Prolonged fasting significantly changes nutrient oxidation and glucose tolerance after a normal mixed meal

TRACY J. HORTON AND JAMES O. HILL
Center for Human Nutrition and Department of Pediatrics, University of Colorado Health Sciences Center, Denver, Colorado 80262

Received 26 October 1999; accepted in final form 3 August 2000

Horton, Tracy J., and James O. Hill. Prolonged fasting significantly changes nutrient oxidation and glucose tolerance after a normal mixed meal. J Appl Physiol 90: 155–163, 2001.—The aim of this study was to establish the experimental paradigm of fasting, followed by refeeding, to investigate individual differences in nutrient partitioning. Eight non-obese men were fed a normal meal (25% of daily energy requirements) on two occasions, after an overnight (13-h) fast and after a prolonged (72-h) fast. During the entire fasting period, subjects were resident in a whole room indirect calorimeter, and blood samples were drawn periodically. Because no other food was consumed over the 12 h after either meal, negative energy balance was observed after the overnight and prolonged fast. Postprandial carbohydrate oxidation was significantly reduced after the 72- vs. 13-h fast (P < 0.0001), whereas fat oxidation was significantly increased (P < 0.0001). Interestingly, carbohydrate balance was positive after the prolonged fast but negative after the overnight fast (24 ± 17 vs. −57 ± 16 g/12 h, respectively; P < 0.001), whereas fat balance was negative under both conditions (−78 ± 7 vs. −47 ± 8 g/12 h, respectively; P < 0.002). With 72 h of fasting, the glucose and insulin excursions in response to the mixed meal were significantly greater compared with the 13-h fast (P < 0.001). In conclusion, prolonged fasting resulted in a significant decrease in carbohydrate oxidation and an increase in fat oxidation, after a normal mixed meal, in healthy men. This was associated with a significant decrease in glucose tolerance. Because circulating free fatty acids were greatly elevated at all times after the prolonged fast, these may be mediating some of the changes in postprandial metabolism.

Understanding factors that determine the balance between fat and carbohydrate oxidation can ultimately improve our understanding of the development of obesity. The energy status of an individual is one important factor that influences the partitioning of ingested lipid and carbohydrate between oxidation and storage. This is relevant because people regularly undergo periods of fasting followed by refeeding. The severity of fasting and the degree of refeeding can vary enormously from day to day and between different individuals. How an individual responds to such changes in energy and nutrient status may have important long-term consequences with respect to the retention of body fat.

Our laboratory previously showed that with excess energy intake (overfeeding) ingested carbohydrate is preferentially oxidized over ingested fat (17). This results in a significantly positive fat balance by virtue of the prioritization of carbohydrate for oxidation. Fasting represents the opposite extreme of overfeeding, where energy requirements are satisfied predominantly through the oxidation of endogenous fat stores (5, 28). Consumption of a meal after a typical overnight fast results in a rapid transition from predominantly fat oxidation to carbohydrate oxidation (13). Prolonged fasting represents an even greater perturbation in energy status. Because glycogen stores are almost completely depleted with extended fasting, there is an even greater dependence on the oxidation of endogenous fat stores (5, 28). Interestingly, ingestion of glucose under these conditions results in a decrease in carbohydrate oxidation compared with an overnight fast (11). Whether this decrease in carbohydrate oxidation is also observed with ingestion of a normal mixed meal after prolonged fasting is not known. This is relevant to our understanding of factors determining nutrient partitioning and ultimately energy balance.

