

## Possible metabolic impact of Ramadan fasting in healthy men

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**Background/aim:** Insulin sensitivity and  $\beta$ -cell function during Ramadan fasting in healthy male subjects have not been investigated so far. We assessed the changes of these and other metabolic parameters to judge the potential metabolic benefits of Ramadan fasting.

**Materials and methods:** Twenty-four healthy males of Turkish origin living in Germany, with normal glucose tolerance, participated in this study during Ramadan of 2009; 19 who completed fasting were analyzed. Blood was drawn at sunset after a period of fasting lasting approximately 15 h on days 0, 16, and 30 of Ramadan, as well as 7 and 28 days later. Insulin sensitivity (Homeostasis Model Assessment, HOMA),  $\beta$ -cell function, and other parameters were assessed.

**Results:** Ramadan fasting was associated with a significant reduction (–) or increment (+) for the following variables: insulin sensitivity (–20%;  $P = 0.04$ ),  $\beta$ -cell function (+10%;  $P = 0.049$ ), high-density lipoprotein cholesterol (–23%;  $P = 0.0003$ ), low-density lipoprotein cholesterol (+14%;  $P = 0.007$ ), nonesterified fatty acids (–62%;  $P < 0.0001$ ), resistin (–20%;  $P = 0.01$ ), adiponectin (+16%;  $P = 0.003$ ), and glucagon (–21%;  $P = 0.01$ ). C-peptide, insulin, leptin, triglyceride, and very low-density lipoprotein cholesterol concentrations were not significantly changed.

**Conclusion:** Ramadan fasting is associated with transiently impaired insulin sensitivity, compensated for by an increased  $\beta$ -cell function. However, the pattern of insulin resistance-mediating adipocytokines suggests a potentially beneficial metabolic effect of Ramadan fasting.

**Key words:** Insulin sensitivity,  $\beta$ -cell function, adiponectin, leptin, resistin, Ramadan fasting

### 1. Introduction

Many efforts have been undertaken to elucidate the changes of metabolic parameters during Ramadan fasting in healthy subjects, including the following: 1) Regarding blood glucose, significant reductions (1,2) or no significant changes (3,4) have been reported. 2) Insulin was not changed (3,5). 3) For high-density lipoprotein (HDL) as well as low-density lipoprotein (LDL) cholesterol concentrations, conflicting data have been published, (6,7), and no significant changes were described for very low-density lipoprotein (VLDL) cholesterol (4,7,8). 4) Concerning triglycerides, conflicting data have been presented (3,4,6,7). 5) Regarding thyroid parameters, Fadaïl et al. reported a significant rise in total thyroxine and a reduction in triiodothyronine (5), and Sajid et al. showed a significant increment in thyrotropin (9). 6) For cortisol, Al-Hadramy et al. reported a reduction and Bahijri et al. reported an increase in concentrations (10,11). 7) With respect to renal parameters, conflicting urea concentrations have been reported (2,3,7), and Maislos

et al. found no significant changes regarding creatinine concentrations (7). Several studies showed a reduction (3,8,12), and other studies no change, in body weight or body mass index (BMI) (4,13) during Ramadan fasting. 8) Blood pressure during Ramadan fasting has been found unchanged (14), or systolic as well as diastolic blood pressures were reported decreased (15). Adlouni et al. reported an increase (6) and Bouhlel et al. a significant decrease (3) in total calorie intake during Ramadan fasting.

All adult Muslims, with some exceptions (e.g., illness, gestation, menstruation, and travel), are requested to fast 30 days from early dawn until sunset during the ninth lunar calendar every year.

Metabolic parameters are usually determined in the morning. However, in studies during Ramadan the parameters are obtained before sunset in the evening. The equivalence/comparability of fasting parameters obtained in the morning and in the evening, respectively, has not been investigated so far.

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To our knowledge, insulin sensitivity,  $\beta$ -cell function, glucagon, nonesterified fatty acids, and adipocytokines (adiponectin, leptin, and resistin) have not been examined during and after Ramadan fasting in healthy subjects with normal glucose tolerance so far.

Therefore, in the present study we investigated the changes in insulin sensitivity,  $\beta$ -cell function, and other metabolic factors during and after Ramadan fasting in healthy men with normal glucose tolerance.

## 2. Materials and methods

### 2.1. Protocol

The study protocol was approved by the ethics committee of the Georg-August University of Göttingen (registration number 23/1/09) on 18 May 2009, and the study was carried out in accordance with the Declaration of Helsinki.

### 2.2. Subjects

We recruited 24 healthy male migrants of Turkish origin in Germany who had decided to participate in Ramadan fasting independent from our study. Inclusion criteria: Healthy male subject with normal oral glucose tolerance, aged 18–75 years. Exclusion criteria: BMI of  $<18.5$  or  $\geq 35$  kg/m<sup>2</sup>, any type of diabetes, impaired glucose tolerance, impaired fasting glucose, endocrine disorders influencing glucose metabolism, treatment with drugs with influence on glucose metabolism (e.g., hydrochlorothiazide,  $\beta$ -blockers, diazoxide, interferon-alpha, cyclosporine, glucocorticoids, nicotinic acid, statins, and fibrates), any interruption of Ramadan fasting, renal insufficiency at screening [estimated glomerular filtration rate (eGFR) of  $<60$  mL/min], and overt hyper- or hypothyroidism.

