Influence of fasting on carbohydrate and fat metabolism during rest and exercise in men

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Knapi, Joseph J., Carol N. Meredith, Bruce H. Jones, Linda Suek, Vernon R. Young, and William J. Evans. Influence of fasting on carbohydrate and fat metabolism during rest and exercise in men. J. Appl. Physiol. 64(5): 1923-1929, 1988.—Metabolic effects of an overnight fast (postabsorptive state, PA) or a 3.5 day fast (fasted state, F) were compared in eight healthy young men at rest and during exercise to exhaustion at 45% maximum O₂ uptake. Glucose rate of appearance (Ra) and disappearance (Rd) were calculated from plasma glucose enrichment during a primed, continuous infusion of [6,6-³H]glucose. Serum substrates and insulin levels were measured and glycogen content of the vastus lateralis was determined in biopsies taken before and after exercise. At rest, whole-body glucose flux (determined by the deuterated tracer) and carbohydrate oxidation (determined from respiratory exchange ratio) were lower in F than PA, but muscle glycogen levels were similar. During exercise, glucose flux, whole-body carbohydrate oxidation, and the rate of muscle glycogen utilization were significantly lower during the fast. In the PA state, glucose Ra and Rd increased together throughout exercise. However, in the F state Ra exceeded Rd during the 1st h of exercise, causing an increase in plasma glucose to levels similar to those of the PA state. The increase in glucose flux was markedly less throughout F exercise. Lower carbohydrate utilization in the F state was accompanied by higher circulating fatty acids and ketone bodies, lower plasma insulin levels, and the maintenance of physical performance reflected by similar time to exhaustion.

EXERCISE during a brief or prolonged fast may be undertaken as therapy for obesity, under circumstances of natural or manmade disasters, in military environments, or under other conditions in which physical exertion is necessary despite an absence of food. Short-term fasting (24 h) results in a depletion of liver glycogen (21, 28) coupled with declining plasma glucose and insulin levels (7, 29). In contrast, intermediate fasting periods of 3–7 days appear to result in glucose homeostasis. Plasma glucose, plasma insulin, and muscle glucose uptake decline over 3 days, then stabilize at a lower level (7, 29, 30). Whole-body glucose flux and hepatic glucose release are also lower, but gluconeogenesis increases compared with both a brief or very prolonged fast (14, 32). Plasma free fatty acid and glycerol levels stabilize at elevated levels compared with a 24-h fast, although ketone body mobilization and oxidation continue to increase (7, 29).

Prolonged low-intensity exercise produces metabolic effects that resemble an accelerated fast. There is increased gluconeogenesis (1), enhanced fatty acid mobilization and oxidation (15, 17), and enhanced hepatic and muscle glycogenolysis (20, 21). In contrast to the fasting state, glucose flux increases (26), reflecting the accelerated glucose utilization rate.

Studies on the combined effects of brief fasting and moderately intense aerobic exercise have shown that the lower carbohydrate oxidation in the fasted state is maintained throughout exercise and that levels of free fatty acids increase even further, but the fast has no apparent sparing effect on muscle glycogen (8, 25). Prolonged self-paced exercise by relatively untrained men is typically performed at a low intensity of about 45% maximum O₂ uptake (VO₂ max) (10). These conditions of exercise combined with a fast of several days may be expected in a military environment. The present study was designed to investigate fuel utilization, by use of an isotopic tracer and conventional techniques, during exercise after an overnight fast and after a 3.5-day fast.

METHODS

Subjects. Eight male soldiers participated in this study after giving written voluntary consent. Their physical characteristics are shown in Table 1. Subjects had performed no regular exercise program before the study. Body fat was estimated from skinfolds (9), muscle mass from 24-h urinary creatinine (18), muscle fiber type from histochemical analysis of myofibrillar ATPase (6), and VO₂ max using a discontinuous incremental cycle ergometer protocol.

Study design. The study was approved by the Human Use Review Committees of the Army Research Institute of Environmental Medicine and the Massachusetts Institute of Technology.