The circulating substrate and hormonal milieu can play a role in determining the partitioning of ingested nutrients between oxidation and storage. After a prolonged fast, there are significant changes in many circulating parameters that could affect postprandial nutrient metabolism. Specifically, extended fasting results in significant elevations in circulating free fatty acids (FFAs), glycerol, and ketone bodies with a fall in blood glucose (5, 28, 41). Lipolytic hormones increase (norepinephrine, cortisol, and growth hormone) (28, 30, 41), while there is an increase in gluconeogenesis (5, 12) and a significant impairment of insulin’s action on lipolysis (19, 39) and glucose metabolism (5, 11, 12). Imposing a refeeding meal on this metabolic environment may, therefore, result in a very different nutrient and hormone response relative to an overnight fast. This could subsequently affect the pattern of postprandial fuel oxidation and nutrient balance.
The purpose of this study, therefore, was to characterize the changes in 1) nutrient oxidation and nutrient balance and 2) the circulating substrate and hormone environment in response to refeeding a normal mixed meal after a 72-h fast vs. an overnight fast (13 h). This study was performed in a group of normal healthy men to establish the experimental paradigm and provide a data set for future comparison to other population groups.

METHODS

Subjects

Eight male subjects, aged 20–40 yr, were recruited for the study. Subjects were excluded if they smoked; had a history of diabetes, cardiovascular disease, or other major health problems; had a body mass index of >27 kg/m²; and/or had percent body fat >27%. The study protocol was approved by the University of Colorado Committee Institutional Review Board for the Protection of Human Subjects. All subjects read and signed a subject consent form before admission into the study. Subject characteristics are shown in Table 1.

Preliminary assessments. Body composition was determined by measuring body density using underwater weighing as previously described (18). Percent body fat was estimated from body density using the revised equation of Brozek et al. (4).

RESTING METABOLIC RATE. Resting metabolic rate (RMR) was measured using indirect calorimetry via a metabolic cart system (model 2900, Sensormedics, Yorba Linda, CA). Subjects were tested in the morning after a 12-h fast. After 30 min of rest, a 15- to 20-min measurement of metabolic rate was made using a ventilated canopy. Gas concentrations were measured in the air exiting the hood. O₂ consumption (VO₂) and CO₂ production were used to calculate metabolic rate (40). The RMR value was used to determine energy intake of subjects during the period of prestudy diet control.

HEALTH AND PHYSICAL EXAMINATION. A health and physical examination was performed on subjects to confirm that there was no medical reason for their exclusion from the study.

INTRAVENOUS GLUCOSE TOLERANCE TEST. A frequently sampled intravenous glucose tolerance test (FSIGTT), using the tolbutamide protocol, was administered to subjects after an overnight fast (42). After baseline blood sampling, a glucose bolus was administered (0.3 g/kg body wt), followed 20 min later by the tolbutamide bolus (100 mg). Twenty-seven blood samples were drawn over the next 3 h for measurement of glucose and insulin concentrations. The FSIGTT gives both the insulin sensitivity index and glucose effectiveness as calculated from the MINIMOD computer program (29).

Premature diet and exercise control. Subjects were fed a controlled diet for 5 days before the study. All food was prepared by the General Clinical Research Center (GCRC) diet kitchen at the University of Colorado Health Sciences Center, and subjects were required to consume breakfast in the GCRC with other food prepared to take away. No other food was permitted, and subjects were required to consume all the food given. The only optional part of the diet was two food modules (840 kJ each), one or both of which the subjects could eat if they were hungry. The diet composition was 30% fat, 15% protein, and 55% carbohydrate, and initial energy intake was calculated at 1.6 × RMR and adjusted, if necessary, to maintain a stable body weight. Subjects were allowed to follow their usual activity routine for the first 4 days of the diet, and on the last day they refrained from planned exercise.