### 2.3. Procedures

The volunteers participated at the following visits: Visit 1, screening in the morning, 0800–0900 hours (between 5 June and 19 August 2009), after a period of at least 12 h without nutrient intake. Visit 2 (day 0), beginning of Ramadan fasting [20 August 2009, 1940–2040 hours, at sunset (“iftar”)] (to enable the participants to take their meal at sunset on the first day of Ramadan fasting (21 August 2009) together with their families at home, we started the fasting 1 day earlier than usual). Visit 3 (day 16), in the middle of the Ramadan fasting period (5 September 2009, 1905–2005 hours). Visit 4 (day 30), end of Ramadan fasting (19 September 2009, 1835–1935 hours). Visit 5 (day 37), 7 days after the end of Ramadan fasting (26 September 2009, 1820–1920 hours). Visit 6 (day 58), 28 days after the end of Ramadan fasting (17 October 2009, 1730–1830 hours). During Ramadan fasting and thereafter the examinations were at sunset after a fasting period.

The duration of the fasting period was from 0440 to 2040 hours on day 0, and thereafter it shifted with the exact timing of dawn and sunset until day 30 of Ramadan,

when fasting started at 0507 hours and finished at 1935 hours. The daily fasting period varied between 16 h in the beginning and 14.5 h at the end of Ramadan fasting.

At screening, an oral glucose tolerance test was performed using an Accu Chek-Dextro O.G-T., containing 75 g of glucose (Roche Diagnostics, Mannheim, Germany).

At every visit insulin sensitivity [homeostasis model assessment (HOMA2-%S; primary endpoint)],  $\beta$ -cell function [HOMA2-%B; HOMA 2 model (16)], insulin, C-peptide, glucose, glucagon, adiponectin, leptin, resistin, cortisol, lipid, renal, thyroid, and anthropometric parametric parameters were assessed.

#### 2.3.1. Blood specimens

For determination of glucose, tubes containing sodium fluoride (Glukose-Fluorid, Sarstedt Monovetten, Sarstedt AG & Co., Nümbrecht, Germany) were used. For the determination of HDL, LDL, and VLDL cholesterol, we separated a serum aliquot, which was stored at 4 °C and studied on the same day or within 2 days. For the determination of other parameters, serum was frozen in aliquots at  $-80$  °C and parameters were analyzed after the last visit (all in the same assay run). Glucose was determined immediately on the day of sample collection; supernatant was stored as aliquots at  $-80$  °C. For glucagon determination per mL of plasma 50  $\mu$ L of aprotinin (Trasylol, 10,000 KIU/mL, Bayer AG, Leverkusen, Germany) was added to inhibit degradation of glucagon.

#### 2.3.2. Laboratory determinations

Glucose was measured using an enzymatic-amperometric method (Biosen S-line, EKF-diagnostic GmbH, Barsleben, Magdeburg, Germany). Insulin, C-peptide, thyrotropin (TSH), free triiodothyronine, free thyroxine, and cortisol were measured using an immunoassay system (Siemens Healthcare Diagnostics ADVIA Centaur CP-System; Siemens Healthcare Diagnostics Ltd., Camberley, UK). Glucagon concentrations in plasma were measured after extraction of plasma with 70% ethanol (v/v, final concentration). The antibody employed (code no. 4305) is directed against the C-terminus of the glucagon molecule and therefore mainly measures glucagon of pancreatic origin (17). Standards were human glucagon and the tracer was monoiodinated human glucagon (both gifts from NovoNordisk, Bagsværd, Denmark). Sensitivity and detection limit are below 1 pmol/L, intraassay coefficient of variation is below 6% at 20–30 pmol/L, and recovery of standard, added to plasma before extraction, is about 100% when corrected for losses inherent in the plasma extraction procedure. Adiponectin and resistin were measured using an enzyme immunoassay system (Mediagnost ELISA; Mediagnost, Reutlingen, Germany). Leptin was measured using a radioimmunoassay system (Mediagnost Leptin RIA CT R44; Mediagnost). Triglycerides, nonesterified fatty acids (NEFAs), urea, and

creatinine were measured using an enzymatic bichromatic endpoint measurement (Siemens Healthcare Diagnostics Dimension clinical chemistry system; Siemens Healthcare Diagnostics Ltd.). Total cholesterol was measured using an enzymatic polychromatic endpoint measurement (Siemens Healthcare Diagnostics Dimension clinical chemistry system; Siemens Healthcare Diagnostics Ltd.). HDL, LDL, and VLDL cholesterol were measured using a kit for determination of lipoproteins (Lipidophor Tris All in 12 (Technoclone GmbH, Vienna, Austria). Sodium and potassium were measured using the Siemens Healthcare Diagnostics Dimension QuickLYTE Integrated Multisensor System Kit (Siemens Healthcare Diagnostics Ltd.). The eGFR was estimated using the Cockcroft–Gault formula.

Insulin sensitivity (HOMA2-%S) and  $\beta$ -cell function (HOMA2-%B) were calculated using the HOMA Calculator [HOMA2 Calculator v2.2, Diabetes Trials Unit, University of Oxford, a software implementation of the HOMA2 model), downloaded from <http://www.dtu.ox.ac.uk/homa> (16)]. Briefly, insulin sensitivity was calculated from simultaneously determined plasma glucose and serum insulin. For the calculation of  $\beta$ -cell function, simultaneously determined plasma glucose and C-peptide were used (16).

#### 2.4. Statistical analysis

Subject characteristics as well as results are presented as mean  $\pm$  standard deviation (SD). The descriptive analysis (mean, SD, range) was performed using IBM SPSS Statistics Release 19.0.0.1 (IBM Corporation, Armonk, NY, USA). Normal distribution for all parameters was checked by the Kolmogorov–Smirnov test. For normally distributed variables, analysis of variance (ANOVA) was performed.

