Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise

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Horowitz, Jeffrey F., Ricardo Mora-Rodriguez, Lauri O. Byerley, and Edward F. Coyle. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E768–E775, 1997.—This study determined if the suppression of lipolysis after preexercise carbohydrate ingestion reduces fat oxidation during exercise. Six healthy, active men cycled 60 min at 44 ± 2% peak oxygen consumption, exactly 1 h after ingesting 0.8 g/kg of glucose (Glc) or fructose (Fru) or after an overnight fast (Fast). The mean plasma insulin concentration during the 50 min before exercise was different among Fast, Fru, and Glc (8 ± 1, 17 ± 1, and 38 ± 5 µU/ml, respectively; P < 0.05). After 25 min of exercise, whole body lipolysis was 6.8 ± 1.3, 4.3 ± 0.3, and 3.7 ± 0.5 µmol·kg⁻¹·min⁻¹ and fat oxidation was 6.1 ± 0.2, 4.2 ± 0.3, and 3.1 ± 0.3 µmol·kg⁻¹·min⁻¹ during Fast, Fru, and Glc, respectively (all P < 0.05). During Fast, fat oxidation was less than lipolysis (P > 0.05), whereas fat oxidation approximately equaled lipolysis during Fru and Glc. In an additional trial, the same subjects ingested glucose (0.8 g/kg) 1 h before exercise and lipolysis was simultaneously increased by infusing Intralipid and heparin throughout the resting and exercise periods (Glc + Lipid). This elevation of lipolysis during Glc + Lipid increased fat oxidation 30% above Glc (4.4 ± 0.3 vs. 4.1 ± 0.5 µmol·kg⁻¹·min⁻¹; P < 0.05), confirming that lipolysis limited fat oxidation. In summary, small elevations in plasma insulin before exercise suppressed lipolysis during exercise to the point at which it equaled and appeared to limit fat oxidation.

—stable isotopes

IT HAS LONG BEEN recognized that carbohydrate ingestion before exercise reduces fat oxidation during a subsequent exercise bout (3). More recently, it has been reported that this suppression persists for at least 4 h after a meal (20). Thus fat oxidation in active people is often under the influence of the insulin response from normal dietary carbohydrate. However, little is known about the mechanisms by which carbohydrate ingestion reduces fat oxidation during physical activity.

Fat oxidation during exercise involves several steps, beginning with the hydrolysis of triglycerides (i.e., lipolysis) to liberate fatty acids that are transported, either via plasma and/or through myoplasm, to the muscle mitochondria for oxidation (30). Triglycerides must first be hydrolyzed before the resultant fatty acids can be oxidized. Therefore, equivalent rates of lipolysis and fat oxidation could imply that lipolysis limits fatty acid oxidation. After an overnight fast, lipolysis exceeds fat oxidation by as much as 50% both at rest and during exercise (25, 32), and thus lipolysis clearly does not limit fat oxidation when a person is fasted. However, administration of the antilipolytic agent nicotinic acid reduces plasma free fatty acid (FFA) concentration and fat oxidation during exercise (13), but it has not been determined if fat oxidation equals lipolysis under these conditions. Like nicotinic acid, small elevations in plasma insulin concentration (i.e., 10–30 µU/ml) dramatically reduce lipolysis at rest (4, 5), yet lipolysis still appears to be in excess of fat oxidation at rest (5). During exercise, however, fat oxidation increases severalfold above resting levels, making it more likely for a suppression of lipolysis to limit fat oxidation.

No study, to our knowledge, has directly quantified lipolysis during exercise after an increase in plasma insulin concentration to determine the relationship between lipolysis and fat oxidation during exercise. This was the primary purpose of the present study. In that lipolysis appears sensitive to even small increases in plasma insulin, we fed subjects small amounts of both a low-glycemic (i.e., fructose) and high-glycemic carbohydrate (i.e., glucose) before exercise to raise plasma insulin concentration only 10–30 µU/ml above fasting levels. To more directly determine if an insulin-induced suppression of lipolysis limits fat oxidation, we also raised lipolysis via intravenous triglyceride and heparin infusion to determine the extent to which this raised fat oxidation.

A reduction in fat oxidation during exercise after the ingestion of carbohydrate requires a compensatory increase in carbohydrate oxidation to maintain energy production. The source of the increase in carbohydrate oxidation (i.e., blood glucose vs. muscle glycogen) is debated (1, 8, 14). Whereas leg glucose uptake and oxidation increase during exercise after a preexercise carbohydrate meal compared with during fasting (1, 2), conflicting evidence indicates that muscle glycogen utilization either increases (8) or remains the same (1, 14).