Study period. Subjects spent the evening before the study in the GCRC and consumed their last meal by 2000. On the morning of the study, subjects were awakened at ~0700. An intravenous flexible catheter (18–20 gauge) was placed in a forearm vein and flushed with normal saline. At ~0755, subjects emptied their bladder and then collected all their urine for the remainder of the study. Subjects entered a whole room indirect calorimeter, located on the GCRC, at ~0800 and remained resident for the next 84 h (3.5 days). Between 0700 and 0800 each day, subjects exited the calorimeter and were allowed to shower but not to leave the GCRC. Calorimetry instrumentation was recalibrated during this time. At 0900 of the first day [time (t) = 0 h; 13-h fasted], subjects received a breakfast meal composed of real food. The energy content of the meal was 25% of each subject’s normal daily energy intake and averaged 3,150 ± 953 kJ. Nutrient composition was 15% protein, 54% carbohydrate, and 31% fat. The meal was consumed within 15 min. No further food was consumed for the next 72 h. Consumption of water was encouraged, and noncaloric, noncaffeinated beverages were permitted. Subjects occupied themselves with sedentary pursuits during the calorimeter stay and performed specified light walking and stepping activities in the afternoon and early evening. At 0900 of the fourth day (t = 72 h), subjects received the same exact breakfast meal they consumed on the first day. This was again consumed within 15 min. At 2300 that evening, subjects exited the calorimeter and the study was ended.

Whole room indirect calorimeter. This has been previously described in detail (37). The calorimeter allows continuous measurement of whole body VO₂ and CO₂ expiration.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Body Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Body Fat, %</th>
<th>Fat-Free Mass, kg</th>
<th>VO₂max, ml·kg⁻¹·min⁻¹</th>
<th>S₇₅, 10⁻⁴·min⁻¹</th>
<th>S₀, 1·µU⁻¹·ml⁻¹</th>
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<td>61.9</td>
<td>40.8</td>
<td>4.35</td>
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</table>

BMI, body mass index; VO₂max, maximal O₂ consumption; S₇₅, insulin sensitivity index; S₀, glucose effectiveness.

BMI, body mass index; VO₂max, maximal O₂ consumption; S₇₅, insulin sensitivity index; S₀, glucose effectiveness.
over the duration of stay. Accuracy of the differential O₂ and CO₂ analyzers is verified by the combustion of propane gas. Propane tests are performed one to two times per month. Measurement of O₂ concentrations are accurate to within ±4%, and CO₂ measurements are accurate to within ±3%.

Energy expenditure and the oxidation of carbohydrate and fat were calculated from respiratory gas exchange (20) from 0900 to 2300 on days 1 and 4. The 1-h preprandial measurements (0800–0900) were excluded because this period allowed for equilibration of the room air in the calorimeter and stabilization of the CO₂ concentration. Respiratory gas calculations were made over the 12-h postprandial period (0900–2300). It was necessary to correct the total V̇O₂ over the 12 h for the accumulation of ketones (35). The accumulation of the most abundant ketone, β-hydroxybutyrate (β-HOB), in blood and urine, was used to estimate the quantity of O₂ that was used to generate ketones that were not subsequently oxidized (35). The corrected total V̇O₂ values were then used in the calculation of energy expenditure and nutrient oxidation. Protein oxidation was estimated from urinary nitrogen excretion measured over the respective calorimeter stay. Results of the indirect calorimetry over the duration of the entire study are not presented. This paper only includes indirect calorimetry measurements coinciding with the monitoring of the systemic postprandial response.

The calorimeter has a port constructed in the wall that allows for drawing blood samples. This port is sealed on both the inner and outer sides of the calorimeter wall by detachable perspex plates connected by a screw post. In between the plates is a series of rubber iris ports. When the perspex plates are removed, the person inside the calorimeter can put his forearm through the rubber iris ports. The layering of these iris ports ensures minimal exchange of air between the calorimeter and the outside.

