Glucose ingestion blunts hormone-sensitive lipase activity in contracting human skeletal muscle

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Watt, Matthew J., Peter Krusstrup, Niels H. Secher, Bengt Saltin, Bente K. Pedersen, and Mark A. Febbraio. Glucose ingestion blunts hormone-sensitive lipase activity in contracting human skeletal muscle. Am J Physiol Endocrinol Metab 286: E144–E150, 2004.—To examine the effect of attenuated epinephrine and elevated insulin on intramuscular hormone sensitivity lipase activity (HSLa) during exercise, seven men performed 120 min of semirecumbent cycling (60% peak pulmonary oxygen uptake) on two occasions while ingesting either 250 ml of a 6.4% carbohydrate (GLU) or sweet placebo (CON) beverage at the onset of, and at 15 min intervals throughout, exercise. Muscle biopsies obtained before and immediately after exercise were analyzed for HSLa. Blood samples were simultaneously obtained from a brachial artery and a femoral vein before and during exercise, and leg blood flow was measured by thermodilution in the femoral vein. Net leg glycerol and lactate release and net leg glucose and free fatty acid (FFA) uptake were calculated from these measurements. Insulin and epinephrine were also measured in arterial blood before and throughout exercise. During GLU, insulin was elevated (120 min: CON, 11.4 ± 2.4, GLU, 35.3 ± 6.9 pM, P < 0.05) and epinephrine suppressed (120 min: CON, 6.1 ± 2.5, GLU, 2.1 ± 0.9 nM; P < 0.05) compared with CON. Carbohydrate feeding also resulted in suppressed (P < 0.05) HSLa relative to CON (120 min: CON, 1.71 ± 0.18, GLU, 1.27 ± 0.16 mmol/min·kg dry mass−1). There were no differences in leg lactate or glycerol release when trials were compared, but leg FFA uptake was lower (120 min: CON, 0.29 ± 0.06, GLU, 0.82 ± 0.09 mmol/min) and leg glucose uptake higher (120 min: CON, 3.16 ± 0.59, GLU, 1.37 ± 0.37 mmol/min) in GLU compared with CON. These results demonstrate that circulating insulin and epinephrine play a role in HSLa in contracting skeletal muscle. fat metabolism; substrate flux; exercise

CARBOHYDRATE INGESTED before and during prolonged low-intensity exercise induces a decrease in fat oxidation, but the precise mechanism(s) mediating the decrease in fat oxidation are not fully understood (1, 8, 10, 14). It has been suggested that the maintenance of plasma insulin levels (8, 9) may account for this response because hyperinsulinemia during exercise is associated with a decrease in adipose tissue lipolysis (6, 10, 21), a possible limitation at carnitine palmitoyltransferase I via increased malonyl-CoA (10, 34), and increased fatty acid (FA) esterification into endogenous lipid pools (12). Insulin increases total free fatty acid (FFA) uptake in the contracting isolated rat soleus (12); however, FFA uptake with high, yet physiological, insulin concentrations have not been examined in humans.

Intramuscular triacylglycerol (IMTG) is regarded as an important metabolic substrate during moderate-intensity exercise (11, 15, 22, 32, 42), although its contribution to energy turnover remains controversial (36, 38). In contrast to the large body of work investigating adipose tissue lipolysis and FA oxidation during exercise with glucose feeding, few studies have examined IMTG metabolism and the mechanisms by which glucose ingestion, and the consequent hormonal and metabolic responses during exercise, affects IMTG metabolism. IMTG is hydrolyzed by the rate-limiting enzyme hormone-sensitive lipase (HSL), which is controlled by both local and hormonal regulators (25, 26, 43, 45, 46). HSL is phosphorylated and activated by protein kinase A (PKA) (20) in adipose tissue. PKA is increased in response to epinephrine via a cAMP-dependent pathway and decreased by insulin via increased phosphodiesterase activity (13). Thus, in adipose tissue, plasma epinephrine increases HSL activity (HSLa) and adipose tissue lipolysis (27, 39), whereas insulin decreases HSLa and lipolysis (13, 40).