The principal aims of this study were to determine 1) how the magnitude of the plasma insulin concentration after ingestion of a low- vs. a high-glycemic carbohydrate meal before exercise influenced lipolysis during a subsequent exercise bout, 2) if the suppression in lipolysis after a relatively small preexercise carbohydrate meal reduced fat oxidation during exercise, and 3) how the relative contributions of blood glucose and muscle glycogen were influenced by a reduction in lipolysis and fat oxidation during exercise after carbohydrate ingestion.

METHODS

Subjects. Six healthy, active males participated in this experiment. Their peak oxygen consumption (V̇O₂peak) while cycling, body weight, and age were 3.91 ± 0.24 l/min (52.8 ±
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Experimental protocol. On four separate occasions, the subjects arrived at the laboratory in the morning after a 12-h fast. Exactly 1 h before exercise, the subjects ingested 0.8 g of carbohydrate/kg body weight (i.e., ~60 g) from 1% glucose (Glc) to elicit a relatively high plasma insulin concentration, 2% fructose (Fru) to elicit a small elevation in plasma insulin concentration, or 3% glucose ingestion followed by an intravenous infusion of a 20% triglyceride emulsion (Intralipid) and heparin (Glc+Lipid) to increase lipolysis and prevent a decline in plasma FFA concentration after glucose ingestion; or they remained fasted (Fast). Both Glc and Fru were provided as a 20% solution in water. During Glc+Lipid, as the subjects ingested the 20% glucose solution 1 h before exercise, we initiated a constant rate infusion of a 20% triglyceride emulsion (i.e., Intralipid) and sodium heparin (after a bolus of 7.1 U/kg) to promote lipolysis of the infused triglycerides. Infusion rates in the first two subjects (0.7 ml·kg^{-1}·h^{-1} of triglyceride and 7.1 U heparin·kg^{-1}·h^{-1}) produced plasma FFA concentrations higher than Fast. A lower infusion rate in the remaining four subjects (0.4 ml·kg^{-1}·h^{-1} of triglyceride and 4.8 U heparin·kg^{-1}·h^{-1}) elicited plasma FFA that closely matched levels during Fast. Exactly 1 h after ingestion, the subjects cycled for 60 min at 44 ± 2% VO_{2peak}. The order of the trials was counterbalanced, and they were separated by a minimum of 48 h.

Isotope infusion. When the subject arrived at the laboratory, Teflon catheters were inserted into a forearm vein of both arms of the subject (one for infusion, the other for blood sampling), and a heating pad was affixed to the forearm and hand of the sampling arm. A blood sample was then withdrawn for determination of background isotopic enrichment, followed by a primed, constant-rate infusion of 6,6-d_{2}-glucose and 3,3-d_{2}-fructose (i.e., 0.41 µmol·kg^{-1}·min^{-1}; prime of 35 µmol/kg) and d_{3}-glycerol (i.e., 0.24 µmol·kg^{-1}·min^{-1}; prime of 3.6 µmol/kg), using calibrated syringe pumps (Harvard Apparatus, South Natick, MA). Subjects were infused for at least 2 h before the start of exercise to allow for attainment of isotopic equilibrium. The rates of isotope infusion were maintained throughout the entire experiment.

Blood sampling and analysis. Blood samples were withdrawn immediately before and every 10 min after ingestion during both rest and exercise. Each blood sample was divided into three different tubes for subsequent analysis and immediately placed in an ice bath until the end of the trial. Three milliliters of each blood sample were placed in evacuated tubes containing 143 USP units of sodium heparin (Vacutainer, Becton Dickinson, Rutherford, N.J.). These samples were later analyzed for isotopic enrichment of the aldonitrile acetate derivative of 6,6-d_{2}-glucose (29) and the tris(hydroxymethyl)amionomethane (Tris)-trimethylsilyl derivative of d_{3}-glycerol (31), via gas chromatography-mass spectrophotometry (GC-MS). An additional 2 ml of each blood sample were placed in a test tube containing 0.2 ml of an Aprotinin (0.5 TIU/ml) and EDTA (82 mM) solution and later analyzed for plasma insulin concentration (radioimmunoassay; ICN Bio- medicals, Costa Mesa, CA). The final 3 ml of each blood sample were placed in a test tube containing 0.15 ml of EDTA (82 mM) for later determination of plasma glycerol [fluorometric assay (12)], glucose (glucose oxidase autoanalyzer, Yellow Springs Instruments, Yellow Springs, OH), and FFA (colorimetric assay (22)). In each tube, plasma was separated by centrifugation (3,000 rpm for 20 min at 4°C), immediately frozen, and stored at −70°C until analysis.