**DETERMINATION OF CIRCULATING HORMONE AND SUBSTRATE LEVELS.**

Blood was sampled immediately before the meal on the first day (t = 0 h) and then every hour up to 6 h postprandially. Subsequent sampling occurred every 12 h up to the refeeding meal on day 4. Blood samples were drawn every 1 h for the next 6 h and then immediately before the subjects exited the calorimeter. Blood samples were drawn from a forearm vein; therefore, the circulating measurements represent both arterial concentrations and venous drainage from the local muscle bed. Approximately 7 ml whole blood were allowed to clot, and the serum was separated after spinning. Whole blood (2.5 ml) was added to 40 µl of preservative (3.6 mg EGTA plus 2.4 mg glutathione in distilled water) for plasma catecholamine determinations. These samples were immediately placed on ice and spun. All plasma and serum samples were stored at −70°C until analysis. Serum glucose was measured enzymatically, in singlecate, using the hexokinase method on an automated Roche COBAS Mira Plus glucose analyzer [intra-assay coefficient of variation (CV) of 0.7%]. Enzymatic assays were used to determine serum glycerol (Boehringer Mannheim Diagnostics, Indianapolis, IN) and FFAs (Wako Chemical USA, Richmond, VA) in singlecate (intra-assay CVs of 7.8 and 1.2%, respectively) using the automated Roche COBAS Mira Plus analyzer. β-HOB (Sigma Diagnostics, St. Louis, MO) was measured in duplicate with intra-assay CV of 10.8%. Catecholamines were determined in duplicate by radioenzymatic assay (intra-assay CVs of 16 and 8% for norepinephrine and epinephrine, respectively) (24). Radioimmunoassays were used to determine serum insulin (Kabi Pharmacia, Piscataway, NJ), cortisol (Diagnostic Products, Los Angeles, CA), and glucagon (Linco Research, St. Louis, MO). Samples were run in duplicate with intra-assay CVs of 10, 6.7, and 9.4%, respectively.

**Data Analysis**

Fasting blood parameters were compared after the 13- vs. 72-h fast, with a paired t-test. The pattern of the postprandial change in blood variables was compared between the 2 days using a repeated-measures analysis of variance with time and day (13- or 72-h fast) as grouping factors. For substrates and hormones, the incremental or decremental area under the curve (AUC) was calculated and compared between meals using a paired t-test. Total energy expenditure, fat, carbohydrate and protein oxidation, and corresponding balances were calculated over the 12-h postmeal and compared between days using a paired t-test.

**RESULTS**

**Body Weight**

Subjects maintained a stable body weight during the period of diet control. Average weight change during this period was <2% of initial body weight (0.65 ± 0.26 kg). All subjects had a significant decrease in body weight from day 1 to day 4 of the fast (average change −3.6 ± 0.5 kg).

**Energy Expenditure and Nutrient Oxidation**

Energy expenditure was not significantly different over the 12-h postprandial period after the prolonged vs. overnight fast (5,792 ± 508 vs. 6,031 ± 462 kJ/12 h, respectively). Because the test meal was the only food consumed during this time, energy balance was negative under both conditions (−2,877 ± 483 vs. −3,079 ± 273 kJ/12 h, respectively). Nutrient oxidation and nutrient balances are shown in Figs. 1 and 2, respectively. There was no difference between the two periods in protein oxidation or protein balance. Carbohydrate oxidation after the 13-h fast plus meal was significantly greater compared with after the 72-h fast (P < 0.001). Consequently, carbohydrate balance was significantly different between the two conditions (P < 0.001), being negative with the 13-h fast (−57 ± 16 g) and positive after the 72-h fast (24 ± 17 g). Postprandial fat oxidation was significantly greater after the prolonged vs. overnight fast (P < 0.002). Fat balance was negative under both conditions but more so after the 72-h fast than after the 13-h fast plus meal (P < 0.002; −78 ± 7 vs. −47 ± 8 g, respectively).

**Circulating Hormone and Substrate Levels**

Table 2 shows the circulating substrate and hormone concentrations after the 13- and 72-h fast. Premeal levels of glucose and insulin were significantly reduced after the 72- vs. 13-h fast, whereas FFA, glucagon, cortisol, norepinephrine, and β-HOB were significantly increased.