Few studies have examined the regulation of HSLa in skeletal muscle. Given the importance of IMTG as a potential energy source in this tissue and the fact that changes to local mediators are more marked in contracting skeletal muscle compared with adipose tissue, more studies are warranted. HSL is activated in contracting isolated rat skeletal muscle (25) independently of changes in the hormonal milieu, and epinephrine increases HSLa independently of muscle contraction in both rodents (26) and humans (46). However, in humans, an increase in epinephrine is not essential to elevate HSLa during exercise, suggesting that contractile mechanisms are powerful mediators of skeletal muscle HSLa (46). On the other hand, exogenous epinephrine infusion during moderate-intensity exercise increases muscle HSLa to a greater degree than during exercise, when plasma epinephrine is low (46). HSLa increases at the onset of exercise (45), reaches a peak at 1 h, and returns toward basal levels by 2 h (43). Paradoxically, the decrease in HSLa at 2 h of exercise occurs concomitantly with marked increases in plasma epinephrine and almost complete suppression of plasma insulin, but due to the absence of a comparison group in this previous study (43), it is not possible to ascertain the importance of these hormonal changes for HSL activity during prolonged exercise.

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In this context, the main aim of the present study was to determine the effect of increased insulin and decreased epinephrine on HSL activity and estimated nonplasma FA (FA) oxidation. We achieved this by having subjects ingest glucose immediately before and during 2 h of moderate exercise. We hypothesized that carbohydrate ingestion would result in decreased HSL activity via alterations in circulating insulin and epinephrine, resulting in concomitant reductions in estimated nonplasma FA oxidation.

METHODS

Subjects. Seven men volunteered to participate as subjects after the procedures and associated risks of the experiment were explained. Their age, weight, and peak pulmonary oxygen uptake (V\textsubscript{O\textsubscript{2} peak}) were 22 ± 4 yr, 81 ± 12 kg, and 53.2 ± 3.9 ml·kg⁻¹·min⁻¹, respectively. The Ethics Committee of Copenhagen Frederiksbergs approved all experimental procedures.

Preexperimental procedures. Subjects visited the laboratory on four occasions. Subjects completed a graded exercise test to volitional exhaustion on a semirecumbent cycle ergometer to determine V\textsubscript{O\textsubscript{2} peak} of the four occasions. Subjects completed a graded exercise test to volitional exhaustion on a semirecumbent cycle ergometer to determine V\textsubscript{O\textsubscript{2} peak} and the exercise power output required for subsequent trials. Subjects returned to the laboratory ≥2 days later to complete a preexercise measure for 45 min at 65% V\textsubscript{O\textsubscript{2} peak}. Subjects were provided with a food package (15.6 MJ/day; 70% carbohydrate, 15% fat, 15% protein), which they consumed for the following 2 days. Subjects were requested to refrain from exercise and alcohol and caffeine ingestion during this period. This protocol was designed to minimize any difference in endogenous substrate availability when trials were being compared.

Experimental protocol. Subjects completed two exercise trials that were separated by ≥10 days. Trials were randomized and counterbalanced. On both occasions, subjects exercised for 5 min at 50% V\textsubscript{O\textsubscript{2} peak} and 115 min at 65% V\textsubscript{O\textsubscript{2} peak} on the previously mentioned semirecumbent cycle ergometer. On one visit, subjects ingested 250 ml of a 6.4% carbohydrate solution (Lucozade Sport; GlaxoSmithKline, Slough, UK) at the onset of, and at 15-min intervals throughout exercise (denoted herein as GLU). During the other trial, subjects ingested an artificially flavored placebo (CON).

Subjects arrived at the laboratory (∼8:00 AM) after a 12-h overnight fast and rested quietly on a bed. A catheter (1 mm ID; 20 gauge) was inserted into the left brachial artery and a second catheter (7-Fr diameter; Cook, Bjaeverskov, Denmark) was inserted into the left femoral vein ∼1–2 cm distal to the inguinal ligament. The femoral vein was secured in the retrograde direction, which prevents contamination from fat deposits outside the active skeletal muscle (41). Catheters were kept patent by intermittent flushing with 0.9% saline. One leg was prepared for percutaneous needle biopsy of the vastus lateralis muscle by making two incisions in the skin and deep fascia under local anesthesia (2% lidocaine). Immediately before exercise, a muscle sample was obtained and rapidly frozen in liquid nitrogen for later analysis. Blood samples were simultaneously collected from the brachial artery and the femoral vein at rest and at 30-min intervals during exercise. Subjects continued to exercise during blood sampling. Arterial and venous blood was later analyzed for FFA, glucose, and lactate. Arterial blood was also analyzed for insulin, epinephrine, and norepinephrine. Femoral venous blood flow was determined by the constant-infusion thermodilution technique (2). Expired respiratory gases were collected for 5 min immediately before blood sampling and were analyzed online using a Medgraphics CPX/D metabolic cart (St. Paul, MN). A second biopsy was obtained and rapidly frozen (<20 s) upon cessation of exercise.