To ensure that in vitro lipolysis of the infused triglycerides during Glc+Lipid did not occur after sampling, we compared plasma FFA concentrations using our blood storage method with two methods reported to prevent in vitro lipolysis during hypertriglyceridemia. These methods were 1) the same method as presently used, with the exception of substituting ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N"-tetraacetic acid (EGTA) for EDTA (11), and 2) blood samples immediately centrifuged and placed in a test tube containing 0.2 ml of a 5 M NaCl solution and incubated for 30 min at 56°C (23). If the plasma FFA concentrations were greater using our present storage method compared with the others, this would suggest that in vitro lipolysis may have occurred during sampling during Glc+Lipid. However, no differences existed in the mean plasma fatty acid concentration among the different methods (i.e., 0.28 ± 0.03, 0.30 ± 0.02, 0.30 ± 0.04 mM for EDTA-, EGTA-, and NaCl-treated samples, respectively), indicating that significant in vitro lipolysis did not presently occur.

Isotope enrichment sample preparation. Plasma samples (1 ml) were deproteinnized by adding 1 ml of 0.3 N Ba(OH)_{2} and 1 ml of 0.3 N Zn(SO)_{4}. Each tube was then vortexed and incubated in an ice bath for 20 min. After centrifugation at 3,000 rpm for 15 min at 4°C, the supernatant was placed in separate tubes for glucose (0.5 ml) and glycerol analysis (1.5 ml) and the water was removed from the tubes via vacuum centrifugation (Savant Instruments, Farmingdale, NY). The aldonitrile acetate derivative of glycerol was prepared by adding 100 µl of hydroxolamine-hydrochloride solution (20 mg/ml in pyridine) to the dried sample. After a 30-min incubation at 100°C, 75 µl of acetic anhydride (Supelco, Bellefonte, PA) were added. The samples remained incubating for an additional hour and then were evaporated under N_{2}. Before injection into the GC-MS, the samples were reconstituted with ethyl acetate. The Tris-trimethylsilyl derivative of glycerol was prepared by reconstituting the dried sample with 30 µl of a trimethylsilyl solution (Tri-Sil, Pierce, Rockford, IL).

Preliminary testing. VO_{2peak} was determined while subjects cycled an ergometer (Monark, model 819, Varberg, Sweden) by using a continuous protocol that lasted 7–10 min. In addition, 2 days before each experimental trial, the subjects performed the experimental exercise protocol (60 min at 45% V˙O_{2peak}) to ensure homogeneity of the last exercise bout. The subjects ate the exact same meals at the same time of day before each of the four trials.

Measurement of gas exchange. As subjects inhaled through a two-way Daniel’s valve, inspired air volume was measured with a Parkinson-Cowan CD4 dry gas meter (Rayfield Equipment, Waitsfield, VT). The expired gases were continuously sampled from a mixing chamber and analyzed for oxygen (Applied Electrochemistry, model SA3, Ametek, Pittsburgh, PA) and carbon dioxide (Beckman, model LB-2, Schiller Park, IL). These instruments were interfaced with a computer for calculations of the rate of oxygen consumption (VO_{2}) and respiratory exchange ratio (RER).

Calculations. Whole body lipolysis was quantified by measuring the rate of glycerol appearance in plasma (R_{a} glycerol). This calculation assumes that one mole of glycerol is liberated for every mole of triglyceride hydrolyzed. It is theoretically possible that R_{a} glycerol may underestimate whole body lipolysis due to phosphorylation of glycerol within the cell and/or by incomplete triglyceride hydrolysis (16). Both of these possibilities would reduce glycerol appearance in the systemic circulation after lipolysis. Conversely, others indicate that neither of these events occur in skeletal muscle or in adipose tissue (21, 31) and that nearly all glycerol liberated via lipolysis mixes
with the circulation after triglyceride hydrolysis, thus suggesting that Ra glycerol provides a reasonably accurate index of lipolysis. Ra glycerol as well as the rate of appearance and disappearance of glucose in plasma (i.e., Ra Glc and Rd Glc) were calculated using the non-steady-state equation of Steele (28), modified for use with stable isotopes:

\[
Ra = \frac{F - V_d[C/(1 + E)(dE/dt)]}{E}
\]

\[
R_d = Ra \frac{V_d[(dC/dt)(1 + E) - C(dE/dt)]}{(1 + E)^2}
\]

where \(F\) is isotope infusion rate, \(V_d\) is volume of distribution (i.e., estimated to be 230 ml/kg for glycerol and 100 ml/kg for glucose), \(C\) is plasma concentration of the tracer, \(E\) is tracer isotopic enrichment, and \(dE/dt\) and \(dC/dt\) are maximum rates of change of enrichment and concentration, respectively, with respect to time. Fat (i.e., triglyceride) and carbohydrate oxidation were calculated from \(V_{O2}\) and \(RER\) (nonprotein respiratory quotient), measured from expired air during the 20- to 30- and 50- to 60-min periods of exercise (17). Muscle glycogen oxidation was calculated as the difference between total carbohydrate oxidation and Ra Glc. Coggan et al. (7) reported that ~90% of Ra Glc is oxidized, and thus it provides a reasonable representation of blood glucose oxidation. These calculations have been used in previous studies (25, 26).

Statistical analysis. Significant differences among trials were identified using a two-way analysis of variance (treatment by time) for repeated measures with Tukey’s post hoc analysis. Planned comparisons for mean values were evaluated using a paired Student’s t-test with a Bonferroni correction factor, \(P < 0.05\).

RESULTS

Plasma glucose concentration. The average plasma glucose concentration during the 50-min period before exercise was high during both Glc and Glc+Lipid (7.3 ± 0.4 and 6.6 ± 0.6 mM, respectively; \(P < 0.05\) vs. Fru and Fast), moderate during Fru (5.4 ± 0.2 mM; \(P < 0.05\) vs. Fast), and remained at basal levels during Fast (4.8 ± 0.2 mM). During exercise, no differences in plasma glucose concentration existed among the trials (Fig. 1).

Plasma insulin concentration. As designed, the plasma insulin concentration was elevated to different levels after the different carbohydrate meals. The mean plasma insulin concentration during the 50-min period before exercise was 38.5 ± 5.4, 17.0 ± 1.3, and 8.2 ± 0.8 µU/ml during Glc, Fru, and Fast, respectively (all \(P < 0.05\); Fig. 2). Insulin concentration decreased during exercise in both Glc and Fru (Fig. 2), and no differences existed among trials by 40 min of exercise.

Ra glycerol. At rest 5 min before exercise, Ra glycerol during Glc was ~60% lower than Fast (1.64 ± 0.11 vs. 3.81 ± 0.7 µmol·kg⁻¹·min⁻¹; \(P < 0.05\); Fig. 3). Fructose ingestion reduced resting Ra glycerol ~30% below Fast (2.75 ± 0.51 µmol·kg⁻¹·min⁻¹). However, this difference did not reach statistical significance. During exercise, Ra glycerol during Fast was significantly greater than both Glc and Fru (\(P < 0.05\)). Ra glycerol during Fru was intermediate to Fast (\(P < 0.05\) at all times) and Glc (\(P < 0.05\) at 10 and 25 min; Fig. 3).

Plasma FFA concentration. The reduction in lipolysis during Glc and Fru lowered (\(P < 0.05\)) plasma FFA concentration immediately before exercise compared with Fast (0.16 ± 0.01, 0.18 ± 0.01, and 0.36 ± 0.06 mM, respectively), and it remained low throughout exercise (Fig. 4). Lipolysis of the infused triglycerides during Glc+Lipid prevented the reduction in plasma FFA concentration after glucose ingestion (Fig. 4). In a subgroup of four subjects, a relatively low Intralipid infusion rate was used, and in these subjects, the plasma FFA concentration during exercise was identical to that observed while subjects fasted (Fig. 4B).