**Postprandial substrate and insulin changes.** After the same mixed meal, the glucose and insulin excursions were significantly greater with 72 vs. 13 h of fasting (Fig. 3; incremental AUC, P < 0.001 for both insulin and glucose, Table 3). In addition, the glucose-to-insulin ratio was significantly lower (P < 0.001) after the 72- vs. 13-h fast. As expected, there was a significant decrease in both circulating FFA and glyc-
erol concentrations in response to meal consumption (Fig. 4). The postprandial decremental AUC for FFA was significantly greater after the 72-h fast vs. 13-h fast (Table 3; $P < 0.01$), whereas there was no significant difference in the decremental AUC for glycerol. Although the absolute postprandial concentrations of FFA fell after the meal after the 72-h fast, the absolute concentrations remained elevated for the entire period, relative to the 13-h fast. The pattern of change in β-HOB, and differences between days, paralleled the changes in circulating FFAs, being elevated postprandially after the 72- vs. 13-h fast (Fig. 4).

Postprandial hormone changes. The elevated glucagon, cortisol, and norepinephrine, after 72 h of fasting, decreased postprandially (Figs. 5 and 6). This contrasted to the postprandial changes after 13 h of fasting where there was little change in cortisol and norepinephrine. Glucagon declined and then increased with the prolonged fast, whereas the opposite was observed after the overnight fast. The insulin-to-glucagon ratio (Fig. 5) increased significantly more after the meal after the 72-h fast compared with the 13-h fast. Although epinephrine levels were higher, both pre- and postprandially after the prolonged vs. overnight fast, results were not significantly different between the two measurements (Fig. 6) because of the high variability in concentrations.

**DISCUSSION**

This study demonstrated that both the nutrient balance and the substrate and hormone response to a normal mixed meal are profoundly affected by the duration of fasting. Compared with an overnight fast, 72 h of fasting resulted in a significant reduction in postprandial carbohydrate oxidation but a significant increase in fat oxidation. Despite a similar degree of negative energy balance, over the 12 h after each meal, carbohydrate balance was positive after the 72-h fast compared with negative after the 13-h fast. In addition, the prolonged fast resulted in a profound elevation in the postprandial glucose and insulin responses. This demonstrated that subjects had become glucose intolerant and suggested a decrease in insulin action.

It appears that the extent to which ingested carbohydrate promotes its own oxidation is partly dependent on the energy status of an individual. In contrast to what is observed with overfeeding (17), or with individuals who have been in energy balance (13, 34), prolonged fasting resulted in a decreased total carbohydrate oxidation after a normal mixed meal. It is unlikely that the reduced carbohydrate oxidation after the 72-h fast was due to a decrease in the oxidation of endogenous carbohydrate, because glycogenolysis and gluconeogenesis would have been depressed. Our
be increased when carbohydrate is refed (26). A decrease in oxidative but not nonoxidative glucose disposal after extended fasting (23) would preserve glucose and possibly increase glucose stores in the event that further nutrient ingestion did not occur. How long it takes, with resumption of a regular meal pattern, for the metabolic response to a meal to be normalized would be of interest.

Factors responsible for the different pattern of postprandial lipid and carbohydrate oxidation after the prolonged fast vs. overnight fast likely relate to differences in the circulating substrate and hormonal milieu and the metabolic environment in oxidative tissues. Significantly elevated circulating FFAs were observed both before and after the mixed meal after the 72- vs. 13-h fast. This suggests increased oxidation of endogenous FFAs before, and possibly after, the meal. Under conditions of elevated insulin, for example, during a euglycemic clamp (2, 33) or during an OGTT (22), it has been observed that acutely increasing circulating FFAs significantly decrease whole body carbohydrate oxidation. The elevated FFAs after a prolonged fast and preexisting high rates of lipid oxidation in tissues may therefore promote high rates of postprandial lipid oxidation and thus decrease the need for carbohydrate to be used as a fuel. Effectively, this represents the glu-

Table 2. Circulating substrate and hormone concentrations before meal consumption

