Effect of intermittent fasting and refeeding on insulin action in healthy men

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2005; doi:10.1152/japplphysiol.00683.2005.—Insulin resistance is
currently a major health problem. This may be because of a marked
decrease in daily physical activity during recent decades combined
with constant food abundance. This lifestyle collides with our ge-
nome, which was most likely selected in the late Paleolithic era
(50,000–10,000 BC) by criteria that favored survival in an environ-
ment characterized by fluctuations between periods of feast and
famine. The theory of thrifty genes states that these fluctuations are
required for optimal metabolic function. We mimicked the fluctua-
tions in eight healthy young men [25.0 ± 0.1 yr (mean $\pm$ SE); body
mass index: 25.7 ± 0.4 kg/m^2] by subjecting them to intermittent
fasting every second day for 20 h for 15 days. Euglycemic hyperin-
sulinemic (40 mU·min^{-1}·m^{-2}) clamps were performed before and
after the intervention period. Subjects maintained body weight
(86.4 ± 2.3 kg; coefficient of variation: $0.8 \pm 0.1\%$). Plasma free fatty
acid and β-hydroxybutyrate concentrations were 347 ± 18 and $0.06 \pm
0.02$ mM, respectively, after overnight fast but increased ($P < 0.05$)
to 423 ± 86 and 0.10 ± 0.04 mM after 20-h fasting, confirming that
the subjects were fasting. Insulin-mediated whole body glucose up-
take rates increased from 6.3 ± 0.6 to 7.3 ± 0.3 mg·kg^{-1}·min^{-1}
($P = 0.03$), and insulin-induced inhibition of adipose tissue lipolysis
was more prominent after than before the intervention ($P = 0.05$).
After the 20-h fasting periods, plasma adiponectin was increased
compared with the basal levels before and after the intervention
($5,922 \pm 991$ vs. $3,860 \pm 784$ ng/ml, $P = 0.02$). This experiment is
the first in humans to show that intermittent fasting increases insulin-
mediated glucose uptake rates, and the findings are compatible with
the thrifty gene concept.

euglycemic clamp; adiponectin

OUR GENOME WAS PROBABLY SELECTED during the Late-Paleolithic
era (50,000–10,000 BC), during a time humans existed as
hunter-gatherers (6). At that time there were no guarantees in
finding food, resulting in intermixed periods of feast and
famine. In addition, physical activity had to be a part of our
ancestors' daily living as forage and the hunt for food must
have been done through physical activity (15). Cycling be-
tween feast and famine, and thus oscillations in energy stores,
as well as between exercise and rest, was characteristic in the
Late-Paleolithic era and might have driven the selection of
genes involved in the regulation of metabolism (30).

Thus our genotype selected centuries ago to favor an envi-
ronment with oscillations in energy stores still exists with few
if any changes. The modern sedentary lifestyle common in the
westernized countries is characterized by constant high food
availability and low physical activity, and it has led to an
imbalance between our genotype and the environment in which
we live today. This may predispose our potential "thrifty"
genes to misexpress metabolic proteins, manifesting in chronic
diseases (e.g., Type 2 diabetes) in the industrialized part of the
world.

It is well known that physical training increases insulin
action (10). The molecular events leading to an exercise-
mediated increase in insulin action are not fully characterized.
In addition, energy usage during each exercise bout in the
training regimen with subsequent eating creates oscillations in
energy stores. These oscillations are probably not as massive as
the oscillations seen between periods of feast and famine for
the Late-Paleolithic people, but some similarities might exist,
and we speculated whether exercise-induced oscillations in
energy stores could be mimicked by intermittent fasting. This
study was undertaken to test the hypothesis that 14 days of
intermittent fasting and refeeding improves insulin-stimulated
glucose disposal.

MATERIALS AND METHODS

Subjects

Eight healthy young Caucasian men (age 25.0 ± 0.1 yr, body mass
index 25.7 ± 0.4 kg/m^2) gave their written consent according the
declaration of Helsinki to participate in the study. The study was
approved by the local Danish ethical committee (KF 01-109/04).

Two days before both clamp experiments (see *Experimental Pro-
cedure*), the subjects were instructed to eat at least 250 g of carbo-
hydrate each day and to avoid strenuous exercise.

Throughout the intervention, the subjects were instructed to uphold
their normal exercise habits, to maintain their usual macronutrient
mixing of their meals, and to eat sufficient quantities of food on the
nonfasting days to ensure that their body weight was stable. The
subject characteristics are given in Table 1.

Experimental Procedure

The subjects were examined on two occasions: before and after 14
days of fasting every second day for 20 h, giving seven fasting
periods. Each fasting period started at 2200 and ended at 1800 the
following day (for protocol see Fig. 1). During the fasting periods the
subjects were allowed to drink water and were instructed to maintain
habitual activities.

On the day of clamp experiments, the subjects arrived at the
laboratory at 0800, after an overnight fast. The subjects were weighed
and had their height measured and were placed in a bed position.

A microdialysis catheter was inserted in the subcutaneous fat on the
abdomen (see below), and a small subcutaneous depot of ^{133}Xe was
placed in close proximity (~5 cm) to the microdialysis catheter. One

Table 1. *Subject characteristics*

	Before the Intervention	After the Intervention
Age, yr	25±1	
Body weight, kg	87.1±2.3	86.2±2.4
BMI, kg/m^2	25.7±0.4	25.5±0.3
%Body fat	20.1±0.8	20.4±1.1
Fasting plasma glucose, mmol/l	5.0±0.1	5.1±0.1
Fasting insulin, pmol/l	34±5	38±7

Data are means ± SE. BMI, body mass index. No significant differences were seen.

catheter (18-gauge, Becton Dickinson, Helsingborg, Sweden) was inserted in the medial cubital vein for infusion of glucose and insulin, and one catheter (18-gauge, Becton Dickinson) was inserted in a superficial hand vein in the retrograde direction. The hand was then placed in a heating pad for sampling of arterialized blood. After basal blood samples were obtained, concentrations of CO_2 and O_2 were measured in expiratory air by a ventilated hood and a muscle biopsy was taken from the thigh (vastus lateralis). Then the clamp was started. During the last 15 min of the 120-min clamp, CO_2 and O_2 in expiratory air were determined. At time $t = 120$ min during the clamp, a second muscle biopsy was taken from the thigh.