Fat oxidation and lipolysis. During Fast, lipolysis was 15–25% in excess of fat oxidation throughout exercise (6.9 ± 0.6 vs. 6.1 ± 0.2 µmol·kg⁻¹·min⁻¹ at 20–30 min and 8.4 ± 0.6 vs. 6.8 ± 1.2 µmol·kg⁻¹·min⁻¹ at 50–60 min; both \(P < 0.05\); Fig. 5). During both Glc and Fru, however, lipolysis and fat oxidation were suppressed to the point at which fat oxidation was closely matched to lipolysis (Fig. 5). Increasing lipolysis after glucose ingestion via lipid-heparin infusion during Glc+Lipid increased fat oxidation 30% above Glc
Fat oxidation during exercise.

At 20–30 min of exercise, fat oxidation was similar when the lipid-heparin infusion increased plasma FFA concentrations to either 0.4 mM (i.e., fat oxidation 5.0 6 0.6 µmol·kg\(^{-1}\)·min\(^{-1}\); n = 4) or 0.9 mM (i.e., fat oxidation 4.1 6 0.4 µmol·kg\(^{-1}\)·min\(^{-1}\); n = 2).

Table 1. Fat oxidation rate

<table>
<thead>
<tr>
<th>Trial</th>
<th>Exercise Time</th>
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<tbody>
<tr>
<td></td>
<td>20–30 min</td>
</tr>
<tr>
<td>Fast</td>
<td>6.1 6 0.2</td>
</tr>
<tr>
<td>Fructose</td>
<td>4.2 6 0.5*</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.1 6 0.3*†‡</td>
</tr>
<tr>
<td>Glucose + Lipid</td>
<td>4.0 6 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE and are in µmol·kg\(^{-1}\)·min\(^{-1}\). *Significantly different from Fast, P < 0.05. †Significantly different from Fructose, P < 0.05. ‡Significantly different from Glucose + Lipid, P < 0.05. See Experimental protocol for description of experimental trials.
Glc and Fru was significantly greater (P < 0.05) than Fast (87 ± 8, 84 ± 9, and 70 ± 8 μmol·kg⁻¹·min⁻¹, respectively; Table 2). During this same period, the increase in lipolysis and the resultant elevation in plasma FFA concentration during Glc+Lipid lowered muscle glycogen oxidation compared with Glc (74 ± 8 vs. 87 ± 8 μmol·kg⁻¹·min⁻¹, respectively; P < 0.05) and there was no difference in muscle glycogen oxidation in Fast compared with Glc+Lipid (70 ± 8 vs. 74 ± 8 μmol·kg⁻¹·min⁻¹, respectively). During the 50- to 60-min period of exercise, no differences in muscle glycogen oxidation were observed among trials (62 ± 7, 64 ± 6, 68 ± 5, and 70 ± 8 μmol·kg⁻¹·min⁻¹ for Fast, Fru, Glc, and Glc+Lipid, respectively).

Energy expenditure. Figure 7 summarizes the estimated relative caloric contribution from fat, blood glucose, and muscle glycogen during the 20- to 30-min period of exercise. The total rate of energy expenditure was similar during all trials (108 ± 11 cal·kg⁻¹·min⁻¹). During Fast, the relative energy expenditure from fat, blood glucose, and muscle glycogen was 44 ± 2, 9 ± 2, and 47 ± 6, respectively (Fig. 7). Compared with Fast, Glc reduced fat oxidation by one-half, representing only 22 ± 2% of the total energy, whereas the contributions of both blood glucose and muscle glycogen increased to 20 ± 2 and 58 ± 6%, respectively (all P < 0.05 vs. Fast). Lipolysis of the infused triglycerides during Glc+Lipid increased the relative contribution of fat to 30% (P < 0.05 vs. Glc) and returned the relative contribution of muscle glycogen back to fasting levels (50 ± 6%; P < 0.05 vs. Glc). During Fru, the relative contributions from fat (30 ± 4%) and blood glucose (14 ± 2%) were intermediate to those during Fast and Glc (P < 0.05 vs. Fast and Glc). However, the relative contribution of muscle glycogen was the same as Glc (56 ± 6%). During the 50- to 60-min period of exercise, the relative contribution of fat during Fast (48 ± 3%) was significantly greater than Glc, Glc+Lipid, and Fru (34 ± 4, 37 ± 3, and 37 ± 5, respectively; P < 0.05 vs. Fast), whereas the contribution from blood glucose during Fast (11 ± 3%) was lower (22 ± 4, 19 ± 4, and 22 ± 4%, respectively; P < 0.05 vs. Fast). However, no differences in fat oxidation or Rd Glc existed among Glc, Glc+Lipid, and Fru (34 ± 4, 37 ± 3, and 37 ± 5%, respectively; P < 0.05 vs. Fast). During this period, the contribution from muscle glycogen was not different among any of the trials (41 ± 5%, 44 ± 4%, 45 ± 5%, and 41 ± 4% for Fast, Glc, Glc+Lipid, and Fru, respectively).