<table>
<thead>
<tr>
<th>Variable</th>
<th>13-h Fast</th>
<th>72-h Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>90 ± 3</td>
<td>69 ± 2</td>
</tr>
<tr>
<td>FFA, μeq/l</td>
<td>378 ± 134</td>
<td>1,234 ± 436</td>
</tr>
<tr>
<td>Glycerol, μmol/l</td>
<td>97 ± 17</td>
<td>139 ± 40</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>6.3 ± 0.7</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>56.5 ± 3.4</td>
<td>76.3 ± 6.7</td>
</tr>
<tr>
<td>Cortisol, μg/dl</td>
<td>10.1 ± 3.6</td>
<td>15.3 ± 5.4</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>60 ± 7</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>269 ± 95</td>
<td>484 ± 171</td>
</tr>
<tr>
<td>Blood β-HOB, mg/dl</td>
<td>0.8 ± 0.2</td>
<td>23.6 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acids; β-HOB, β-hydroxybutyrate. Significantly different from 13-h fast: *P < 0.001, †P < 0.002, ‡P < 0.003, †P < 0.005, ‡P < 0.02.

Table 3. Postprandial area under the curve for circulating substrate and hormones

<table>
<thead>
<tr>
<th>Variable</th>
<th>13-h Fast</th>
<th>72-h Fast</th>
</tr>
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<tr>
<td>Glucose, mg/dl 1-360 min⁻¹</td>
<td>1,628 ± 1,204</td>
<td>15,923 ± 2,168</td>
</tr>
<tr>
<td>Insulin, μU/ml 1-360 min⁻¹</td>
<td>4,230 ± 788</td>
<td>17,768 ± 2,774</td>
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<tr>
<td>FFA, μeq1⁻¹-360 min⁻¹</td>
<td>-5,603 ± 1,619</td>
<td>-206,010 ± 63,246</td>
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<td>Glucagon, μmol1⁻¹-360 min⁻¹</td>
<td>-3,953 ± 3,593</td>
<td>-8,325 ± 9,439</td>
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Values are means ± SE. Significantly different from 13-h fast: *P < 0.001, †P < 0.02.

Fig. 3. A: postprandial changes in circulating glucose. There was significant effect of time (P < 0.0001), day (P < 0.0009), and day-by-time interaction (P < 0.0001). B: postprandial changes in circulating insulin. There was significant effect of time (P < 0.0009), day (P < 0.0001), and day-by-time interaction (P < 0.0002). C: postprandial changes in the circulating glucose-to-insulin ratio. There was significant effect of time (P < 0.03), day (P < 0.0001), and day-by-time interaction (P < 0.0002).
cose-fatty acid cycle as first proposed by Randle et al. (32). In the context of the preceding metabolic state, conservation of carbohydrate would seem a priority with refeeding after prolonged fasting. Increased FFA oxidation may therefore facilitate glycogen storage. This is supported by data under hyperinsulinemic-euglycemic clamp conditions where acutely elevating FFAs had no effect on nonoxidative glucose disposal (2), whereas glycolysis was decreased (21).

One of the other significant observations made in this study was the dramatically elevated postprandial glucose and insulin excursions after the prolonged vs. overnight fast. Previous studies have utilized either the hyperinsulinemic-euglycemic clamp technique or an OGTT to investigate the effect of prolonged fasting on glucose metabolism. These studies have shown that insulin-mediated glucose disposal (7, 23) and oral glucose tolerance (5, 11, 15) are both impaired with prolonged fasting. The results of the present investigation also demonstrate a significant impairment of glucose tolerance in the context of a normal mixed meal. The greater postprandial insulin excursion after the prolonged fast also indirectly suggests impaired insulin action with respect to glucose metabolism. Adding further to the concept of the glucose-fatty acid cycle (32) is the fact that elevated FFAs and/or elevated lipid oxidation can inhibit insulin action (2, 22, 33) due to effects on insulin signaling and/or glucose transport (10, 33). Therefore, the significantly increased FFA concentration and/or fat oxidation with the prolonged fast, both pre- and postprandially, could be mediating the impaired glucose tolerance, and potential insulin resistance, after the mixed meal.