All eight subjects were tested in both a postabsorptive (PA) state (14 h without food) and a fasted (F) state (3.5 days without food). Four men were studied first in the PA state, and four were studied first in the F state. Testing in these two states were separated by 14–35 days. Four days before each test subjects consumed a balanced
TABLE 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Ht, cm</th>
<th>Wt, kg</th>
<th>Body Fat, %</th>
<th>Muscle Mass, %</th>
<th>Type II Muscle Fibers, %</th>
<th>( \overline{\text{\text{Vo}}_2\text{max}} ) l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.5±2.5</td>
<td>172.5±5.4</td>
<td>76.8±14.1</td>
<td>16.4±4.6</td>
<td>43.1±15.8</td>
<td>70.3±7.9</td>
<td>3.25±0.62</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \overline{\text{Vo}}_2\text{max} \), maximum oxygen uptake.

diet consisting of 12% protein calories, 53% carbohydrates calories, and 34% fat calories. Meals were provided by a registered dietitian, and subjects' consumption was monitored by the investigators.

Before testing in the PA state, subjects spent the night in a metabolic ward and were studied the following morning. Before testing in the F state subjects lived in the metabolic ward for 3 days and were studied on the morning of the 4th day. Subjects were under the constant supervision of the nursing staff and were allowed to consume only distilled water and selected herbal teas. A 24-h urine sample was collected in the first 32 h of fasting and analyzed for urinary creatinine.

Testing in the PA and F state was identical, and the design is shown in Fig. 1. Subjects rested in bed for 3 h, then exercised on a cycle ergometer at 45% \( \overline{\text{Vo}}_2\text{max} \) until exhaustion. Throughout rest and exercise, subjects received a continuous intravenous infusion of \([6,6-^2\text{H}]-\)glucose (0.28 \( \mu\)mol·kg\(^{-1}\)·min\(^{-1}\)) after a bolus dose of 22.4 \( \mu\)mol/kg (80:1 ratio between the primer and infusion rate). Isotopic enrichment of the infused tracer was 98%. Samples of expired gas and venous blood were obtained at intervals shown in Fig. 1. At rest, a single expired gas sample was collected for 5 min in a vinyl Douglas bag. During exercise duplicate gas samples were collected for 30 s and averaged. Near the end of the rest period a muscle biopsy sample was obtained from the vastus lateralis, and a second biopsy was obtained from the same site 30 min after exercise.

Analysis of samples. Expired gas samples were analyzed for \( \text{O}_2 \) (Applied Electrochemistry, model S-3A) and \( \text{CO}_2 \) concentration (Beckman, model LB 2). Gas volumes were measured using a tissot spirometer. The respiratory exchange ratio (R) was calculated as \( \overline{\text{V}}\text{CO}_2/\overline{\text{Vo}}_2 \) without correction for urinary nitrogen loss.

Metabolites and insulin were analyzed in aliquots of blood, plasma, or serum. Lactate was determined on whole blood (Roche AutoAnalyzer). Glucose was determined by the glucose oxidase method (Beckman glucose analyzer). Serum aliquots were analyzed for glycerol (40), alanine (24), \( \beta \)-hydroxybutyrate (\( \beta \)-OHB,41) and free fatty acids (FFA, 23). Insulin was determined by radioimmunoassay (Serono Laboratories kit). Isotopic enrichments of plasma glucose were measured in butyl-borurate acetate derivatives by electron impact gas chromatography and mass spectrometry (4).

Muscle biopsy samples were rapidly separated into three to five smaller samples and were stored in liquid nitrogen until analyzed for glycogen content (31). A sample from the PA session was sectioned and stained for myofibrillar ATPase for muscle fiber type classification (6).

Calculation and statistical analysis. Glucose flux was defined as the sum of glucose production and utilization (39). Whole-body rates of glucose appearance and disappearance were determined by use of the non-steady-state equations of Steele (38) modified by Radziuk et al. (34)

\[
Ra = \frac{i \cdot p \cdot V \cdot ((G_{t1} + G_{t2})/2)[(I_{Et1} - I_{Et1})/\Delta t]}{(I_{Et1} + I_{Et2})/2}
\]

and

\[
Rd = Ra - [pV(G_{t2} - G_{t1})/\Delta t]
\]

where Ra is the rate of appearance (\( \mu\)mol·kg\(^{-1}\)·min\(^{-1}\)); Rd is the rate of disappearance (\( \mu\)mol·kg\(^{-1}\)·min\(^{-1}\)); i is