ANOVA with repeated measures was performed for the primary endpoint (insulin sensitivity) as well as for the secondary endpoints (other variables) for visit 2 to visit 6 using Statistica (StatSoft Inc., Hamburg, Germany, Version 10.0). If significant, values at single time points were compared to the value measured on day 0 using Duncan's post hoc test. For linear regression analyses we used GraphPad Prism, version 5.02 (GraphPad Software Inc., La Jolla, CA, USA). A 2-sided P value of  $<0.05$  was taken to indicate significant differences.

### 3. Results

Thirty-one volunteers were screened. Seven of them had to be excluded because of violations of inclusion/exclusion criteria. Twenty-four subjects participated in the study. Five of them could not be analyzed because of incomplete data or interruption of Ramadan fasting. Nineteen subjects completed the study and were analyzed.

Characteristics of the volunteers at screening (before Ramadan fasting, blood drawn in the morning) and at the beginning of Ramadan fasting (day 0, blood drawn at sunset) are presented in the Table.

#### 3.1. Anthropometric parameters

Four weeks after the beginning of Ramadan fasting, body weight decreased ( $-1\%$ ,  $P = 0.046$ ). Body mass index significantly increased ( $+1\%$ ,  $P = 0.04$ ) 4 weeks after the end of Ramadan fasting. Waist measurement and waist-to-hip ratio did not significantly change during and after Ramadan fasting.

#### 3.2. Comparability of fasting metabolic parameters obtained in the morning and at sunset

There were some significant changes between determinations at dawn (screening) and sunset (day 0) of some parameters. At sunset, insulin sensitivity ( $+70\%$ ), NEFAs ( $+150\%$ ), and HDL cholesterol ( $+43\%$ ) were higher, while insulin ( $-31\%$ ), C-peptide ( $-23\%$ ), leptin ( $-19\%$ ), and TSH ( $-33\%$ ) decreased. A comparison of all parameters and the results of linear regression analyses are presented in the Table. We found a significant positive linear correlation between the results in the morning and sunset for body weight, BMI, waist measurement, and the waist-to-hip ratio. There was a significant difference between concentrations in the morning and at sunset for body weight and BMI. For the waist measurement and waist-to-hip ratio, we found no significant difference between concentrations in the morning and at sunset. We found a significant positive linear correlation between the results in the morning and at sunset for glucagon,  $\beta$ -cell function, insulin, and C-peptide (Figure 1). There was a significant difference between concentrations at dawn and sunset for plasma glucose, insulin, C-peptide, and insulin sensitivity (HOMA2-%S). For  $\beta$ -cell function (HOMA2-%B) and glucagon we found no significant difference between concentrations at dawn and sunset. We found significant linear correlations between the results in the morning and at sunset for free thyroxine. There was no significant difference between concentrations in the morning and at sunset for free thyroxine. We found a significant positive linear correlation between the results at dawn and sunset for serum creatinine and eGFR. There was a significant difference between concentrations in the morning and at sunset for serum creatinine, eGFR, and sodium. For urea and potassium we found no significant difference between concentrations in the morning and at sunset (Table).

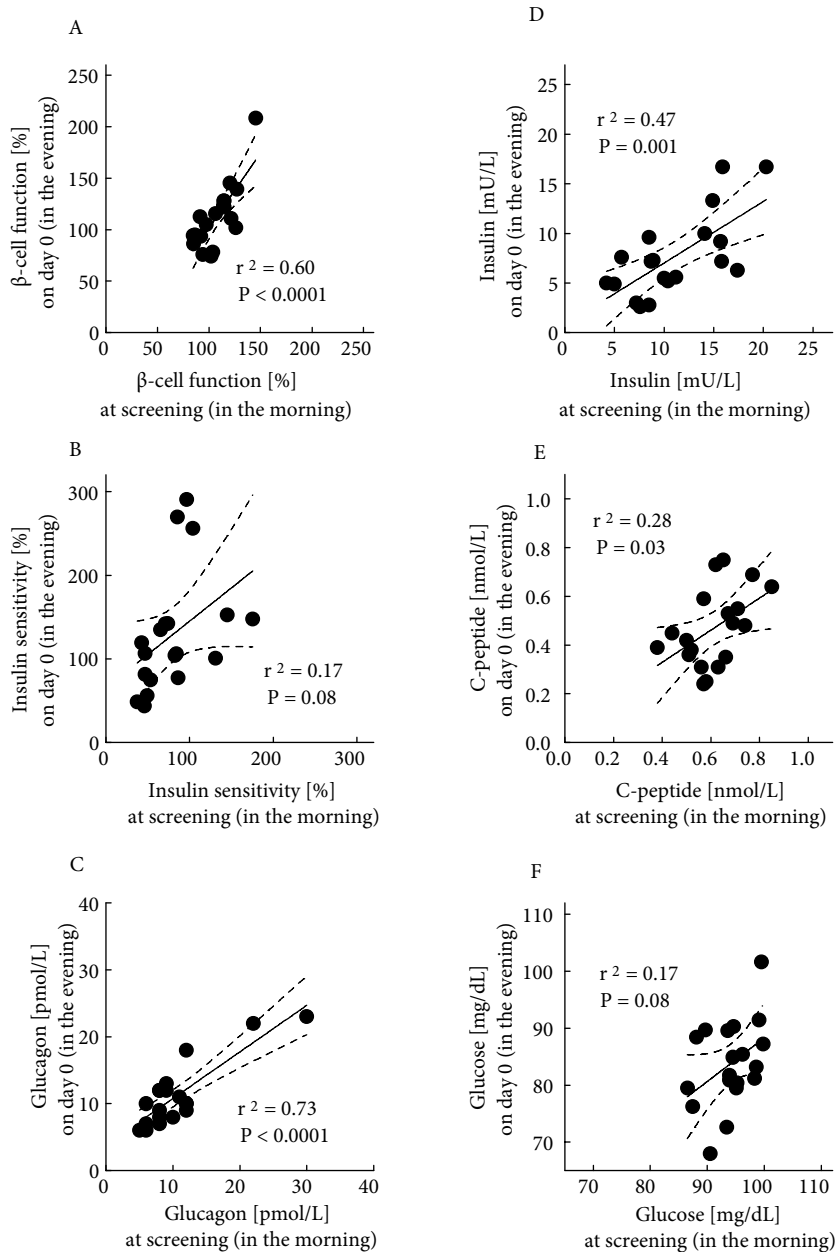
#### 3.3. Parameters characterizing insulin sensitivity and secretion, glucose metabolism, and glucagon