During the intervention period the subjects recorded their heart rate (Ultima, Cardiosport, Denmark) 24 h a day and measured their body weight in the morning before breakfast (on nonfasting days).

During the intervention period the subjects came to the laboratory at 1700 three times (*days 6, 10,* and *14*) for weight measurement, venous blood sampling, and measurement of expiratory gases. In addition, on *day 10* a muscle biopsy (see below) was taken.

Finally, the subject's body composition was measured by dual-energy X-ray absorption scanning before and after the intervention period.

Microdialysis. Microdialysis was performed as described previously (44). At 08.30 a single microdialysis catheter (CMA 60, CMA, Microdialysis AB) was placed in the abdominal subcutaneous adipose tissue. At sites of perforation the skin was anesthetized. The catheter was connected to a high-precision syringe pump (CMA 100 syringe pump, CMA/Microdialysis AB). For determination of interstitial glycerol concentrations, the catheter was perfused with a fluid containing an isotonic ringer acetate buffer with 2 mM glucose, ^{14}C-glycerol (5 kBq/ml, PerkinElmer) at a speed of 1 μl/min. The relative recovery was determined by the internal reference calibration technique (37). The relative recovery was calculated as $(dpm_p - dpm_d)/dpm_p$, where dpm_p and dpm_d are the ^{14}C activity in the perfusate and dialysate, respectively.

Euglycemic hyperinsulinemic clamp. For each subject, a 50-ml insulin infusate had been prepared from insulin (100 IU/ml Atrapid, Novo Nordisk, Copenhagen, Denmark), 2.5 ml of the subject's own plasma, and saline. Each clamp started with a 2-ml insulin infusate bolus followed by a constant infusion (40 mU·min^{-1}·m^{-2}) for 120 min. Plasma glucose concentrations were maintained at a preexperimental level by frequent analysis of arterialized blood samples (ABL-system 700, Radiometer) with subsequent adjustments of the glucose infusion rate.

Blood flow. Subcutaneous blood flow was determined by the standard local ^{133}Xe washout method (5, 26) in the abdominal subcutaneous adipose tissue in close proximity to the microdialysis catheter. The tissue-blood partition coefficient was set to 10 (5).

Muscle biopsies. Muscle biopsies were obtained from the middle portion of the vastus lateralis before and in the end of each clamp experiment. After administration of local anesthesia, an incision of 10 mm was made and the biopsy was taken (Bergström needle method modified to apply suction). In addition, smaller biopsies were obtained from the mid portion of vastus lateralis after the fourth fasting period (i.e., *day 10*). The biopsy was then obtained with a Tru-core I biopsy needle and instrument (Medical Device Technology, Gainesville, FL).

Muscle biopsies were quickly cleaned from visible blood and frozen in liquid nitrogen (within 15 s) and stored at −80°C until further analysis.

Before analysis, the biopsies were freeze dried and carefully dissected free from connective tissue, blood, and fat. A sample of the muscle powder was used to determine glycogen content by the hexokinase method (25). Another part of the muscle powder was used to determine intramuscular triglyceride (IMTG) content by the chemical extraction method (18, 33). Briefly, the samples were homogenized in methanol and chloroform and the supernatant containing the lipids was removed and mixed with water. The lipids contained in the chloroform phase were then removed and hydrolysis was accomplished by adding tetraethylammoniumhydroxide (20%) and ethanol (1:28). After 30 min at 60°C, the reaction was stopped with HCl. The released acyl-glycerol was finally determined on a CMA 600 analyzer (CMA/microdialysis) and the triacylglycerol content was calculated.

GLUT4 expression. Expression of GLUT4 protein was measured by Western blot in a muscle biopsy obtained during the fasting condition before each clamp. Muscle biopsies were quickly cleaned from visible blood and/or fat, frozen in liquid nitrogen, and stored at −80°C. The muscle tissue was subsequently homogenized with a Polytron PT 3100 (Kinematica, Littau-Luzern, Switzerland) at maximum speed for ~10 s in 10 vol of ~55°C buffer (4% SDS, 10 mM pyrophosphate, 2 mM sodium orthovanadate, 10 mM EDTA, 25 mM Tris·HCl, pH 6.8). Samples were sonicated for ~5 s to break DNA strands, and total protein concentrations were determined by the bicinchoninic acid method using BSA as standard. For Western blot, 10 μg of protein were separated by SDS-PAGE on 10% gels (Criterion system, Bio-Rad, Hercules, CA) and electrophoretically transferred to polyvinylidene difluoride membranes for 45 min at 100 V by using a tank buffer system (Bio-Rad). Transfer buffer contained 25 mM Tris, 192 mM glycine, and 20% methanol. Membranes were
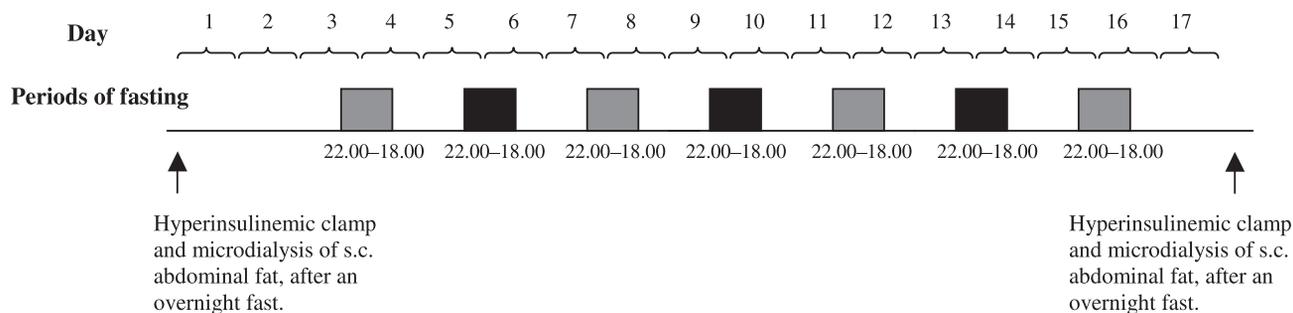

