

Figure 3: The correlation between TEE and weight during and after Ramadan in 10 participants. TEE, Total Energy Expenditure. There was no significant difference between Ramadan and post-Ramadan regression lines (ANCOVA; $t = 0.35$, $P = 0.727$); the main factor influencing TEE was body weight ($t = 2.72$, $P = 0.015$)

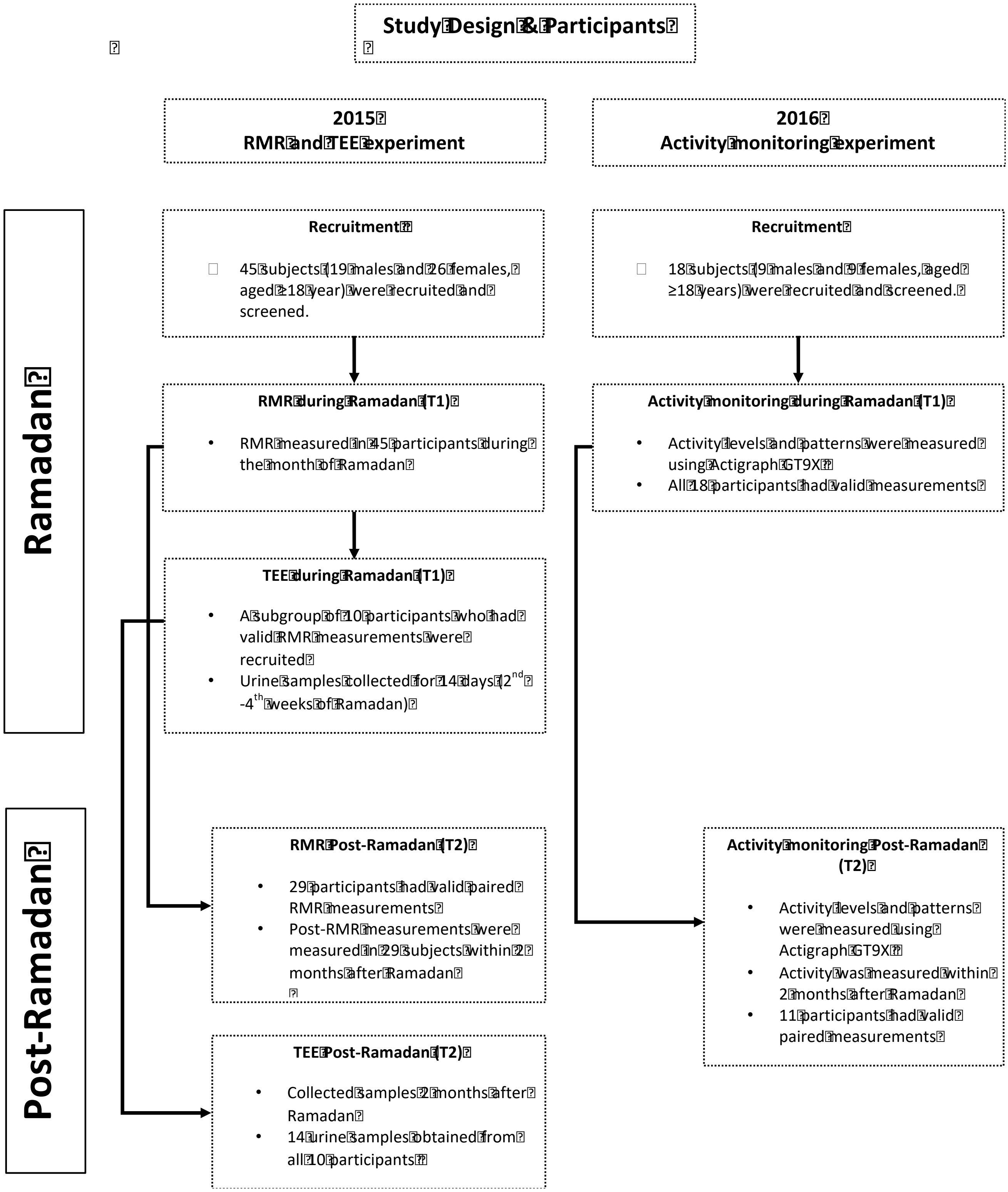
Online Supplemental Material

Supplemental Table 1: Daily energy expenditure measured using the Doubly Labelled Water technique during and after Ramadan.