![Fig. 1. Test design in both postabsorptive and fasted states. EXH, exhaustion; \( \text{Vo}_2 \), \( \text{O}_2 \) uptake; \( \text{Vco}_2 \), \( \text{CO}_2 \) production; \( \overline{\text{Vo}}_2\text{max} \), maximum \( \text{O}_2 \) uptake.](http://jap.physiology.org/content/225/22/1924/F1)

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the infusion rate (μmol·kg⁻¹·min⁻¹); p is the pool fraction (a constant = 0.65); V is the volume distribution of glucose [taken at 25% of body wt (34)] (ml/kg); Gt₁ is the serum glucose at time 1 (μmol/ml); Gt₂ is the serum glucose at time 2 (μmol/ml); IE₀ is the plasma isotopic enrichment of [6,6-²H]glucose at time 1 [atoms excess (AE)]; IE₂ is the plasma isotopic enrichment of [6,6-²H] glucose at time 2 (AE); and Δt is the time over which measurements occurred (min).

Resting and exercising data were analyzed separately using appropriate repeated measures analysis of variance statistics. When significant differences were found in multilevel variables, Tukey’s test was used to isolate the differences. The 0.05 level was chosen to indicate statistical significance. Values are reported as means ± SE.

RESULTS

Exhaustion occurred at 139 ± 13 min in the PA state and 118 ± 18 min in the F state (22). This difference was not statistically significant (P < 0.09).

Muscle glycogen values are shown in Fig. 2. PA and F muscle glycogen levels did not differ before (P < 0.41) or after (P < 0.17) exercise. However, there was a difference in the percent change in muscle glycogen from before exercise to after exercise: there was a 44 ± 6% decrease in the PA state and a 28 ± 4% decrease in the F state (P < 0.01). Average glycogen utilization rates during exercise, calculated as the difference in glycogen before and after exercise divided by the time to volitional fatigue on the cycle ergometer, were 0.31 ± 0.04 μmol·g⁻¹·min⁻¹ in the PA state and 0.19 ± 0.02 μmol·g⁻¹·min⁻¹ in the F state (P < 0.01).

The pattern of glucose enrichment at rest and during exercise is shown in Fig. 3. The data show that isotopic steady state was achieved during the hour of rest before exercise in both the PA and F states. During exercise, analysis of glucose enrichment over time showed that subjects were not at isotopic steady state and non-steady-state equations were used to calculate glucose kinetics (34, 38).

Glucose flux was consistently lower in the F state compared with the PA state, as shown in Fig. 4. For statistical purposes exercise was arbitrarily divided into a period of 0–60 min and a period of 60 min to exhaustion. At rest, the Ra and Rd were ~23% lower in the F state compared with the PA state (P < 0.001). During exercise in the F state, the rate of increase in glucose flux was lower (P < 0.001) and the pattern of change in the Ra and Rd was different compared with the PA state. In the PA state the Ra and Rd changed together at all times. In the F state the Ra and Rd were of similar magnitude at rest and during the 2nd h of exercise; however, during the 1st h of exercise the Ra was greater than the Rd (P < 0.01).

Figure 5 depicts the changes in substrates in the PA and F sessions over time. During the fast, blood glucose at rest was 3.93 ± 0.18 mM, which was 18% lower than in the PA state (P < 0.001). During PA exercise blood glucose remained constant through the 1st h, then de-

![Fig. 2. Mean muscle glycogen content in postabsorptive (PA) and fasted (F) states before and after exercise. Vertical bars, ±SE.](image-url)
CARBOHYDRATE METABOLISM DURING FASTING AND EXERCISE

FIG. 5. Mean changes in serum or blood substrates in postabsorptive (PA) and fasted (F) states at rest and during exercise. Vertical bars, ±SE. Negative times, time before exercise. ----, Time to exhaustion differed for each subject. △, Glycerol; ○, free fatty acids; ●, β-hydroxybutyrate; △, alanine; ○, lactate; ●, glucose.

decreased at exhaustion to below resting values ($P < 0.01$). Conversely, in the 1st h of F exercise, glucose increased progressively, and at 60 min blood glucose values were similar in the F and PA states. Glucose had again decreased by exhaustion but not below resting values for the F state.