Fig. 1. Experimental protocol. Eight men were subjected to fasting (marked with bars) every second day for a total of 7 fasting periods. Before and after this intervention euglycemic clamps and microdialysis were performed. Blood samples and expiratory gas measurements were performed after the fasting on the days marked with black bars (*days 6, 10,* and *14*). Additionally, after the fasting on *day 10* a muscle biopsy was taken for measurement of glycogen and IMTG.

Table 2. *Primers for real-time RT-PCR*

mRNA	Sense Primer	Antisense Primer
PGC-1α	TCAGACCTGACACAACACGGACA	TCAAGAGCAGCAAAAGCATCACA
GAPDH	CCTCCTGCACCACCAACTGCTT	GAGGGGCCATCCACAGTCTTCT
RPLP0	GGAAACTCTGCATTCTCGCTTCCT	CCAGGACTCGTTTGTACCCGTTGM

blocked in 1% defatted milk powder plus 5% BSA in TS buffer [10 mM Tris (pH 7.4), 150 mM NaCl], incubated for 90 and 60 min with primary and horseradish peroxidase-labeled secondary antibodies, respectively, and diluted in blocking solution. Antigen-antibody complexes were visualized and quantitated by a LAS 3000 imaging system (Fuji Film). Monoclonal antibody F-27 was used for detection of GLUT4 (35). Signals were normalized against amount of desmin by reprobing the polyvinylidene difluoride membrane with a monoclonal antibody against desmin (DakoCytomation, Glostrup, Denmark).

Real-time RT-PCR. Total RNA was isolated from muscle biopsies by phenol extraction (TriReagent, Molecular Research Center) as previously described (7). Intact RNA was confirmed by denaturing agarose gel electrophoresis. Five hundred nanograms total RNA were converted into cDNA in 20 μl by using the OmniScript reverse transcriptase (Qiagen) according to the manufacturer's protocol. For each target mRNA, 0.25 μl cDNA was amplified in a 25-μl SYBR Green PCR reaction containing 1$\times$ Quantitect SYBR Green Master Mix (Qiagen) and 100 nM of each primer (Table 2). The amplification was monitored in real time using the MX3000P real-time PCR machine (Stratagene). The threshold cycle values were related to a standard curve made with the cloned PCR products. The quantities were normalized to mRNA for the large ribosomal protein P0 (RPLP0). RPLP0 was chosen as internal control, assuming RPLP0 mRNA to be constitutively expressed (14). To validate this assumption, another unrelated "constitutive" RNA, GAPDH mRNA, was measured and normalized for RPLP0. No changes in the ratio were observed.

Blood sampling and analysis. Arterialized blood for measurement of hormones, metabolites, and cytokines was sampled from the catheter in the hand vein at basal and at termination of the clamp. Blood was collected in iced tubes and immediately centrifuged at 4°C. Blood for determination of free fatty acids (FFA), glycerol, and β-hydroxybutyrate was stabilized with 10 IU heparin/ml blood. Blood for determination of insulin, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), leptin, and adiponectin was stabilized with 500 kalikrein inhibitory units aprotinin (Trasylol) and 10% EDTA. All plasma samples were stored at -20°C, except those for FFA, IL-6, TNF-α, leptin, and adiponectin, which were stored at -80°C.

Plasma concentrations of insulin, IL-6, TNF-α, leptin, and adiponectin were measured by sandwich ELISA and performed according to the manufacturer's instructions (insulin: DakoCytomatics; adipokines: R&D Systems, Minneapolis, MN).

Plasma FFA analysis was performed by an enzyme color assay (ACS-ACOD, WAKO) and performed according to manufacturer's instructions. β-Hydroxybutyrate was determined by a modification of the method of Olsen (31). The concentration of glycerol in plasma was determined by a spectrophotometric method (automatic analyzer Hitachi 912, Roche, Glostrup, Denmark).

Indirect calorimetry. Expiratory gases were measured on the Oxycon Pro Online Ventilated hood system (Jaeger). The measured values were averaged over 10 min of steady state.

Statistics. All data are presented as means $\pm$ SE, except muscle mRNA and protein as well as plasma hormones (excluding insulin), which were log transformed before statistical analysis and are presented as geometric means $\pm$ back-transformed SE.

Two-way ANOVA for repeated measurements was used for detection of differences between the glucose infusion rates before and after

the intermittent fasting. When a significant main effect was observed, the Student-Newman-Keuls test was used post hoc. In comparison of a single parameter before, during, and after the experiment, a one-way ANOVA for repeated measures was used. Comparison of a single parameter before and after the fasting intervention was performed with Student's paired t-test. The SigmaStat version 2.03 software package was used for all statistical analysis. $P < 0.05$ was considered statistically significant in two-tailed testing.

RESULTS

Weight, Body Composition, and Indexes of Physical Activity

The body weight was maintained stable throughout the experiment (86.4 $\pm$ 2.3 kg, 0.8 $\pm$ 0.1% coefficient of variation) and percent body fat was also unchanged before compared with after the fasting intervention (Table 1).