Table 2. Total carbohydrate oxidation, rate of glucose disappearance, and muscle glycogen oxidation

<table>
<thead>
<tr>
<th>Trial</th>
<th>Total carbohydrate oxidation</th>
<th>Rd Glucose</th>
<th>Muscle glycogen oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>85 ± 9</td>
<td>15 ± 1</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>Fructose</td>
<td>105 ± 8*</td>
<td>21 ± 2‡</td>
<td>84 ± 9*</td>
</tr>
<tr>
<td>Glucose</td>
<td>117 ± 6*‡</td>
<td>30 ± 3‡</td>
<td>87 ± 8‡</td>
</tr>
<tr>
<td>Glucose + Lipid</td>
<td>107 ± 7*</td>
<td>33 ± 2‡</td>
<td>74 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE and are in μmol·kg⁻¹·min⁻¹. Rd Glucose, rate of glucose disappearance. *Significantly different from Fast, P < 0.05. †Significantly different from Fru, P < 0.05. ‡Significantly different from Glucose + Lipid, P < 0.05.
DISCUSSION

The present study is the first to our knowledge to quantify lipolysis during exercise after an elevation in plasma insulin concentration and to demonstrate that fat oxidation was suppressed to levels closely matching the rate of lipolysis. The notion that fat oxidation can be “limited” by a low lipolytic rate stems from the fact that a triglyceride must first be hydrolyzed before the resultant fatty acids can be oxidized. Because there is a very small pool of unesterified fatty acids within the human body (~10–40 µmol/kg), the rate of fat oxidation cannot exceed the rate of lipolysis for more than a few minutes during exercise. It follows that when lipolysis is suppressed to low levels, the rate of fat oxidation may be limited by the reduced availability of unesterified fatty acids due to the low lipolytic rate.

Lipolysis exceeds fat oxidation both at rest and during exercise when subject is fasted (25, 32). In the present study, lipolysis exceeded fat oxidation by 15–25% when subjected was fasted. Thus, because more fatty acids are liberated via lipolysis than are oxidized, fat oxidation is not limited by lipolysis when subjects are fasted (25, 32). It has been reported that lipolysis was suppressed ≥60% at rest after an elevation in plasma insulin concentration to only ~30 µU/ml (5). This suppression, together with the high rate of triglyceride reesterification at rest (5, 32), appears to account for the observation of Campbell et al. (5) that small elevations in plasma insulin concentration (e.g., to only ~30 µU/ml) in resting subjects reduced the rate of FFA appearance in plasma, plasma FFA concentration, and the rate of FFA disappearance from plasma (R_{FFA}) to the point that R_{FFA} equaled fat oxidation. Therefore, at rest, a reduction in mobilization of FFA from adipose tissue after a relatively small increase in plasma insulin concentration may limit fat oxidation, whereas whole body lipolysis remains in excess of fat oxidation.

During exercise, both lipolysis and fat oxidation increase above resting levels (25, 32). However, we have presently reported that a relatively small elevation in plasma insulin concentration before exercise (9 µU/ml during Fru and 30 µU/ml during Glc) attenuated the rise in lipolysis during exercise to the point that the rate of lipolysis was similar to the rate of fat oxidation (Fig. 5). Thus, because fat oxidation cannot exceed the rate of lipolysis, a close matching of lipolysis and fat oxidation suggests that the lipolytic suppression was sufficient to limit fat oxidation. However, lipolysis and fat oxidation were measured using different methods (i.e., isotope dilution and indirect calorimetry, respectively). As such, the close matching of these measurements provides only indirect evidence that lipolysis limited fat oxidation during exercise after carbohydrate ingestion. Direct support for the idea that lipolysis did indeed limit fat oxidation was obtained from our observation that fat oxidation increased 30% with an increase in lipolysis (i.e., via Intralipid and heparin infusion) after glucose ingestion. However, this increase in lipolysis did not restore fat oxidation to fasting levels, suggesting that carbohydrate ingestion has additional effects on muscle that concomitantly reduced fat oxidation.