It is possible that factors other than the elevated FFA and/or fat oxidation could have caused the decrease in postprandial glucose tolerance after the prolonged fast. In this context, the increased lipid oxidation after meal consumption could be a consequence of...
rather than a contributory factor to, the impairment in insulin action. Other possible mediators of impaired insulin action after the 72-h fast include the elevated cortisol (25) and ketone bodies (38). However, the cortisol concentration declined rapidly after the refeeding meal such that similar postprandial levels were observed after the 72- and 13-h fast. Although we did not measure growth hormone concentrations, it is likely that these would also have been elevated by the prolonged fast and could have decreased insulin action. Future studies, therefore, are required to define the mechanism causing the decreased glucose tolerance after mixed-meal consumption after extended fasting. It has been reported that insulin's suppression of lipolysis is also impaired after a prolonged fast (19, 39). In the present study, there was a significant decrease in the postprandial FFA and glycerol concentrations after the mixed meal under both fasting conditions, suggesting suppression of lipolysis. However, we observed no difference between the two fasting periods in the postprandial decline (decremental AUC) in glycerol concentrations, despite the much greater insulin response after the extended fast. Circulating glycerol concentrations are considered indicative of changes in whole body lipolysis (3) because there is little reutilization of glycerol within adipose tissue (6) (the major site of glycerol release). This contrasts to FFAs, which can be reesterified within adipocytes. Although data show that muscle can potentially reutilize glycerol produced from intramuscular lipolysis (6), this is likely a very minor contributor to whole body lipolysis under fasting conditions. The present study, therefore, would suggest impairment of insulin's antilipolytic action based on circulating glycerol changes. However, circulating concentrations do not indicate rates of substrate production and removal and only represent the balance between the two. This can only be addressed with the use of isotopic-tracer techniques.

The present study demonstrated a significant increase in the insulin-to-glucose ratio after the mixed meal after the prolonged vs. overnight fast. This suggests that, for a given change in glucose concentration, there was a greater insulin secretion. However, we only measured insulin at hourly intervals and did not measure C-peptide concentrations, a better marker of insulin secretion (31). An alternative explanation for the greater insulin concentrations could be a decrease in insulin clearance. The elevated FFA concentrations before the meal after the 72- vs. 13-h fast could have impacted either insulin secretion and/or clearance. Indeed, elevated FFAs have been shown to be important for both non-glucose- (1, 9) and glucose-stimulated (9, 36) insulin secretion, especially after fasting (8, 36). The elevated β-HOB could also contribute to an increased insulin secretion (27). It could be postulated that the FFAs act as a "signal," indicating that a greater insulin secretion is required to overcome the impairment in insulin action that has developed as a result of the prolonged fasting. Because elevated FFAs may be an important factor causing the impaired insulin action, they may play an important role in determining both the substrate and hormone response to fasting and refeeding.

The typical hormonal changes resulting from a prolonged fast were observed in the present study, that is, an increase in norepinephrine, cortisol, and glucagon with a decrease in insulin (28, 30, 41). We did not observe a significant increase in epinephrine, although levels were elevated. However, it has been reported that the sensitivity to the lipolytic action of epinephrine is increased after prolonged fasting (41), therefore diminishing the need for a large increase in this catecholamine. Postprandially, there was a decrease in the hormones associated with substrate mobilization, that is, norepinephrine, cortisol, and glucagon. Interestingly, postprandial norepinephrine and epinephrine were still somewhat elevated after the 72- vs. 13-h fast. This elevation in catecholamines could have helped

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**Fig. 6.** A: postprandial changes in cortisol. There was significant effect of day ($P < 0.01$). B: postprandial changes in norepinephrine. There was significant effect of time ($P < 0.0009$), day ($P < 0.0001$), and day-by-time interaction ($P < 0.0002$). C: postprandial changes in epinephrine (nonsignificant differences).
maintain a higher rate of postprandial lipolysis after the 72-h fast, thus opposing the antilipolytic action of the greatly elevated insulin.