The level of habitual daily physical activity did not decrease during fasting days. Thus the average heart rate during daytime was not different during fasting (79 $\pm$ 3 min^{-1}) compared with nonfasting days (80 $\pm$ 3 min^{-1}).

Whole Body Glucose Metabolism

Plasma glucose concentration during both clamps was kept constant (Fig. 2), with a coefficient of variance of 4.4% $\pm$ 1.3% mmol/l during the last hour of the clamps.

The glucose infusion rate was significantly increased during the last 30 min (from 6.3 $\pm$ 0.6 to 7.3 $\pm$ 0.3 mg·min^{-1}·kg^{-1}) after the fasting intervention compared with before, respectively ($P = 0.03$) (Fig. 2).

Glycerol Metabolism in Adipose Tissue

There was no effect of intermittent fasting in either the adipose tissue blood flow (2.4 $\pm$ 0.5 vs. 2.9 $\pm$ 0.7 ml·100 g^{-1}·min^{-1} at basal and 2.6 $\pm$ 0.5 vs. 3.1 $\pm$ 0.5 ml·100 g^{-1}·min^{-1} at the insulin-stimulated state) or the absolute interstitial glycerol concentrations (Fig. 3A) during the clamps. However, the interstitial glycerol concentrations decreased exponentially with the insulin infusion ($R^2 = 0.96$ before and $R^2 = 0.99$ after the fasting intervention), and the negative slopes of the curves were larger after the fasting intervention compared with before ($P = 0.05$) (Fig. 3B). This indicates that insulin had an enhanced inhibitory effect on lipolysis after intermittent fasting compared with before.

Substrates and Metabolites

Fasting (8 h) plasma glucose concentrations were similar before (5.0 $\pm$ 0.1 mM) and after (5.1 $\pm$ 0.1 mM) the intermittent fasting period. After 20-h fasting, i.e., *days 4, 6*, and *10*, plasma glucose concentrations were lower (4.6 $\pm$ 0.1, 4.6 $\pm$ 0.1, and 4.7 $\pm$ 0.1 mM, respectively) compared with the shorter fasting periods (8 h) ($P < 0.05$).
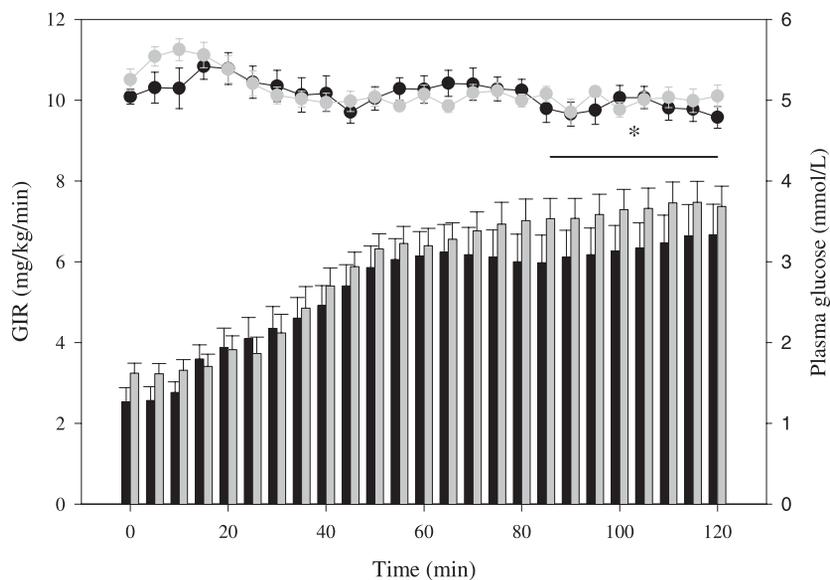
Fig. 2. Glucose infusion rate and glucose concentrations during the clamps before and after intermittent fasting for 15 days. *Left* axis (bars) shows the glucose infusion rate (GIR) necessary to maintain euglycemia during the both clamps. *Right* axis (dots) shows the arterialized plasma glucose concentrations during both experiments. Black bars and dots represents data from the clamp before the fasting intervention; gray bars and dots are data from after the fasting intervention. *$P = 0.03$ in GIR (taken as an average over the last 30 min of the clamps) before and after the fasting intervention. Data are means ± SE.
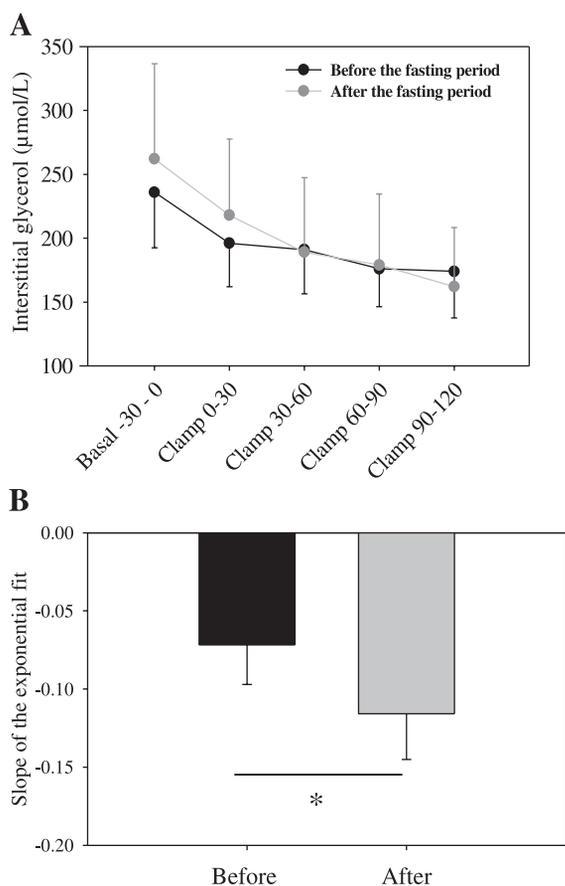

Fig. 3. *A*: insulin-mediated decrease in interstitial glycerol concentrations in subcutaneous abdominal adipose tissue before and after a period of intermittent fasting as measured by microdialysis. Basal values denote microdialysis fluid collected the last 30 min before the clamp started. During the clamp, microdialysis fluid was collected in 30-min periods. With insulin stimulation the interstitial glycerol concentrations followed an exponential drop. *B*: slope of this exponential drop. *$P = 0.05$ between before and after the intervention. Data are means ± SE.