We have attributed the suppression in lipolysis to the elevation in plasma insulin concentration despite an elevation in plasma glucose concentration and a likely fall in plasma glucagon concentration after carbohydrate ingestion. Previous reports indicate that alterations in glucose (6) and glucagon concentration (19) do not affect lipolysis when plasma insulin concentration does not change even slightly. In addition, we recognize the possibility that the measurement of R_{glycerol} may underestimate whole body lipolysis. Potential causes for this underestimation include some activity of glycerol kinase within adipose and/or muscle tissue and incomplete hydrolysis of a triglyceride into di- or monoglycerides (16). Both of these events would reduce glycerol appearance in the systemic circulation after lipolysis. To the contrary, reports indicate that neither of these phenomena occur within skeletal muscle or in adipose tissue (21, 31). However, despite the possible underestimation of whole body lipolysis in the present study, we have clearly demonstrated that lipolysis was suppressed during exercise after carbohydrate ingestion, compared with an overnight fast. Additionally, by demonstrating that an increase in lipolysis during Glc+Lipid increased fat oxidation 30% above Glc, we established that this suppression in lipolysis during exercise after glucose ingestion was sufficient to limit fat oxidation.

Increasing lipolysis during Glc+Lipid significantly raised fatty acid oxidation from 3.1 ± 0.3 to 4.0 ± 0.4 µmol·kg^{-1}·min^{-1}, but it did not restore fat oxidation to levels observed during Fast (6.1 ± 0.2 µmol·kg^{-1}·min^{-1}). This occurred despite the fact that plasma FFA concentration was elevated to a level as great (0.4 mM for n = 4; Fig. 3B) or greater than when fasted (0.9 mM for n = 2). Thus the remaining reduction in fat oxidation below fasted levels was not likely a result of an inadequate increase in plasma FFA concentration. This indicates that the lower fat oxidation during Glc+Lipid vs. Fast was due to an inhibition within the exercising muscle. Unfortunately, we were unable to differentiate between adipose and intramuscular triglyceride lipolysis in the present study. Because infusion of Intralipid and heparin only increases lipolysis in the vasculature, it is possible that a reduction in intramuscular triglyceride lipolysis after the ingestion of glucose may have reduced fat oxidation during Glc+Lipid compared with Fast (9). We have also recently found that acute hyperglycemia, hyperinsulinemia, and the resultant increased glycolytic flux reduce long-chain fatty acid transport and oxidation within muscle mitochondria (9). Therefore, it is possible that carbohydrate ingestion regulates fat oxidation through the combined effects of a reduction in whole body lipolysis (i.e., both adipose and intramuscular) superimposed on an inhibition of long-chain fatty acid oxidation in muscle.

The factors that regulate the relative contribution of fat and carbohydrate to total energy production are not well understood. More than 30 years ago, Randle and co-workers (24) first proposed the classic hypothesis...
that fat oxidation regulates carbohydrate oxidation (i.e., "glucose-fatty acid cycle"). This theory states that increases in mitochondrial acetyl-CoA and cytosolic citrate concentrations, secondary to an increase in fat oxidation, inhibit pyruvate dehydrogenase and phosphofructokinase and subsequently reduce glycolysis and glucose uptake (24). However, little evidence is available to support the functional significance of this mechanism in human skeletal muscle during exercise (10). Investigations have failed to detect elevations in muscle acetyl-CoA, citrate, and glucose 6-phosphate concentrations coincident to an elevation in fat oxidation during exercise after an increase in plasma FFA concentration (10, 11). Additional evidence for the absence of this regulatory mechanism in exercising humans was the present observation that a 30% increase in fat oxidation during Glc + Lipid compared with Glc did not reduce $R_d$ Glc (i.e., glucose uptake), which agrees with the recent finding of Romijn et al. (26). Carbohydrate ingestion and the resultant increase in plasma insulin concentration suppressed lipolysis while additionally increasing $R_d$ Glc. These are two powerful mechanisms by which carbohydrate metabolism regulates fat oxidation.