	During Ramadan						Post Ramadan					
subject	BM (kg)	K_o	K_d	N_o (mols)	N_d (mols)	DEE [Kcal/day]	BM (kg)	K_o	K_d	N_o (mols)	N_d (mols)	DEE [Kcal/day]
1	53.1	0.0038	0.0031	1027.27	1073.03	1015.0	51.4	0.0041	0.0031	1314.26	1366.44	1577.4
2	84.1	0.0037	0.0028	2201.75	2293.38	2667.0	82.7	0.0037	0.0032	2397.20	2466.38	1367.1
3	49.6	0.0063	0.0054	1423.76	1471.11	1508.1	50.2	0.0064	0.0057	1493.34	1537.09	1018.1
4	83.5	0.0040	0.0031	2066.65	2160.38	2499.6	84.8	0.0035	0.0029	2236.90	2303.53	1773.4
5	80.1	0.0049	0.0041	1720.24	1772.11	1799.5	80.5	0.0072	0.0061	1597.76	1654.00	2182.1
6	68.1	0.0050	0.0040	1823.56	1917.95	2177.0	65.9	0.0052	0.0043	1759.20	1783.95	1759.0
7	71.7	0.0038	0.0031	1918.50	1947.43	1615.9	71.1	0.0048	0.0037	1989.23	2042.14	2688.8
8	84.5	0.0049	0.0042	2367.38	2427.68	2205.4	82.2	0.0062	0.0052	2385.23	2459.14	2944.5
9	59.4	0.0049	0.0041	1905.60	1980.81	1691.5	59.1	0.0061	0.0050	1866.39	1901.85	2332.6
10	77.4	0.0058	0.0051	2489.74	2576.20	2092.9	74.5	0.0040	0.0032	2714.57	2767.49	2695.9

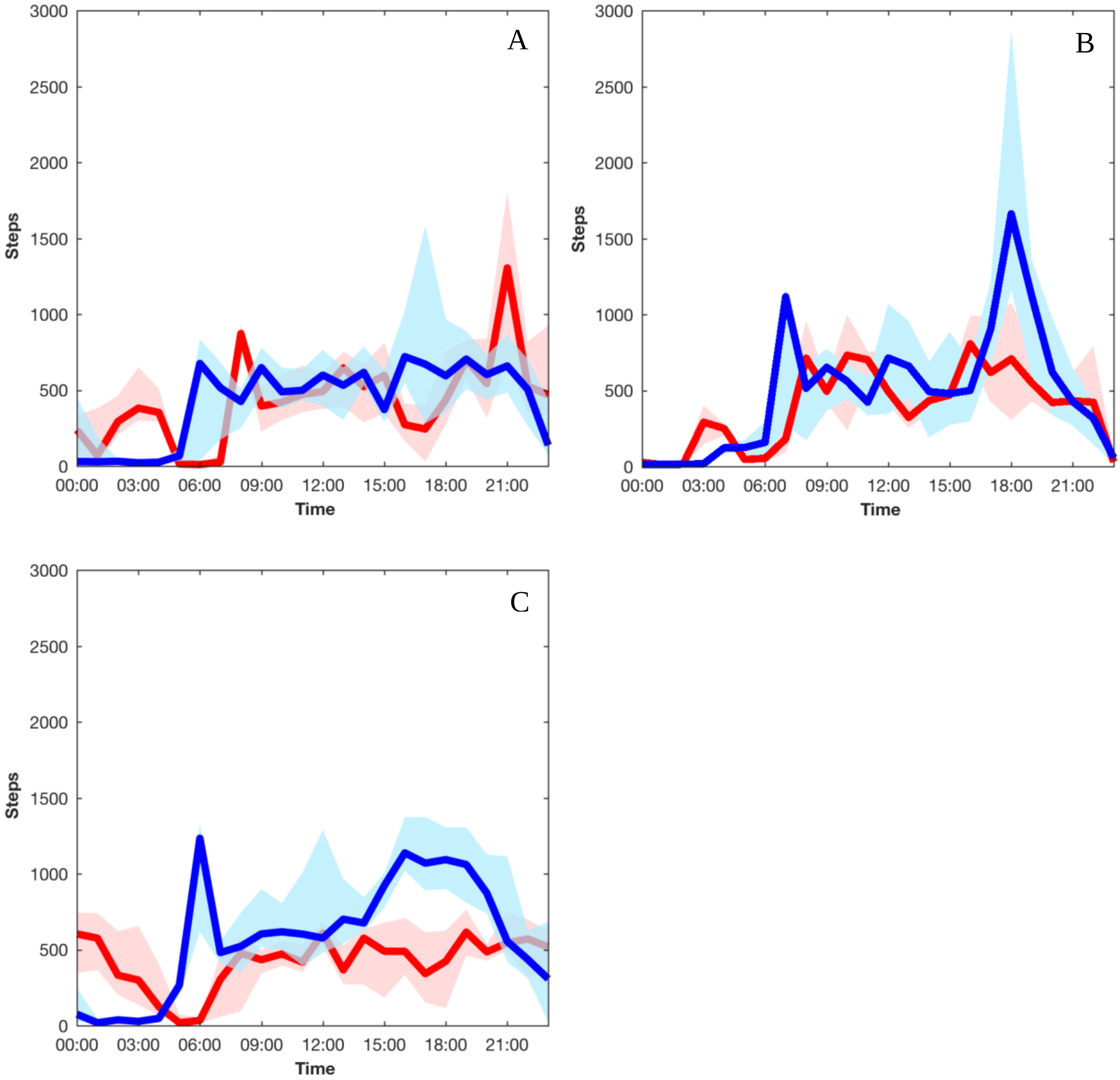
Individual estimates of N_o, N_d, K_o and K_d are provided. K_o and K_d are the rates at which Oxygen18 and Deuterium are flushed from the body. N_o and N_d are the isotope dilution spaces calculated using oxygen18 and deuterium. This is an estimate of body water as a percentage of body mass.