Fasting (8 h) plasma β-hydroxybutyrate, FFA, and glycerol concentrations were similar before and after the intermittent fasting period, and all decreased ($P < 0.05$) with insulin infusion (Fig. 4). After 20-h fasting, i.e., *days 4, 6*, and *10*, plasma FFA and glycerol concentrations were increased compared with the shorter fasting periods ($P < 0.05$) whereas the increase in β-hydroxybutyrate did not attain statistical significance ($P = 0.07$) (Fig. 4).

Hormones

Fasting (8 h) plasma insulin concentrations were similar before (33 ± 5 pM) and after (38 ± 7 pM) the intermittent fasting period, and concentrations increased ($P < 0.05$) with insulin infusion (to 439 ± 63 and 404 ± 18 pM, respectively). After 20-h fasting, i.e., *days 4, 6*, and *10*, plasma insulin concentrations were unchanged (24 ± 4, 24 ± 5, and 16 ± 4 pmol/l) compared with the shorter fasting period (Fig. 4).

Plasma adiponectin concentrations did not change with insulin infusion and were similar on the 2 clamp days (Fig. 5). However, after 20-h fasting (*days 6, 10*, and *14*) a 37% increase was seen compared with the shorter fasting days ($P = 0.02$).

Plasma leptin concentrations were similar on the 2 clamp days and did not change with insulin infusion (Fig. 5). However, after the 20-h fasting days (*days 6, 10*, and *14*) plasma leptin concentrations decreased compared with the shorter fasting days ($P = 0.02$) (Fig. 5).

No significant differences were observed in either TNF-α or IL-6 concentrations during this study (Fig. 5).

Muscle Triglyceride, Glycogen, GLUT4, and PGC-1α mRNA

No overall changes were observed in concentrations of IMTG ($P = 0.11$), glycogen ($P = 0.26$), or mRNA content of PGC-1α ($P = 0.18$) when measured before and after each clamp and after fasting on *day 10* (Figs. 6 and 7). However, with insulin stimulation (data from both clamps are included), we observed a significant decrease ($P = 0.04$) in the IMTG

Fig. 4. Plasma concentrations of β-hydroxy-butyrate (*A*), insulin (*B*), free fatty acids (FFA) (*C*), and glycerol (*D*), before and after clamps performed before and after 15 days of intermittent fasting, and after 20 h of fasting on *days 6*, *10*, and *14* of the intermittent fasting intervention. *$P < 0.05$ decrease during the clamp; †$P < 0.05$ increase during the clamp; ‡$P < 0.05$ between the sample taken after 20 h of fasting compared with basal samples taken after an overnight fast (8 h). Data are means ± SE.
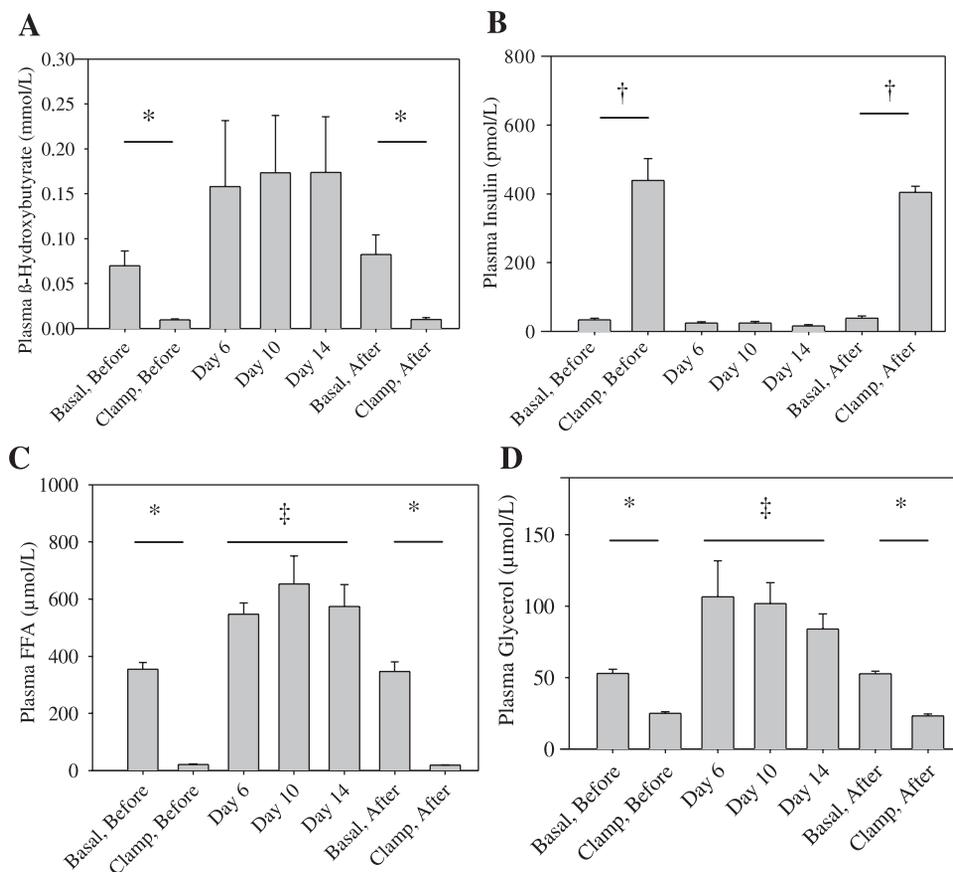
concentration. Furthermore, total muscle GLUT4 protein content did not change with the fasting intervention ($P = 0.66$) (Fig. 7).