Four weeks after the beginning of Ramadan fasting, insulin sensitivity decreased ( $-20\%$ ) significantly from  $129.2 \pm 71.8\%$  (baseline) to  $103.1 \pm 43.2\%$  ( $P = 0.04$ ). Four

**Table.** Anthropometric data and laboratory parameters of completers (healthy men, n = 19) at screening and on day 0 of Ramadan fasting.

Parameter [unit]	Normal range	Screening (in the morning)		Day 0 (in the evening)		Difference of mean (%)	P value <sup>†</sup>	Linear regression	
		Mean ± SD	Range	Mean ± SD	Range			r <sup>2</sup>	P value <sup>‡</sup>
Age [years]		36 ± 9	20–49	36 ± 9	20–49	0.0			
Body weight [kg]		89 ± 12	71–108	88 ± 11	71–106	-1.1	0.009	0.99	<0.0001
BMI [kg/m <sup>2</sup> ]	18.5–24.9	28.8 ± 3.4	22.9–34.6	28.9 ± 3.2	23.0–34.1	0.3	0.007	0.99	<0.0001
Waist measurement [cm]	<94	97 ± 9.8	81–120	96 ± 8.6	82–115	-1.0	0.27	0.93	<0.0001
Hip measurement [cm]	<102	99 ± 7.8	88–112	98 ± 7.0	88–113	-1.0	0.26	0.82	<0.0001
Waist-to-hip ratio	<0.9	0.98 ± 0.05	0.9–1.1	0.98 ± 0.04	0.9–1.1	0.0	0.79	0.79	<0.0001
Systolic blood pressure [mmHg]	<140	122 ± 10	98–138	124 ± 13	100–149	2.5	0.32	0.09	0.22
Diastolic blood pressure [mmHg]	<90	76 ± 11	61–97	78 ± 10	60–97	2.6	0.79	0.34	0.11
Heart rate [beats/min]	60–80	70 ± 10	56–93	77 ± 11	63–99	10.0	0.002	0.38	0.005
Fasting glucose (in plasma) [mg/dL]	<100	94 ± 4	87–100	84 ± 8	68–102	-10.6	<0.0001	0.17	0.08
2-h glucose in plasma [mg/dL]	<140	89 ± 20	61–135						
Insulin [mU/L]	3–25	11.1 ± 4.6	4.2–20.3	7.7 ± 4.1	2.6–16.7	-30.6	0.0005	0.47	0.001
C-peptide [nmol/L]	0.27–1.27	0.61 ± 0.12	0.38–0.85	0.47 ± 0.16	0.24–0.75	-23.0	0.0004	0.28	0.03
Glucagon [pmol/L]		10.6 ± 6.0	5–30	11.2 ± 4.9	6–23	5.7	0.43	0.73	<0.0001
Insulin sensitivity (HOMA2-%S) [%]	100	76.1 ± 16.3	53.4–119.7	129.2 ± 71.8	44–291	69.8	0.004	0.17	0.08
β-cell function (HOMA2-%B) [%]	100	107.4 ± 16.7	84.6–145.3	112.5 ± 31.2	74–208	4.7	0.31	0.60	<0.0001
HDL cholesterol [mg/dL]	>35	23.2 ± 8.7	10–39	33.2 ± 7.7	16–45	43.1	0.0002	0.15	0.11
LDL cholesterol [mg/dL]	<130	135 ± 43	56–249	149 ± 43	96–291	10.9	0.01	0.73	<0.0001
VLDL cholesterol [mg/dL]		27 ± 18	6–70	21 ± 15	7–57	-25.2	0.06	0.38	0.005
Nonesterified fatty acids [mmol/L]	0.1–0.6	0.3 ± 0.1	0.1–0.6	0.7 ± 0.2	0.3–1.2	150.0	<0.0001	0.18	0.07
Triglycerides [mg/dL]	50–150	227 ± 165	46–753	208 ± 159	71–685	-8.4	0.32	0.77	<0.0001
Adiponectin [µg/mL]	1.3–11.6	3.0 ± 1.3	0.9–5.9	3.1 ± 1.4	0.8–5.8	3.3	0.38	0.80	<0.0001
Leptin [ng/mL]	1–56	5.7 ± 3.8	1.8–15.6	4.6 ± 3.8	1.0–13.4	-19.3	0.003	0.89	<0.0001
Resistin [ng/mL]	2.8–12.1	3.4 ± 1.3	1.9–7.2	3.9 ± 1.4	1.7–6.6	14.7	0.07	0.30	0.02
Cortisol [µg/dL]	4.3–22.4	17.2 ± 5.6	7.9–26.1	11.8 ± 5.9	2.1–22.9	-3.4	0.004	0.04	0.36
TSH [mU/L]	0.35–5.5	1.5 ± 0.8	0.4–3.2	1.0 ± 0.5	0.2–1.8	-33.3	0.009	0.22	0.04
fT <sub>3</sub> [pg/mL]	2.3–4.2	4.1 ± 0.4	3.3–4.8	4.0 ± 0.3	3.4–4.5	-2.4	0.40	0.37	0.005
fT <sub>4</sub> [ng/dL]	0.9–1.8	1.5 ± 0.2	1.1–1.9	1.5 ± 0.3	1.0–1.8	0.0	0.72	0.69	<0.0001
Creatinine [mg/dL]	0.8–1.3	0.9 ± 0.1	0.6–1.1	1.0 ± 0.1	0.7–1.2	11.1	0.04	0.62	<0.0001
*eGFR [mL/min]	>60	139 ± 34	98–228	132 ± 32	90–199	-5.0	0.04	0.82	<0.0001
Urea [mg/dL]	15.0–38.5	30.3 ± 6.5	21–43	32 ± 5	26–46	5.6	0.43	0.006	0.76
Sodium [mmol/L]	136–148	140 ± 2	137–144	142 ± 2	140–148	1.4	0.006	0.003	0.82
Potassium [mmol/L]	3.5–5.5	4.2 ± 0.3	3.7–4.8	4.4 ± 0.3	3.8–4.9	4.8	0.09	0.16	0.09