Analysis. Skeletal muscle was freeze-dried overnight, carefully dissected free of nonmuscle contaminants, and powdered for the analyses of glycogen, lactate, and HSLa. Glycogen and lactate were analyzed using enzymatic analyses with fluorometric detection, as previously described (37). Skeletal muscle was analyzed for HSLa, as described (26, 45). Briefly, the powdered muscle was homogenized on ice in 20 volumes of homogenizing buffer with a rotating Teflon pestle on glass. After centrifugation, the supernatant was removed and stored on ice for immediate analysis of HSLa. A substrate consisting of 5 mM triolein, 14 × 10⁻⁶ M"{H}triolein, 0.6 mg of phospholipid (phosphatidylincholine-phosphatidylinositol: 3:1, w/w), 0.1 M potassium phosphate, and 20% BSA was emulsified by sonication. The muscle homogenate supernatant (14 μl) was incubated at 37°C with enzyme dilution buffer (86 μl) and 100 μl of triolein substrate. The reaction was stopped after 20 min with the addition of 3.25 ml of a methanol-chloroform-heptane (10:9.7, vol/vol/vol) solution, and 1.1 ml of 0.1 M potassium carbonate-0.1 M boric acid were added to facilitate the separation of the organic and aqueous phases. The mixture was mixed on a vortex and spun in a centrifuge at 1,100 ×g for 20 min, and 1 ml of the upper phase containing the released FA was removed for determination of radioactivity on a beta spectrometer (Tri-Carb 1500; Packard, Canberra, Australia). Total creatine was determined from an aliquot of the HSL homogenate by enzymatic fluorometric methods (3). HSLa was normalized to the highest total creatine content from the four samples obtained for each subject to normalize for nonmuscle contamination. The creatine normalization averaged 1.08 ± 0.03 (mean ± SE). All measurements were made in triplicate, and the mean of these values is reported. It is important to note that the measurement of muscle creatine content is affected by methods and does not allow for the observation of allosteric effects.

Arterial and venous whole blood was placed in tubes containing heparin and spun in centrifuge, and the plasma was removed for enzymatic end point determination of FFA, glucose, and lactate by use of an automated analyzer (Cobas Fara, Roche, Switzerland). Arterial blood for insulin and catecholamine determinations was added to tubes containing 30 μl of 200 mM EGTA and 30 μl of a mixture of 200 mM glutathione (GSH) and 250 mM EGTA, respectively, and spun in centrifuge at 9,000 ×g for 3 min, and the plasma was stored at −80°C until analyzed. Plasma insulin was analyzed by radioimmunoassay (Insulin RIA 100; Pharmacia, Uppsala, Sweden). Plasma epinephrine and norepinephrine were analyzed by high-performance liquid chromatography as described previously (4).

Calculations. Net glucose, lactate, and glycerc exchange by the leg were calculated using the Fick principle (femoral arteriovenous difference × leg blood flow). For FFA uptake, the arteriovenous difference was calculated by the plasma flow, which was calculated as blood flow × (1 − hematocrit)/100. Total carbohydrate and FA oxidation were calculated using stoichiometric equations (31). Plasma FA and glucose uptakes were converted to their molar equivalents (triaclyglycerol, 860 g/mol; glucose, 180.1 g/mol). Oxidation of FA and glucose uptake was assumed to be 90% for FA and 100% for glucose on the basis of previous observations (24, 33). The rate of nonplasma-derived FA oxidation was estimated as total leg fat oxidation minus estimated leg plasma FA oxidation.