Respiratory Exchange Ratios

Respiratory exchange ratios (RER) were similar at basal (after 8-h fasting) on the 2 clamp days. With insulin stimulation RER increased at both occasions (Fig. 8). No differences were observed in RER values between the overnight and the 20-h fasted state (Fig. 8).

DISCUSSION

In the present study we have used a very simple intervention protocol with the aim of mimicking the perturbations in energy stores that are inherent in a physical active lifestyle with regular exercise sessions. In a wider perspective we have tried to unravel the significance of genes that may be responsible for an evolutionary selection process, i.e., the thrifty genes. In this context the used intervention seems inevitably small. Nevertheless, by subjecting healthy men to cycles of feast and famine we did change the metabolic status to the better, implying that the mismatch between our ancient genotype and the lifestyle of the westernized individual of today became smaller. To our knowledge this is the first study in humans in which an increased insulin action on whole body glucose uptake and adipose tissue lipolysis has been obtained by means of intermittent fasting. This result is in accordance with previously reported in rodents (2, 32). In these studies, fasting every second day increased the insulin sensitivity approximately

sevenfold according to the homeostatic model assessment (2) and decreased the incidence of diabetes (32).

Prolonged fasting for 72 h with minimal physical activity has previously been shown to increase IMTG levels in humans (46). With the present fasting protocol and maintenance of habitual daily physical activity in the fasting periods, we had expected to detect a decrease in IMTG content in the skeletal muscle. The fact that this was not seen and that muscle glycogen content was unchanged could suggest that skeletal muscle is not immediately involved in recognition of acute energy oscillations. There is no doubt, however, that fasting for 20 h while maintaining normal daily physical activity must cause a temporary negative energy balance larger than normally experienced in a daily basis. This is also indicated by our finding of decreased plasma glucose concentrations after 20-h fasting. We did not have the possibility to estimate the hepatic glycogen stores, but from animal studies (17) we must infer that liver glycogen probably also decreased considerably during the 20-h fasting periods. It has previously been suggested that usage of muscle energy depots during fasting would be an evolutionary disadvantage, because it would lessen the capacity for physical performance and hence the ability to provide food (i.e., to hunt and gather) during periods of fasting (6, 45). The present findings support this view.

In contrast to the findings in skeletal muscle, the adipose tissue responded to the changes in energy balance as intermittent fasting changed the plasma concentrations of the adipocyte-specific hormones leptin and adiponectin. However, because we did not measure the energy stores in the adipose
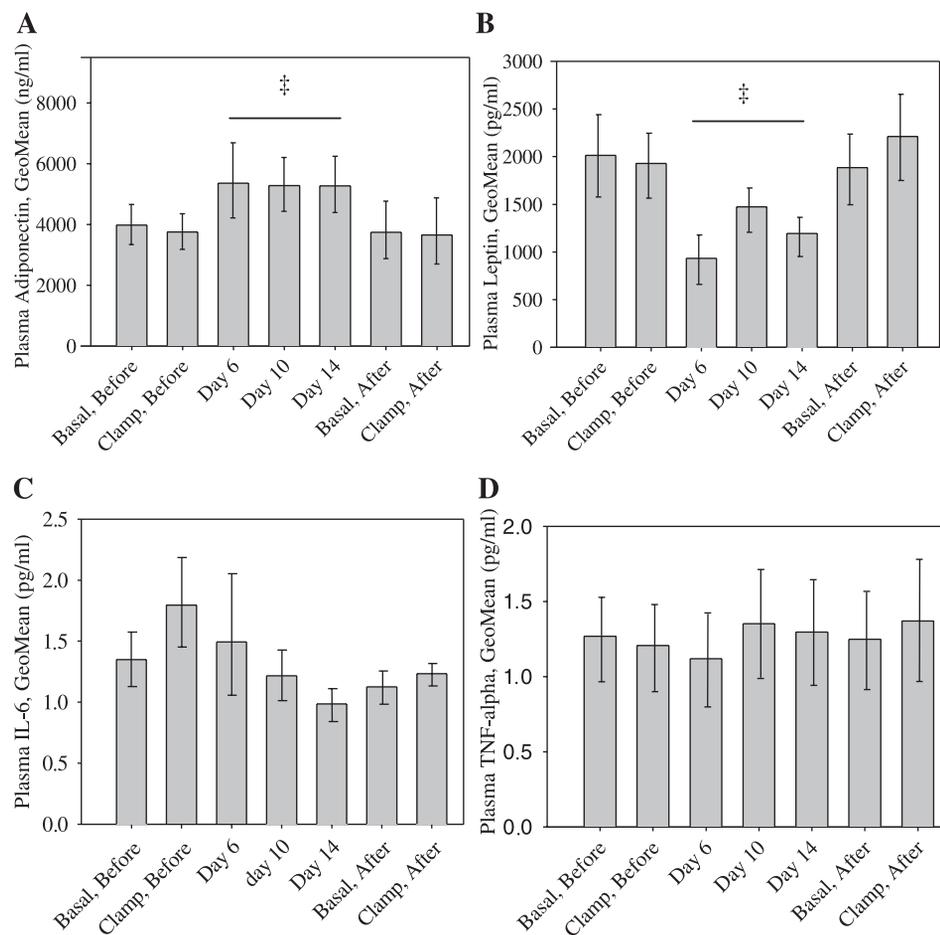
Fig. 5. Plasma concentrations of adiponectin (*A*), leptin (*B*), IL-6 (*C*), and TNF-α (*D*) before and after clamps performed before and after 15 days of intermittent fasting, and after 20 h of fasting on *days 6*, *10*, and *14* of the intermittent fasting intervention. ‡$P < 0.05$ between the sample taken after 20 h of fasting compared with basal samples taken after an overnight fast (8 h). Data are geometric means (GeoMean) ± back-transformed SE.

tissue during the intervention (e.g., by fat cell size), we cannot determine whether the change in adipokine release is merely a secondary response to intermittent fasting or whether the adipose tissue is an active recognizer of energy oscillations.