Blood lactate levels were higher in the F state than in the PA state at rest and throughout exercise ($P < 0.01$). This difference became greater within the first 10–30 min of exercise. Serum alanine did not differ between the PA and F states at rest or during exercise. However, in the F condition there was a progressive rise in alanine; levels became greater than resting values at 30 min ($P < 0.05$) and stayed elevated over resting values for the rest of the exercise period. There was a significant correlation between serum alanine and blood lactate during exercise in the PA state ($R = 0.85; P < 0.01$) and in the F state ($R = 0.96; P < 0.001$).

Serum FFA, glycerols, and β-OHB values were higher in the F state compared with the PA state both at rest and during exercise ($P < 0.01$). For FFA the pattern of change in the two conditions was identical although of greater absolute magnitude in the F state. At 10 min of exercise FFA declined from $0.30 \pm 0.05$ to $0.23 \pm 0.04$ mmol/l in the PA state and from $0.78 \pm 0.07$ to $0.53 \pm 0.07$ mmol/l in the F state. For β-OHB there was no change in the PA state until exhaustion, when values were elevated ($P < 0.01$). During F exercise β-OHB declined sharply in the first 10 min ($P < 0.001$) and remained depressed throughout exercise.

Serum insulin values were lower in the F state than in the PA state both at rest and during exercise ($P < 0.03$), as shown in Fig. 6. The R was also consistently lower in the F state both at rest and exercise ($P < 0.05$), as shown in Fig. 7. The final R at exhaustion in the F state was higher than the 60-min value ($P < 0.04$).

DISCUSSION

The major finding of the present study was that the substantial alterations that have been described in whole-body fuel utilization with fasting (3, 7, 14, 32) were maintained during submaximal exercise. By use of isotopically labeled glucose together with other techniques, it was shown that during F exercise there was a greater use of fat as a substrate and decreased production and utilization of carbohydrates with no significant effect on
endurance time.

Metabolism of carbohydrates and fats at rest. Carbohydrate utilization at rest was markedly decreased by the 3.5-day fast. However, the muscle glycogen level was not significantly reduced. This contrasts with the 24% muscle glycogen decline reported for a single subject after a 3-day fast (20).

Whole-body glucose Ra and Rd decreased by ~23% after the 3.5-day fast, whereas circulating glucose decreased 18% and insulin 16%. The glucose Ra in the F state was 434 ± 26 μmol·kg⁻¹·h⁻¹, which is similar to values obtained in another tracer study (32) but is higher than the value of 353 μmol·kg⁻¹·h⁻¹ calculated for splanchnic output from arteriovenous difference in subjects fasted for 3 days (14). Since liver glycogen stores are essentially depleted after the first day without food (28), glucose appearance in the F state would be the product of hepatic and possibly renal gluconeogenesis (14, 29). In the present study fasting gluconeogenesis supported a glucose Ra equal to 77% of the Ra in the PA state.

It is likely that increased fatty acid availability was the main factor accounting for the lower glucose utilization and oxidation. The decrease in glucose Rd was accompanied by a lower rate of whole-body carbohydrate oxidation suggested by a decrease in the R from 0.84 ± 0.02 to 0.79 ± 0.02. Circulating glycerol, FFA, and β-OHB were elevated about 1.6, 2.6, and 4.6 times, respectively, over PA values. Studies of arteriovenous differences across peripheral tissues have shown that glucose uptake declines as the availability of these substrates increases (30). Even in a postabsorptive state, a 4-wk adaptation to a ketogenic diet, producing similarly high levels of FFA, ketone bodies, and lactate, has been shown to lead to a 29% reduction in the rate of whole-body [¹³C]glucose oxidation (33).

Metabolism of carbohydrates and fats during exercise. Previous studies on moderately intense exercise after a 24-h fast have found increased reliance on fats and decreased utilization of carbohydrates as inferred from changes in plasma substrates and the R (8, 25); however, there was no change in muscle glycogen utilization (25). The present study on low-intensity exercise after a 3.5-day fast has quantified a 32% reduction in glucose Ra, a 40% reduction in Rd, and a 40% reduction in the rate of muscle glycogen utilization. In addition, the declines in FFA and β-OHB early during exercise were of greater magnitude than those found by Dohm et al. (8), suggesting the longer fasting period resulted in greater reliance on lipids (15, 26) despite the lower exercise intensity.