Methodological considerations. Tracer methodology was not used in the present study, and this may have an impact on any conclusions regarding the proportion of plasma and nonplasma FA oxidized. Measuring arteriovenous differences and blood flow allows for the accurate determination of net substrate uptake/release; however, these methods cannot determine whether the metabolite of interest has been degraded to produce energy or stored within the muscle. Although glucose feeding has been shown to have no effect on the percentage of glucose rate of disappearance (R\textsubscript{d}) oxidized (24), it is possible that the percentage of FFA R\textsubscript{d} oxidized is altered with glucose ingestion. Specifically, increased insulin alters the partitioning of FA in contracting muscle from oxidation toward esterification (12). It is important to acknowledge, however, that there are no data in humans confirming these data. It is also possible that “net” FAA and lactate exchange data will underestimate the “true” uptake rates because of the simultaneous uptake and release of FAA and lactate by skeletal muscle. An underestimation of FFA uptake will result in an overestimation of nonplasma-derived FA oxidation. Thus the percentage of
the FFA oxidized may be altered by glucose ingestion and may influence the estimated plasma and subsequently calculated non-plasma-derived FA oxidation values presented herein.

Statistics. Data are expressed as means ± SE. Differences between trials and time points were performed by a repeated-measures two-way analysis of variance. Where a significant F ratio was obtained, specific differences were located with a Student-Newman-Keuls post hoc test. Significance was accepted at P < 0.05.

RESULTS

Physiological responses during exercise. \( \dot{V}O_2 \) was not different between trials and averaged 61.7 ± 0.4% \( \dot{V}O_2 \)-peak throughout exercise in both trials. There was no time effect for \( \dot{V}O_2 \). Heart rate (HR) increased during exercise in both trials, reaching significance (P < 0.05) by 120 min (Table 1). HR was higher at 120 min in GLU compared with CON.

Arterial plasma metabolite responses to exercise. Arterial plasma FFA were not different between trials at rest and averaged 0.85 ± 0.15 mM at rest (Table 2). After 30 min of exercise, arterial plasma FFA levels were decreased (P < 0.05) in both trials. Plasma FFA levels remained constant throughout exercise in GLU but were increased (P < 0.05) at 90 and 120 min in CON. Arterial plasma glycerol demonstrated a similar response to plasma FFA (Table 2). Arterial plasma glucose tended to decrease during exercise in CON and was significantly (P < 0.05) lower than rest by 120 min (Table 2). During GLU, plasma glucose was increased from rest at 30 min, returned to resting values by 60 min, and was maintained throughout exercise. Plasma glucose concentrations were higher (P < 0.05) in GLU at all exercise time points (Table 2).

Hormonal responses during exercise. Plasma insulin was not different at rest between trials (Fig. 1). Insulin levels progressively decreased (P < 0.05) during exercise in CON.

Glucose ingestion resulted in a marked elevation (P < 0.05) in plasma insulin at 30 min, which was largely maintained throughout exercise (Fig. 1). Plasma insulin during GLU was greater (P < 0.05) than in CON throughout exercise. Arterial epinephrine was not different between trials at rest and did not increase during exercise in either trial until 90 min (Fig. 2A). Arterial epinephrine was increased (P < 0.05) from rest during the 2nd h of exercise, although the increase was lower (P < 0.05) in CON. Plasma arterial norepinephrine was increased from rest throughout exercise and was not different between trials (Fig. 2B).

HSLa. HSLa at rest was similar between trials and averaged 1.31 ± 0.13 mmol·min⁻¹·kg dry mass⁻¹ (dm; Fig. 3). HSLa was increased (P < 0.05) at 120 min during CON, and the exercise-induced increase was blunted (P < 0.05) in GLU.

Net limb substrate exchange. Net FFA uptake was observed throughout exercise in both trials (Fig. 4A). Net FFA uptake was not different between trials for the 1st h of exercise but was about threefold greater (P < 0.05) during CON in the 2nd h compared with GLU. Net glycerol release was not different between trials and did not change from resting levels during exercise (Fig. 4B). Net glucose release increased (P < 0.05) to a similar extent in both trials during the 1st h of exercise (Fig. 5A). Net glucose uptake was greater (P < 0.05) in the 2nd h during GLU compared with CON. Leg lactate release did not increase during exercise and was not different between trials (Fig. 5B). Leg blood flow was not different between trials and was increased (P < 0.05) by 60 min and remained unchanged during exercise.