Blood sampling for measurement of adipokines at basal levels before and after the fasting intervention was performed at 1000 whereas the three samples on *days 6*, *10*, and *14* were taken at 1700. The amount of circulating adiponectin is constant or slightly decreased during daytime (20). Hence, the boosts of 37% we observed after each fasting period are not due to nocturnal variation. Because the plasma adiponectin concentration is positively correlated to insulin sensitivity in humans (8, 23, 29) and adiponectin administration in rodents increases insulin action (9, 38, 48), it seems likely that our finding of increases in circulating adiponectin after each fasting period would be able to exert an insulin-sensitizing effect.

Skeletal muscle content of GLUT4 protein after the overnight fast did not differ before and after the fasting intervention. Future studies will have to determine whether the insulin signaling, e.g., phosphorylation of the insulin receptor substrate, is influenced by fluctuations in energy stores and thereby accounts for the increase insulin action as measured by the clamp method reported herein.

Because 36 h passed between the last fasting period and the last clamp, it seems most likely that the potential insulin-sensitizing effects of adiponectin were due to adiponectin-induced changes in gene expression. This could in turn be mediated through an AMPK activation that further activates

several transcription factors including myocyte enhancing factor that increases GLUT4 expression (24, 27). Another possibility is that the adiponectin boosts peroxisome proliferated-activated receptor-γ (PPAR-γ) expression as seen in 3T3-L1 adipocytes (1). In addition, because PPAR-γ induces adiponectin expression (16), it can be speculated that fasting starts a positive feedback loop that results in increased levels of both circulating adiponectin and PPAR-γ. Both are known to increase the insulin sensitivity.

A considerable increase in plasma FFA concentrations (5-fold) may raise the amount of circulating adiponectin slightly (43), and glucocorticoids positively regulate adiponectin gene expression (21). FFA and glucocorticoid increase during fasting, but in previous studies no effect of fasting on circulating adiponectin was seen (19, 49). Apart from differences in increases of FFA and glucocorticoids, different analysis methods used [RIA vs. ELISA (present study)] may recognize different isoforms of adiponectin and thereby account for the discrepancy.

Leptin exhibits nocturnal differences with a peak during the night (2400–0800), whereas there is no difference between 1000 and 1700; if anything, plasma leptin concentrations are slightly higher at 1000 (40). In accordance with previous findings (19, 41, 49), we found a decrease in circulating leptin after 8–20 h of fasting. This decrease most likely reflects a state of energy deficiency and is probably not involved in the increased insulin action we have found in the present study.
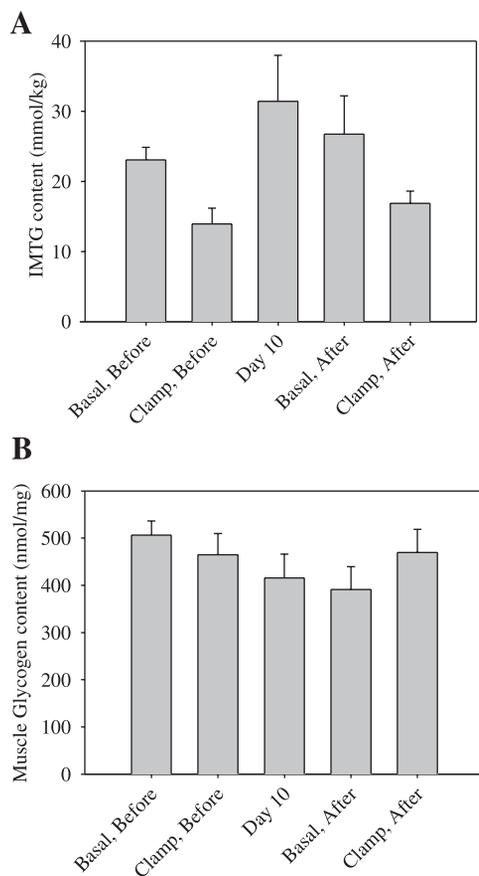
METABOLIC EFFECTS OF INTERMITTENT FASTING

A

B

Fig. 6. Muscle content of triacylglycerol (IMTG) (*A*) and glycogen (*B*) at both basal and insulin-stimulated state before and after intermittent fasting for 15 days, as well as after 20-h fasting on *day 10*. Average concentrations of IMTG decreased ($P = 0.04$) with insulin stimulation when data from both clamps are included. Data are means $\pm$ SE.

A

B

Fig. 7. Muscle content of PGC-1α mRNA normalized to large ribosomal protein P0 (RPLP0; *A*) at both basal and insulin-stimulated state before and after intermittent fasting for 15 days, as well as after 20-h fasting on *day 10*. Muscle content of GLUT4 protein normalized to desmin (*B*) in the basal state before and after the intermittent fasting intervention. AU, arbitrary units. Data are geometric means $\pm$ back-transformed SE.