The use of respiratory gas-exchange data to calculate nutrient oxidation assumes that all the O₂ and CO₂ consumed and produced, respectively, are derived from the oxidation of fat, carbohydrate, and protein. In the context of prolonged fasting, this may not be true because of the generation and utilization of ketones and increased gluconeogenesis (5, 12, 27). This is mainly a problem when there is an imbalance between the production of substrates and their oxidation (35). With respect to ketones, we measured the blood and urine accumulation of the main ketone body, β-HOB, and corrected the postprandial V̇O₂ values based on these measurements. The actual V̇O₂ estimated to be diverted to this process was small, even after the 72-h fast. Therefore, not measuring acetoacetate likely had minimal effects on the V̇O₂ data. With respect to gluconeogenesis, we had no ability to estimate rates of this during the refeeding period. However, it is unlikely that this process would have significantly impacted the respiratory gas-exchange values after the 72-h fast plus meal because gluconeogenesis would have been rapidly suppressed. This is supported by the greatly elevated insulin-to-glucagon ratio after the meal after the prolonged fast.

The present study suggests that the pattern of postprandial nutrient oxidation, after refeeding after a fast, may be partly determined by changes in the substrate and hormone environment. In addition, it could be related to differences in prefasting insulin sensitivity. Thus it could be hypothesized that the lesser the impairment of glucose tolerance after fasting, the smaller the decrease in carbohydrate oxidation and the smaller the increase in fat oxidation. In the present study, however, we observed no significant correlation between the initial insulin sensitivity index, as measured by Bergman’s minimal model, and measures of nutrient oxidation, nutrient balance, and postprandial substrate and hormone responses. However, these data were collected on a small group of male, nondiabetic, relatively nonobese (body fat <27%) subjects. This gave a limited range in the insulin sensitivity of subjects and in the insulin and glucose responses to the meal. It would be of interest to determine the substrate and hormone response to refeeding a mixed meal in other populations, including obese individuals and individuals with Type 2 diabetes. Such individuals differ markedly from their lean counterparts in indexes of insulin action as well as the circulating substrate and hormone environment. Imposing a prolonged fast on an obese person and/or an individual with Type 2 diabetes may thus result in a very different pattern of change in the postprandial nutrient oxidation. Indeed, it has been reported that the reduction in intravenous glucose tolerance, observed in normal subjects after fasting, is less severe in obese individuals (15) and not present in diabetic subjects (5).

The extent to which individuals change from predominantly fat oxidation (after fasting) to predominately carbohydrate oxidation (with consumption of a meal) may be very different after long- vs. short-term fasting. An individual who maintains a higher rate of carbohydrate oxidation after refeeding may be more prone to net fat accumulation. Individual variation in this response may not be detectable after a normal overnight fast but may be exaggerated after a more extended fasting period. Ultimately, retention of body fat requires a positive energy balance, but this could be exacerbated in an individual who favors a higher rate of carbohydrate oxidation between and after meals. Indeed, a greater fasting or 24-h respiratory quotient has been reported to be predictive of weight gain (24, 43). If this were a constitutive characteristic of the preobese individual, rather than a consequence of the obese state, then it may exacerbate body fat accumulation. Such individuals may be more prone to fat accumulation, especially when undergoing prolonged periods between meals. This may be particularly exacerbated if most of the daily energy intake was consumed in one, large, high-fat meal.

In conclusion, this study showed that, in normal healthy men, prolonged fasting resulted in a significant decrease in postprandial carbohydrate oxidation and an increase in fat oxidation. This was associated with a significant impairment in glucose tolerance and a significant elevation in the postprandial insulin excursion. Because circulating FFAs were significantly elevated at all times after the prolonged fast, this could be one factor determining the differences in postprandial nutrient oxidation, and glucose and insulin responses.

We thank all the subjects who volunteered for this study and greatly appreciate their time and cooperation. We also thank all the GCRC staff at University Hospital for help with the study.

This investigation was supported by National Institute of Diabetics and Digestive and Kidney Diseases Grants DK-42549 and DK-48520, and by National Institute of Health Division of Research Resources Grant 5-01-RR-0051.

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