Table 1. Physiological responses during 120 min of cycle exercise at 65% \( \dot{V}O_2 \)-peak

<table>
<thead>
<tr>
<th></th>
<th>Trial 30 min</th>
<th>Trial 60 min</th>
<th>Trial 90 min</th>
<th>Trial 120 min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>VO2, mL·kg⁻¹·min⁻¹</td>
<td>%VO2peak</td>
<td>HR, beats/min</td>
<td>VO2, mL·kg⁻¹·min⁻¹</td>
</tr>
<tr>
<td>CON</td>
<td>32.6±2.2</td>
<td>62±0.0</td>
<td>144±4</td>
<td>32.6±2.2</td>
</tr>
<tr>
<td>GLU</td>
<td>32.0±2.2</td>
<td>63±0.0</td>
<td>148±4</td>
<td>32.0±2.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. VO2peak, peak pulmonary oxygen uptake; HR, heart rate; GLU, ingesting 250 mL of a 6.4% carbohydrate drink; CON, ingesting a sweet placebo drink. *Significant difference from CON (P < 0.05); †significant difference from 30 min of the same trial (P < 0.05).

Table 2. Arterial plasma metabolite responses during 120 min of cycle exercise at 65% \( \dot{V}O_2 \)-peak

<table>
<thead>
<tr>
<th></th>
<th>Trial 0 min</th>
<th>Trial 30 min</th>
<th>Trial 60 min</th>
<th>Trial 90 min</th>
<th>Trial 120 min</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FFA, mM</td>
<td>Glycerol, mM</td>
<td>Glucose, mM</td>
<td>Lactate, mM</td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td>0.8±0.2</td>
<td>79±14</td>
<td>5.4±0.2</td>
<td>0.7±0.2</td>
<td></td>
</tr>
<tr>
<td>GLU</td>
<td>0.87±0.15</td>
<td>134±20</td>
<td>5.4±0.2</td>
<td>0.7±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. FFA, free fatty acid. *Significant difference from corresponding value for CON (P < 0.05); †significant difference from 0 min of the same trial (P < 0.05).
throughout the remainder of the exercise bout (30 min 5.87 ± 0.47, 120 min 6.19 ± 0.49 l/min).

**Estimated substrate oxidation during exercise.** Total fat oxidation increased \((P < 0.05)\) from 13.2 ± 2.7 kJ/min at 30 min to 25.1 ± 3.3 kJ/min at 120 min in CON. Fat oxidation during GLU increased to 18.7 ± 3.2 kJ/min by 120 min (Table 3). There were no differences in total fat oxidation between trials. Total carbohydrate oxidation was not different at 30 and 60 min but was increased \((P < 0.05)\) in the 2nd h of exercise during GLU compared with CON (Table 3). Carbohydrate oxidation was reduced \((P < 0.05)\) at 120 min compared with 30 min in CON. Net glycogen use was not different between trials (CON 263 ± 43, GLU 290 ± 28 mmol/kg dm). Muscle lactate accumulation was not affected by either exercise or treatment (data not shown). Estimated plasma glucose oxida-

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**Fig. 2.** Plasma epinephrine (A) and norepinephrine (B) at rest and during 120 min of cycle exercise at 65% \(\dot{V}O_{2\text{peak}}\). Values are means ± SE; \(n = 7\). ●, CON; *significant difference from corresponding value for CON \((P < 0.05)\); †significant difference from 0 min of the same trial \((P < 0.05)\).

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**Fig. 3.** Hormone-sensitive lipase (HSL) activity at rest and immediately following 120 min of exercise at 65% \(\dot{V}O_{2\text{peak}}\) with or without carbohydrate ingestion. Values are means ± SE; \(n = 7\). *Significant difference from corresponding value for CON \((P < 0.05)\); †significant difference from 0 min of the same trial \((P < 0.05)\).

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**Fig. 4.** Net leg free fatty acid (FFA) uptake (A) and net leg glycerol release (B) at rest and during 120 min of cycle exercise at 65% \(\dot{V}O_{2\text{peak}}\). Values are means ± SE; \(n = 7\). *Significant difference from corresponding value for CON \((P < 0.05)\).