Exercise and fasting are effective means to increase fat oxidation. Thus exercise is often prescribed to individuals attempting to reduce body fat. Our present findings indicate that when exercise is preceded by a carbohydrate meal, lipolysis and fat oxidation are reduced. How this affects body fat and weight regulation over periods of days to months is not clear. It has recently been reported that total fat oxidation was reduced ~30% over an 8-h period when carbohydrate was ingested before exercise, compared with ingestion after exercise (27). Reducing lipolysis and impairing daily fat oxidation by ingesting carbohydrate before exercise increases the likelihood of being in a positive lipid balance and thus may increase fat deposition. Presently, we have demonstrated that ingestion of either a low-glycemic carbohydrate (i.e., fructose) or a high-glycemic carbohydrate (i.e., glucose) suppresses lipolysis and fat oxidation, at least during the 2-h period of this study. Due to the sensitivity of lipolysis to even small elevations of insulin, it appears that to maintain high rates of fat oxidation at rest and during subsequent exercise, people should not eat even small amounts of carbohydrate before exercise.

The reduction in lipolysis and fat oxidation after carbohydrate ingestion necessitates a compensatory increase in carbohydrate oxidation to maintain energy production during exercise. Carbohydrate ingestion and the resultant insulin response increased blood glucose uptake and presumably oxidation during exercise, as previously reported (1, 2). However, in the present study, the increase in blood glucose uptake during the 20- to 30-min period of exercise during Glc compared with Fast (12 cal·kg⁻¹·min⁻¹; $P < 0.05$) was only about one-half as great as the reduction in fat oxidation (23 cal·kg⁻¹·min⁻¹; Fig. 7), and, as a result, the calculated rate of muscle glycogen oxidation appeared to increase 11 cal·kg⁻¹·min⁻¹ (i.e., 22%; $P < 0.05$ Glc vs. Fast). Therefore, muscle glycogen oxidation increased in response to a reduced supply of energy from the combination of fat (i.e., from both plasma FFA and intramuscular stores) and blood glucose. Although AMP and P_i concentrations were not measured in the present study, it has recently been reported that low plasma FFA concentrations during exercise, similar to those reported during Glc in the present study (i.e., <0.2 mM), increased muscle AMP and P_i concentrations (10). Because AMP and P_i increase glycogen phosphorylase activity, this may explain the greater rate of muscle glycogen oxidation during Glc compared with Fast.

The transient decline in plasma glucose concentration during the first 20 min of exercise is a phenomenon characteristic of preexercise carbohydrate ingestion (1, 8). Obviously this reflects an imbalance between $R_d$ Glc and $R_a$ Glc (i.e., $R_d$ Glc > $R_a$ Glc). However, limited information is available describing this imbalance (18). Presently, the reduction in plasma glucose concentration during the first 20 min of exercise after carbohydrate ingestion was due to a large increase in $R_a$ Glc without a compensatory increase in $R_a$ Glc. Muscle contraction and exercise both increase glucose uptake (15), resulting in the relatively large increase in $R_d$ Glc at the onset of exercise when subjects are fed carbohydrate. However, $R_a$ Glc did not increase markedly with the onset of exercise. After 10 min of exercise $R_d$ Glc declined, likely due to the rapid fall in plasma glucose and insulin concentration during exercise (18). After 25 min of exercise, $R_a$ Glc slightly (not significant) exceeded $R_d$ Glc and thereafter plasma glucose concentration returned to fasted levels.

In summary, when fasted and plasma insulin concentrations are at basal levels (<10 µU/ml), lipolysis is in excess of fat oxidation during exercise. However, only a relatively small increase in plasma insulin concentration before exercise (~10 µU/ml after fructose ingestion and ~30 µU/ml after glucose ingestion) reduced lipolysis to the point that it equaled fat oxidation. The idea that lipolysis did indeed limit fat oxidation was demonstrated by the fact that fat oxidation increased 30% with an increase in lipolysis (i.e., via Intralipid and heparin infusion) after glucose ingestion. However, this increase in lipolysis did not restore fat oxidation to fasting levels, suggesting that carbohydrate ingestion has additional effects within skeletal muscle that concomitantly reduced fat oxidation. The increase in lipolysis and plasma FFA concentration during Glc + Lipid, however, did increase fat oxidation sufficiently to reverse the increase in muscle glycogen oxidation observed during exercise after glucose ingestion alone. The present findings demonstrate that carbohydrate ingestion before exercise resulting in a 10–30 µU/ml elevation in plasma insulin concentration reduced fat oxidation primarily by suppressing lipolysis during exercise.

We greatly appreciate the technical support of Dr. Andrew Coggan and Michael Sullivan. We additionally appreciate the assistance from Paul Below, Melissa Domenick, Pete Flatten, Ricardo Fritzschke, Wes
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