The present study using untrained subjects found exercise times to exhaustion did not differ between the PA and F states. Loy et al. (25), using cycle exercise at >65% VO₂ max, found that a 24-h fast significantly reduced time to fatigue in trained cyclists. Training state alone cannot explain this difference, since Dohm et al. (8), using trained subjects, found no difference in treadmill exercise time to exhaustion at 70–75% VO₂ max after a 24-h fast. One important cause of fatigue is muscle glycogen depletion (20). Loy et al. (25) found that a 24-h fast did not change muscle glycogen utilization despite a reduction in exercise time. In contrast, the present study showed significantly less muscle glycogen utilization after the 3.5-day fast despite similar exercise time to fatigue. Phinney et al. (33) reported that exercise times to exhaustion at 65% VO₂ max after 4 wk of adaptation to a eucaloric ketogenic diet were unchanged despite preexercise muscle glycogen values that were substantially reduced. It should be noted that postexercise glycogen values in the present study (50–60 μmol/g) were similar to those found at fatigue in trained men in other studies (25, 33), supporting the hypothesis that muscle glycogen was a determinant of fatigue.

In the present study, the 3.5-day fast changed the magnitude and pattern of glucose appearance and disappearance during exercise. In the PA state, glucose Ra and Rd increased by a factor of 2.5 by the end of 90 min of exercise and remained high until fatigue. However, in the F state, glucose Ra and Rd at fatigue had increased only by a factor of 1.9 compared with already low resting values. Early in F exercise, the increase in Rd did not match the increase in Ra: glucose Rd did not change significantly from resting values, whereas glucose Ra increased 52%, leading to blood glucose levels 20% higher than at rest. Similar increases in circulating glucose in the first 30 min of exercise have been reported in the postabsorptive state after adaptation to a ketogenic diet (33) or after a 24-h fast in subjects adapted to a normal diet (8). Also, during the 1st h of F exercise, there was an increase in glycerol, a marked decline in free fatty acids and ketone bodies, and no increase in peripheral glucose uptake. These changes suggest that the higher energy needs of exercising muscles were met mainly by increased lipid mobilization, uptake, and oxidation (3, 15, 17). During F exercise, the R remained lower than in the postabsorptive state, supporting an increased reliance on fat oxidation in the F state. However, since gluconeogenesis and ketone body utilization respectively decrease or increase the R (12), the effects of fasting plus exercise on substrate oxidation estimated from respiratory data must be interpreted with caution.

The high levels of blood lactate and alanine observed during F exercise are consistent with increased availability and oxidation of FFA and ketone bodies. In muscles with a large capacity for oxidizing fats, high levels of FFA, and ketones can inhibit glucose oxidation (35, 36). Acetyl-CoA from fat oxidation inhibits pyruvate oxidation, forcing pyruvate carbons into alternate metabolic pathways and resulting in production of alanine, lactate, and other substances (35).

In the F state, the glucose Ra increased during the 1st h of exercise, despite unchanging glucose utilization. This could be due to an increased availability of gluconeogenic precursors coupled with enhanced gluconeogenic capacity. Gluconeogenesis in liver and kidney increases with a greater supply of precursors (5, 11). Liver gluconeogenesis is accelerated by both short-term fasting (14) and exercise (27): activities of the key hepatic enzymes pyruvate carboxylase (13) and phosphoenolpyruvate carboxykinase (37) are increased. Renal gluconeogenesis is stimulated by the metabolic acidosis (2) that can be induced by fasting (16) or acute exercise (19).
After the 1st h of F exercise, the balance between the glucose Ra and Rd was restored. Despite a further increase in circulating FFA and decrease in insulin, glucose uptake, and whole-body carbohydrate oxidation tended to increase. The reasons for this are not entirely clear but may be related to the normalization of blood glucose by the end of the 1st hour of exercise.

At an exercise intensity of 45% \( \dot{V}O_2 \text{max} \), a 3.5-day fast did not lead to hypoglycemia or early fatigue. The reduced utilization of muscle glycogen and blood glucose during exercise, together with the exercise-induced increase in gluconeogenesis, tended to shift circulating glucose levels toward values found in the postabsorptive state. This study showed that low-intensity exercise can be maintained after several days without food by a substantial change in fuel utilization involving increased fat utilization, muscle glycogen sparing, and reduced glucose flux.

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