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**Fig. 5.** Net leg glucose uptake (A) and lactate release (B) at rest and during 120 min of cycle exercise at 60% \(\dot{V}O_{2\text{peak}}\). Values are means ± SE; \(n = 7\). *Significant difference from corresponding value for CON \((P < 0.05)\); †significant difference from 0 min of the same trial \((P < 0.05)\).
tion was not different between trials at 30 min but was elevated ($P < 0.05$) at 60 min and thereafter during GLU. Similarly, estimated plasma FFA oxidation was not different from 0 to 1 h but was decreased ($P < 0.05$) in GLU during from 1 to 2 h. Estimated nonplasma FA oxidation did not increase with exercise duration and was not different between trials (Fig. 6).

**DISCUSSION**

The major finding of the present study was that increased plasma insulin and decreased plasma epinephrine induced via carbohydrate ingestion were associated with the attenuation of the exercise-induced increase in HSLa. These results suggest that, even in circumstances where local factors that influence HSLa are important (43), insulin and epinephrine nonetheless regulate this enzyme. Despite the decreased HSLa, estimated nonplasma FA oxidation tended to be augmented with glucose ingestion, suggesting that other factors are important for IMTG degradation.

**Regulation of HSL activity.** Few studies have examined the acute regulation of HSLa in human skeletal muscle. HSLa is increased at the onset (1 min) of exercise and is largely independent of exercise intensity (45). Previous work has demonstrated that HSLa increases with exercise duration, reaching the highest rates at 1 h, after which they decline toward basal rates by 2 h (43). There is evidence from adipose tissue preparations that suggests that factors influencing PKA, such as epinephrine and insulin, are the primary regulators. In this regard, we investigated the hypothesis that glucose ingestion would prevent the normal exercise-induced increase in HSLa secondary to elevated plasma insulin and suppressed plasma epinephrine. The finding of significantly lower (~25%) HSLa activity after 2 h of exercise with carbohydrate ingestion compared with CON supports our hypothesis. These data indicate, but do not confirm, that the large elevation in plasma insulin and relatively small increase in plasma epinephrine were sufficient to inhibit the normal exercise-induced increase in HSLa.

It is possible that local regulatory factors mediated the decreased HSLa with glucose ingestion. Indeed, in a previous study, HSLa was reduced from 2-h rates by 2 h despite marked increases in plasma epinephrine and almost complete suppression of plasma insulin, suggesting that intramuscular factors override hormonal factors during prolonged exercise (43). 5-AMP-activated protein kinase (AMPK) is proposed to prevent HSL activation in adipocytes (16), although studies in skeletal muscle have yet to confirm this finding. It is unlikely that cellular calcium, a known inhibitor of HSLa (49), would be different given that the exercise power output was identical between trials. HSL is a substrate of extracellular regulated kinase (ERK), and evidence in adipocytes (17) and skeletal muscle (46) demonstrates increased HSLa when the ERK pathway is activated. ERK is activated by insulin and contractions via independent pathways (30, 48), and it is likely that ERK activity would be elevated above exercise alone during exercise and glucose ingestion. Thus, despite the possibility of increased ERK activity, the exercise-induced increase in HSLa was attenuated in GLU. Given that HSLa was not elevated above resting rates in GLU, we suggest that, in contrast to control conditions where intramuscular factors (e.g., ERK) are likely to override hormonal regulators during exercise (43), the marked increase in insulin combined with suppression of epinephrine observed with glucose feeding is likely to be more critical than the putative intramuscular effectors for HSLa.

**Substrate utilization during exercise.** To examine the relationship between HSLa and estimated fat oxidation, we estimated nonplasma FA oxidation as the difference between estimated leg FA oxidation and plasma FFA oxidation by use of precise arteriovenous balance measurements. IMTG was not directly measured with muscle biopsy and chemical extraction, because small net changes were expected over 2 h of exercise, and the variability (~25%) observed in untrained individuals (47) precludes the detection of significant changes and meaningful conclusions (44). Although we acknowledge the limitations of calculating apparent IMTG oxidation (i.e., nonplasma FA oxidation; see METHODS), the other non-FA source of FA, plasma triglycerides, are thought to account for a maximum of 5–10% of energy turnover during exercise under normal dietary conditions (18, 19). Hence, we feel justified that our calculated data with respect to nonplasma FA oxidation provide a reasonable index of IMTG use during exercise.