Online Supplemental Material



Supplemental Figure 1: Study design

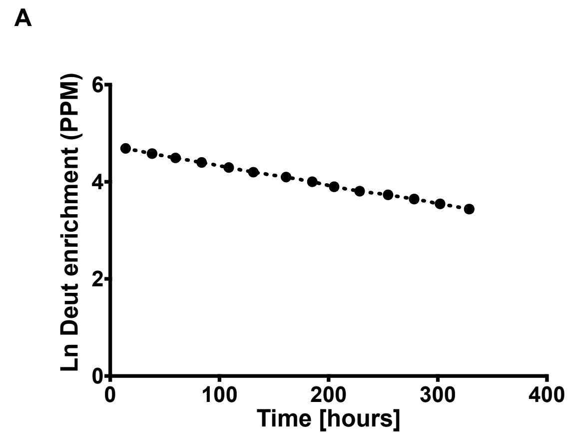
Online Supplemental Material

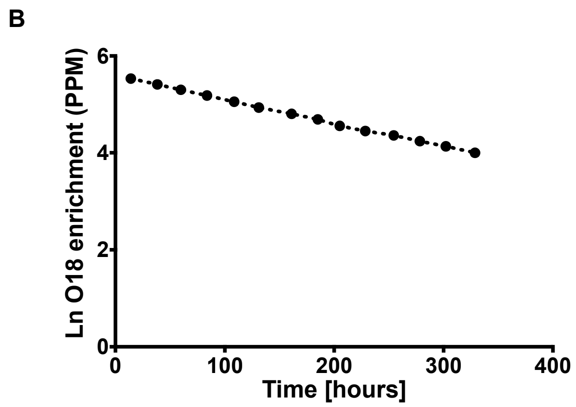


Supplemental Figure 2: Activity profiles of three different participants during and after Ramadan.

24-hour activity profiles during (red) and after Ramadan (blue) represented as the total number of steps per hour for three different subjects (A, B and C). The three participants has had activity records for several days; highlighted areas represent the 25 and 75 percentiles of all measurement days for one subject with the solid lines denoting the median. Subject A stays awake during Ramadan nights and sleeps for a few hours after suhoor. There is less activity during the day compared to the evening. Subject B sleeps before suhoor, wakes up to have his meal or performs his prayers and goes back to sleep after that. This subject is more active in non-Ramadan days, especially in the evening. Subject C stays awake during Ramadan nights and sleeps for one hour after suhoor. There is low activity during the whole day compared to non-Ramadan days.

Online Supplemental Material





Supplemental Figure 3: Typical isotope elimination curves for deuterium and oxygen18 for one subject. To characterize the elimination, pattern a semi-log plot was generated of the logged difference between the enrichment and the background enrichment over time. This process linearized the relationship. The gradient of this line represents the isotope washout rate for each isotope.