\*: eGFR with Cockcroft–Gault formula. SD = Standard deviation. †: P-value for screening versus day 0. ‡: P-value for the correlation between screening and day 0.



**Figure 1.** Regression analysis of data obtained in the morning (at screening, fasting) and in the evening (on day 0 of the Ramadan fasting) in 19 healthy men for  $\beta$ -cell function (A), insulin sensitivity (B), glucagon (C), insulin (D), C-peptide (E), and glucose (F). The P-value indicates the significance of the coefficient of determination ( $r^2$ ) for the linear regression.

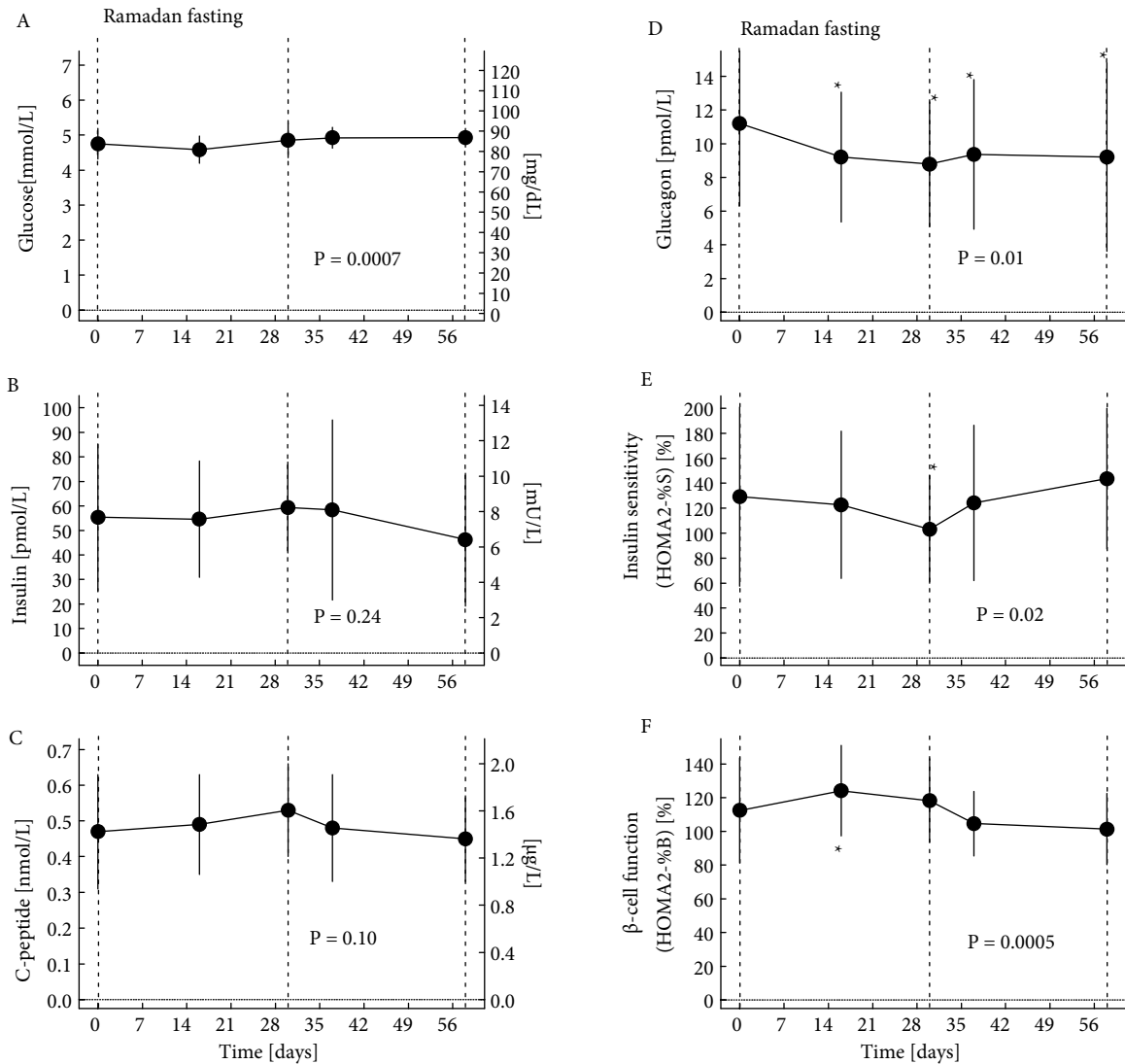
weeks after the end of Ramadan fasting, insulin sensitivity increased to  $143.6 \pm 56.4\%$  ( $P = 0.22$  compared to day 0). Fourteen days after the beginning of Ramadan fasting,  $\beta$ -cell function (HOMA2-%B) increased significantly (+10%) from  $112.5 \pm 31.2\%$  to  $124.2 \pm 26.8$  ( $P = 0.049$ ).