The lowering of total FA oxidation during GLU was the result of a marked decrease in estimated plasma FFA oxidation in the 2nd h. In the 2nd h of exercise, estimated plasma FFA uptake progressively increased during CON, whereas estimated FFA uptake was decreased with carbohydrate ingestion, resulting in an approximately threefold difference between trials by 2 h. The increased estimated plasma FFA uptake in CON completely accounted for the elevated total fat oxidation observed between 1 and 2 h. In contrast, total fat oxidation tended to increase toward the cessation of exercise in GLU despite no change in estimated plasma FFA uptake. It is difficult for us to compare our data with those of others due to
the paucity of studies that have examined the effect of glucose ingestion on fat metabolism in exercising humans. However, compared with a control trial, a 27% reduction in the calculated IMTG oxidation was reported with carbohydrate ingestion during 40 min of exercise at 50% \( \text{VO}_{2\text{peak}} \) with the use of whole body indirect calorimetry and stable-isotope tracer methodology (10). In contrast with these data, we observed no change in estimated nonplasma FA oxidation with carbohydrate ingestion during prolonged exercise despite marked increases in plasma insulin. In fact, the estimated nonplasma FA oxidation was greater during GLU in five of the seven subjects (total estimated nonplasma FA oxidation: 37.1 ± 9.6 vs. 33.2 ± 10.7 kJ/kg for GLU and CON, respectively). Moreover, the difference in estimated nonplasma FA oxidation between trials would be even greater if the esterification of plasma FFA uptake were augmented with elevated insulin, as has been demonstrated in rat skeletal muscle during in vitro contractions (12). The differences between the data from this study and those from the study by Coyle et al. (10) are difficult to reconcile but may relate to training status (endurance trained vs. untrained), duration (40 min vs. 2 h), and intensity (50 vs. 65% \( \text{VO}_{2\text{peak}} \)) of exercise. Finally, it is noteworthy that the calculated nonplasma FA oxidation, which increases, does not closely match the net glycerol release (no change) during GLU. This highlights previous suggestions (41) that using net limb glycerol release to estimate IMTG utilization has limitations, because \( I \) glycerol can be metabolized in the muscle, and 2) measuring the arteriovenous difference when blood flow is high (e.g., during exercise) is technically difficult and detecting subtle changes may be beyond the precision of this technique.

**HSLa and nonplasma FA oxidation: an apparent paradox?**

It seems paradoxical that estimated nonplasma FA utilization tends to increase despite a return of HSL to basal activity rates. It must be recognized, however, that the HSL assay represents net HSL phosphorylation and not catalytic flux in vivo. In this regard, allosteric inhibition of HSL by long-chain fatty acyl-CoA (LCFA-CoA) has been reported in adipose preparations (23). LCFA-CoA accumulates during prolonged exercise when plasma FFA availability is high and may explain the low rates of IMTG oxidation under such conditions (43). The findings of an apparent mismatch between HSLa and estimated nonplasma FA utilization also support the findings in adipose tissue that processes other than HSL phosphorylation are important events in triacylglycerol hydrolysis. These include translocation of HSL from a cytosolic site to the lipid droplet containing the triacylglycerol (5, 7) and phosphorylation of proteins (e.g., perilipins) embedded on the lipid droplet surface (35). A high rate of adipose tissue lipolysis is closely associated with a low perilipin protein content (29), and data from isolated rodent fat cells indicate that HSL catalytic activity is not important for lipolysis and that translocation of HSL to its substrate controls lipolysis (28). It is not determined yet whether these processes are important in the control of skeletal muscle triacylglycerol hydrolysis; however, the existence of multiple control points may explain the apparent mismatch between HSLa and estimated nonplasma FA utilization.

In conclusion, we have demonstrated that HSLa is blunted during exercise when glucose is ingested, most likely mediated by increased insulin and decreased epinephrine. Hence, our data demonstrate that, even during muscle contraction when local mediators of HSL are transiently elevated, the hormonal milieu plays an important role in activating this key fat-regulatory enzyme. The findings of an apparent mismatch between HSLa and estimated nonplasma FA utilization also support the notion that processes other than HSL phosphorylation are important events in IMTG degradation.

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