The mechanism by which physical training increases whole body insulin sensitivity is not known in detail. It has previously been shown that in muscle the effect is mediated via local contraction dependent mechanisms (11–13), and this could include exercise-induced oscillations in local energy stores. However, the insulin-sensitizing effects of exercise and intermittent fasting may not exert their effects via the same pathway. Although the local effect of exercise is well proven (there is no transfer of training-induced increase in insulin sensitivity to nontrained muscle), it is less likely that the effect of intermittent fasting is a local, muscle phenomenon. Thus even though we were not able to detect changes in muscle glycogen and triglyceride content after 20-h fasting, the intervention may still have exerted the effects via oscillations in other energy stores (e.g., in adipose tissue or liver). The finding of decreased leptin concentrations corresponding to the intermittent fasting verifies that adipocyte metabolism was influenced by the intervention.

We did not find an effect of intermittent fasting on muscle PGC-1α mRNA levels. In contrast, PGC-1α mRNA increases with acute exercise (34, 47) and is suggested to be involved in the enhancement of insulin-mediated glucose uptake after exercise training (28, 39). Thus PGC-1α may represent a step at which the insulin enhancement actions of exercise training and intermittent fasting diverge.
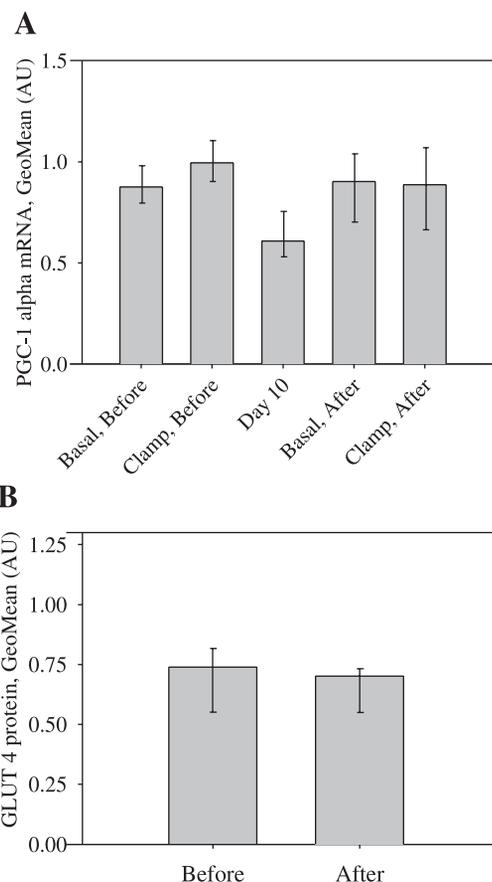
Whole body insulin-mediated glucose uptake was estimated by the euglycemic hyperinsulinemic clamp technique. Even though this method is a standard for measuring insulin action, day-to-day coefficient of variation has been reported to vary between 2.4 and 15% (4, 36, 42). Part of the observed effect of
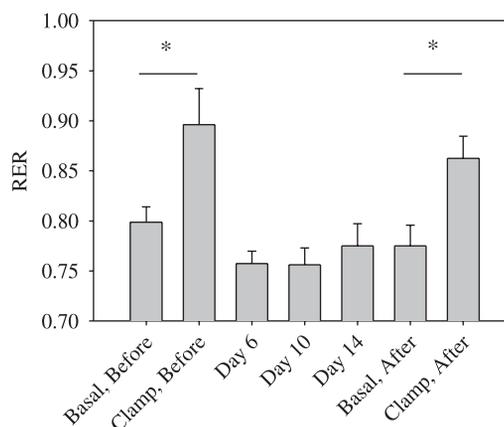

Fig. 8. Respiratory exchange rate (RER) at the basal and insulin-stimulated state before and after a period of intermittent fasting, and after 20 h of fasting on *days 6*, *10*, and *14*. *$P < 0.05$ increase with insulin stimulation. Data are means $\pm$ SE.

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the intervention may therefore be due to biological and instrumental variation.

It is important to note that, in the present study, the subjects maintained their body weight throughout the intervention period, and percent body fat did not change with intermittent fasting. Thus, in contrast to previous studies using alternate-day fasting (22), the subjects in the present study kept their body weight by following the dietary instructions of eating abundantly every other day. It is well known that insulin sensitivity can be influenced by long-term profound changes of macronutrients in the diet. However, because the subjects were instructed to maintain their usual diet habits (although increasing the amount of food), it is unlikely that eventual minor changes in the macronutrient mix during 8 nonconsecutive days (i.e., the nonfasting days) would influence insulin sensitivity.

Furthermore, the increased insulin action after the intervention was not the result of the last fasting period because from the last fasting period until the beginning of the overnight fast the subjects were allowed to eat for 30 h during which they consumed at least 250 g of carbohydrates. Muscle glycogen was not different between the pre- and postintervention clamps, testifying that carbohydrate loading was sufficient before each clamp experiment.

In keeping with previous findings (3), we observed a decrease in IMTG with insulin stimulation. At first glance this seems counterintuitive. However, during insulin stimulation the FFA supply to the skeletal muscle decreases dramatically, and because some skeletal muscle FFA oxidation is still present (RER values of 0.90 ± 0.04 before and 0.86 ± 0.02 after the fasting intervention), it seems arguable that FFA is provided by the IMTG pool, which accordingly will decrease.

In conclusion, the findings that intermittent fasting increases insulin sensitivity on the whole body level as well as in adipose tissue support the view that cycles of feast and famine are important as an initiator of thrifty genes leading to improvements in metabolic function (6). We suggest that a fasting-induced increase in circulating adiponectin is at least partly responsible for this finding. The change in adiponectin, together with changes in plasma leptin with fasting, underlines the important role of the adipose tissue in recognizing the oscillation in energy stores. Finally, the data indicate that intermittent fasting and physical training may increase insulin action via different mechanisms because muscle energy stores did not change with the present fasting intervention.

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