Glucagon concentrations decreased within 2 weeks (-18%,  $P = 0.01$ ) after the beginning of Ramadan fasting

and remained significantly under baseline until 4 weeks after the end of Ramadan fasting (Figure 2).

### 3.4. Lipid parameters

Nonesterified fatty acids significantly decreased 2 weeks after the beginning of Ramadan fasting from  $0.71 \pm 0.23$  mmol/L to a minimum of  $0.27 \pm 0.14$  mmol/L (-62%;  $P < 0.0001$ ) and this reduction below baseline concentrations



**Figure 2.** Parameters characterizing insulin sensitivity and  $\beta$ -cell function and glucagon during and after Ramadan fasting in 19 healthy men (A–F). Data are presented as mean  $\pm$  SD. The P-value indicates differences over time (ANOVA repeated measures); asterisks show significant difference ( $P < 0.05$ , Duncan’s post hoc test) compared to the beginning of Ramadan fasting, day 0.

persisted significantly throughout the follow-up period. Four weeks after the beginning of Ramadan fasting, LDL cholesterol significantly increased (+14%,  $P = 0.007$ ) and HDL cholesterol significantly decreased (–23%,  $P = 0.0003$ ) (Figure 3). VLDL cholesterol and triglyceride concentrations did not change significantly.

**3.5. Adipocytokines**

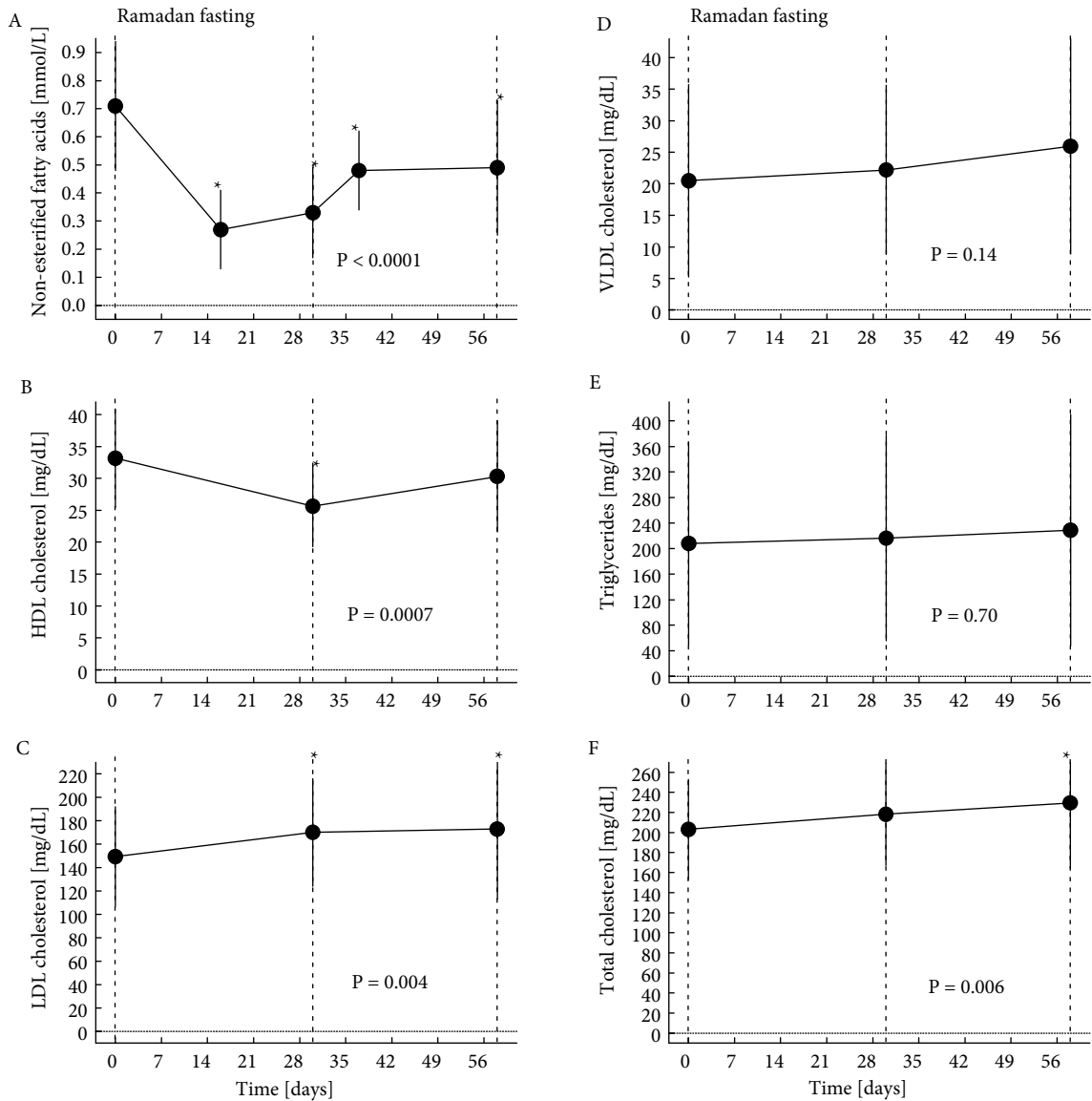
Two weeks after the beginning of Ramadan fasting, resistin decreased significantly (–18%,  $P = 0.01$ ) and remained significantly below baseline during the follow-up period. Adiponectin increased significantly 1 week after the end of Ramadan fasting (+16%,  $P = 0.003$ ). Leptin concentrations did not change significantly during and after Ramadan fasting (Figure 4).

**3.6. Thyroid hormones**

Two weeks after the beginning of Ramadan fasting, TSH significantly increased (+20%,  $P = 0.03$ ) and free thyroxine significantly decreased (–7%,  $P = 0.007$ ). Free triiodothyronine concentrations did not significantly change during Ramadan fasting. One week after the end of Ramadan fasting, cortisol decreased (–27%,  $P = 0.01$  compared to day 0).

**3.7. Circulatory parameters**

Systolic (–6%,  $P = 0.005$ ) and diastolic (–8%,  $P = 0.01$ ) pressure decreased significantly 4 weeks after the beginning of Ramadan fasting.



**Figure 3.** Lipid parameters and adipocytokines during and after Ramadan fasting in 19 healthy men (A–F). Data are presented as mean ± SD. The P-value indicates differences over time (ANOVA repeated measures); asterisks show significant difference ( $P < 0.05$ , Duncan’s post hoc test) compared to the beginning of Ramadan fasting, day 0.

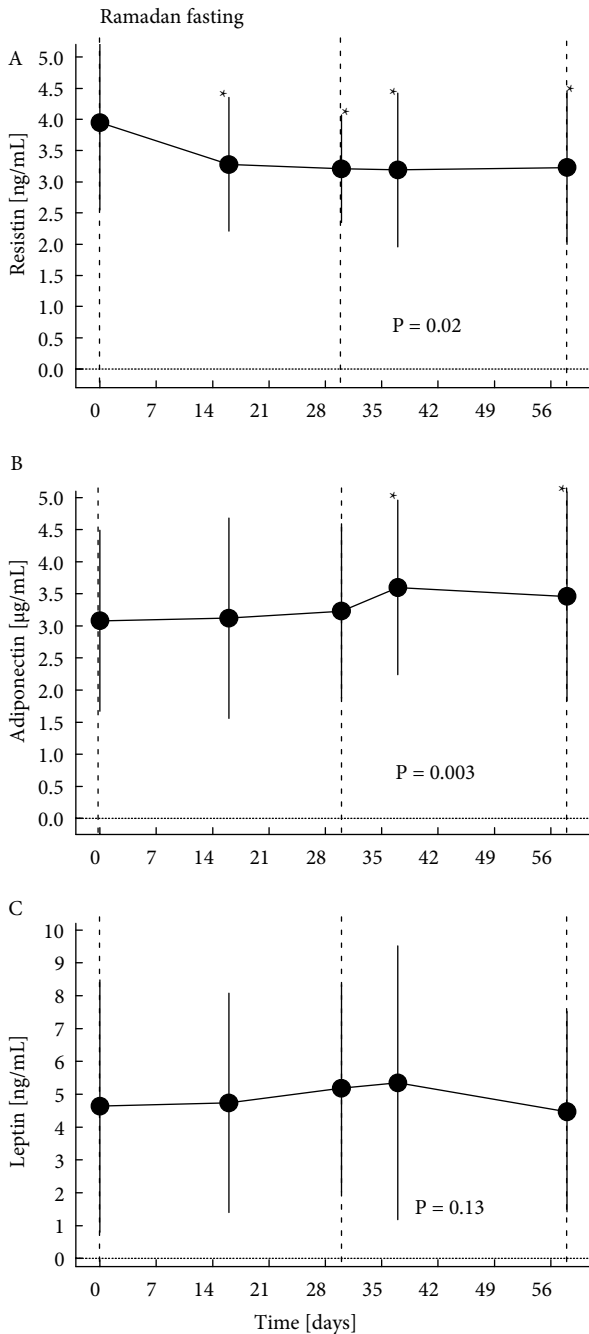
**3.8. Renal parameters**

One week after the end of Ramadan fasting, plasma creatinine significantly decreased (–5%,  $P = 0.01$ ) and remained significantly below baseline concentrations (Table) until 4 weeks after the end of Ramadan fasting. The eGFR significantly increased (+6%,  $P = 0.03$ ) 1 week after the end of Ramadan fasting and reached a maximum 4 weeks after the end of Ramadan fasting. Urea (–16%,  $P = 0.01$ ) and sodium (–1%,  $P = 0.002$ ) significantly decreased 2 weeks after the beginning of Ramadan fasting.

**4. Discussion**

The fasting results that we observed in the morning and at sunset were not generally different, so reliable results can be obtained after Ramadan fasting in the evening. The ratios (in the morning versus at sunset) for insulin (1.4) and C-peptide (1.3) were not identical. However, we cannot explain the small difference.

In this study we showed, for the first time, that compared to baseline concentrations at the begin of Ramadan fasting, insulin sensitivity significantly decreased



**Figure 4.** Resistin (A), adiponectin (B), and leptin (C) concentrations during and after Ramadan fasting in 19 healthy men. Data are presented as mean ± SD. The P-value indicates differences over time (ANOVA repeated measures); asterisks show significant difference (P < 0.05, Duncan's post hoc test) compared to the beginning of Ramadan fasting, day 0.

(-20%) against expectations, β-cell function significantly increased (+10%), and glucagon (-21%) and resistin (-20%) significantly decreased during Ramadan fasting, while adiponectin increased (+16%) 1 week after the

end of Ramadan fasting and leptin showed no significant changes during as well as after Ramadan fasting in healthy male subjects.

Shariatpanahi et al. found that Ramadan fasting is associated with improvement in insulin sensitivity (1/HOMA-IR) in patients with metabolic syndrome (18). Yarahmadi et al. described that insulin resistance significantly decreased during Ramadan fasting in patients with type 2 diabetes (19).

Heilbronn et al. investigated the insulin sensitivity in healthy volunteers after 3 weeks of alternating fasting. They found a significant increase in insulin sensitivity after the end of such a fasting pattern (20). Fasting periods seem to influence insulin sensitivity beneficially. However, it has been shown that uninterrupted 72-h fasting (21) and 4-day fasting (22) lead to worsening insulin resistance. It is believed that insulin resistance arising in skeletal muscle induces an impairment of glucose tolerance after fasting for long periods (22,23).

Regarding insulin sensitivity, our results in healthy subjects are in line with the findings of Bahijri et al. obtained in healthy subjects (10), with the findings of Shariatpanahi et al. obtained in subjects with metabolic syndrome (18), with the findings of Unalacak et al. obtained in nonobese healthy male subjects (24), and with the results of Yarahmadi et al. in patients with type 2 diabetes (19).

The reason for the impairment of insulin sensitivity during Ramadan fasting, although NEFAs and glucagon concentrations significantly decreased, is not obvious. One potential explanation may be that our method of assessing insulin sensitivity (HOMA2 model) was not optimal. More elaborate methods (e.g., the hyperinsulinemic euglycemic clamp) might elicit different results.

During Ramadan fasting we found a temporary increase of β-cell function, possibly compensatory to the decreased insulin sensitivity. This result is consistent with the previous findings of hyperinsulinemia in patients with insulin resistance (25).

During Ramadan fasting we found a continuous decrease of glucagon, lasting after the Ramadan fasting. The relation of the dramatic decrease in NEFA concentrations and glucagon is not understood yet; there are conflicting data. In various in vitro studies with pancreatic islets it was shown that NEFAs lead to an impairment of glucagon secretion in rats (26) and in mice (27). Gerich et al. found that the heparin-induced elevation of NEFA leads to an impairment of glucagon in healthy subjects as well as in subjects with type 1 diabetes (28). Recently, in an in vitro study, it was shown that NEFAs stimulate glucagon secretion in pancreatic islets (29). Regarding NEFAs during Ramadan fasting in healthy volunteers, there are no data in the literature. In the present study we found an impressive decrease of NEFAs 2 weeks after the beginning of Ramadan



fasting. We suppose that this pattern of NEFAs has a beneficial influence on carbohydrate metabolism, since increased NEFA concentrations may induce peripheral as well as hepatic insulin resistance (30,31). This can be explained by the following mechanisms (31): 1) NEFAs are increased in most obese subjects, 2) temporarily increased NEFAs can suppress the insulin-stimulated glucose intake in healthy subjects as well as in subjects with diabetes (31). In the Randle cycle, NEFAs are competing with glucose and influencing the glucose metabolism unfavorably (32). Furthermore, NEFAs have disadvantageous effects regarding insulin uptake into the liver and can lead to an enhancement of hepatic glucose release (33). Due to the impressive decrease of NEFAs in our study it may be supposed that Ramadan fasting can be beneficial in the sense of helping prevent metabolic syndrome in healthy men, because this reduction in NEFA concentrations is probably associated with an enhanced insulin sensitivity in adipose tissue and the liver. The pathway leading to a decrement of NEFAs is probably one of the important points for a potentially beneficial effect of Ramadan fasting. Regarding LDL cholesterol concentrations during Ramadan fasting in healthy subjects, conflicting data have been published (3,6,7). The reason for the increase of LDL cholesterol and the decrease of HDL cholesterol in our study is unclear. However, the findings of Ziaee et al. (8) are in line with our LDL and HDL cholesterol results.

We hypothesize that Ramadan fasting, with daily fasting periods of approximately 15 h, leads to an enhancement of the insulin sensitivity in adipose tissue and in the liver, which may not be measured adequately by the HOMA2 parameters.

We found a significant rise in adiponectin concentrations 1 week after the end of Ramadan fasting. Adipose tissue is involved relevantly in the regulation of energy balance and secretes biologically active adipocytokines, e.g., adiponectin (34), leptin (35), and resistin (36). Adiponectin improves insulin sensitivity (34), e.g., it has an insulin-sensitizing effect in the liver (37,38) and in skeletal muscle (39). Regarding leptin concentrations, our results in healthy subjects are in line with the findings of Khafaji et al. obtained in stable cardiac patients (40). They found no significant changes of leptin concentrations during and after Ramadan fasting. Leptin serum concentrations are positively correlated with body fat mass and with BMI, as well as with fasting insulin

concentrations (41). Resistin can cause or worsen insulin resistance (32). In subjects with metabolic syndrome, resistin concentrations are elevated (42). In obese mice (diet-induced) with insulin resistance and hyperglycemia, administration of antiresistin antibodies is associated with a decrease of blood glucose and an improvement of insulin sensitivity, suggesting a causal role of resistin in the genesis of insulin resistance (32). The decreased resistin concentrations during and after Ramadan fasting in our study tend to support our primary hypothesis that Ramadan fasting may improve insulin sensitivity. However, direct measurement of insulin sensitivity did not confirm this assumption.

A limitation of our study is that we did not document the daily calorie intake and the composition of the meals. In further studies on Ramadan fasting, we recommend the acquisition of such data.

In conclusion, contrary to our original assumption, Ramadan fasting led to a transient reduction in insulin sensitivity, compensated for by improved  $\beta$ -cell function, with no significant change in glucose concentrations. However, favorable changes of insulin resistance mediating adipocytokines, lasting up to 4 weeks after Ramadan fasting, suggest a potentially beneficial metabolic effect. Further studies are needed to explain the mechanism of reduced insulin sensitivity despite a fall in NEFAs and reduction in glucagon.

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