Manipulation of dietary carbohydrate and muscle glycogen affects glucose uptake during exercise when fat oxidation is impaired by β-adrenergic blockade

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First published August 17, 2004; doi:10.1152/ajpendo.00302.2004.—We have recently reported that, during moderate intensity exercise, low muscle glycogen concentration and utilization caused by a high-fat diet is associated with a marked increase in fat oxidation with no effect on plasma glucose uptake (Rd glucose). It is our hypothesis that this increase in fat oxidation compensates for low muscle glycogen, thus preventing an increase in R4 glucose. Therefore, the purpose of this study was to determine whether low muscle glycogen availability increases Rd glucose under conditions of impaired fat oxidation. Six cyclists exercised at 50% peak O2 consumption (V̇O2 peak) for 1 h after 2 days on either a high-fat (HF, 60% fat, 24% carbohydrate) or control (CON, 22% fat, 65% carbohydrate) diet to manipulate muscle glycogen to low and normal levels, respectively. Two hours before the start of exercise, subjects ingested 80 mg of propanolol (βB), a nonselective β-adrenergic receptor blocker, to impair fat oxidation during exercise. HF significantly decreased calculated muscle glycogen oxidation (P < 0.05), and this decrease was partly compensated for by an increase in fat oxidation (P < 0.05), accompanied by an increase in whole body lipolysis (P < 0.05), despite the presence of βB. Although HF increased fat oxidation, plasma glucose appearance rate, Rd glucose, and glucose clearance rate were also significantly increased by 13, 15, and 26%, respectively (all P < 0.05). In conclusion, when lipolysis and fat oxidation are impaired, in this case by βB, fat oxidation cannot completely compensate for a reduction in muscle glycogen utilization, and consequently plasma glucose turnover increases. These findings suggest that there is a hierarchy of substrate compensation for reduced muscle glycogen availability after a high-fat, low-carbohydrate diet, with fat being the primary and plasma glucose the secondary compensatory substrate. This apparent hierarchy likely serves to protect against hypoglycemia when endogenous glucose availability is low.

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subjects consumed either a control (CON) or HF diet of equal caloric content while exercising for 2 h at 65% $V_{\text{O}2\text{peak}}$ on day 1 followed by 1 h at 65% $V_{\text{O}2\text{peak}}$ on day 2. In our previous report using similar subjects, we observed that this 2-day exercise and dietary regimen yields twofold differences in muscle glycogen concentrations the morning of day 3 (716 ± 68 vs. 348 ± 36 mmol/kg dry wt, CON vs. HF) (38). On day 3 of each trial, after a 12-h overnight fast, subjects exercised at 50% $V_{\text{O}2\text{peak}}$ for 1 h while metabolism was monitored with stable isotope tracer infusion ([6,6-$2\text{H}_2$]glucose and [1,1,2,3,3-$2\text{H}_5$]glycerol) and indirect calorimetry. Two hours before exercise, subjects consumed 80 mg of propanolol, a nonselective $\beta$-adrenergic receptor blocker (βB). Propanolol is a potent inhibitor of lipolysis and fat oxidation during exercise (20). The two trials are referred to as control diet with βB (βB CON) and HF diet with βB (βB HF).

Diet. Subjects were provided all their food for the CON and HF diets, and they were asked to carefully and honestly report any food not eaten as well as food eaten in addition to that provided. CON was composed of 22% fat (1 g/kg · day$^{-1}$), 65% carbohydrate (9 g/kg · day$^{-1}$), and 13% protein, and the equal caloric HF was 60% fat (3 g/kg · day$^{-1}$), 24% carbohydrate (3 g/kg · day$^{-1}$), and 16% protein. On the evening before the experimental trial on day 3, subjects consumed a 0.5 g/kg snack of high-glycemic carbohydrate before initiating a 12-h overnight fast before exercise. This final meal was maintained constant for both trials to ensure that the metabolism measured on day 3 was a reflection of the cumulative effects of the 2-day diet and not the effect of the last meal before the overnight fast on metabolism (29).

Experimental trial (day 3). Subjects arrived at the laboratory in the morning before the standardized last meal described above. Upon arrival, tetol catheters were inserted into an antecubital vein in each arm for infusion and blood sampling, respectively. Blood was sampled (8 ml) for determination of background isotopic enrichment. Subjects then ingested 80 mg of propanolol 2 h before exercise. Primed constant-rate infusions of [1,1,2,3,3-$2\text{H}_5$]glycerol (prime = 3.7 μmol/kg, constant = 0.25 μmol·kg$^{-1}$·min$^{-1}$) and [6,6-$2\text{H}_2$]glucose (prime = 35 μmol/kg, constant = 0.40 μmol·kg$^{-1}$·min$^{-1}$; Isotec, Miamisburg, OH) were initiated using calibrated syringe pumps (Harvard Apparatus, South Natick, MA) and maintained for 1.5 h before exercise and during 1 h of exercise. Subjects exercised on a cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands) for 1 h at 50% $V_{\text{O}2\text{peak}}$.

Blood sampling and analysis. Blood was sampled (∼10 ml) at rest and every 10 min during exercise for determination of plasma glycerol, free fatty acids (FFA), insulin concentrations, and rates of plasma glucose and glycerol kinetics and placed in tubes chilled on ice. Eight milliliters were placed into tubes containing 0.4 ml of ethylene diamine tetracetic acid solution (EDTA, 25 mg/ml) and 2 ml were placed in tubes with 0.2 ml of EDTA (25 mg/ml) and aprotinin (0.5 TiU/ml). Plasma was separated by centrifugation (i.e., 1,000 g for 20 min at 4°C) and frozen at −80°C until analysis. Plasma FFA (22) and glucose concentrations (Trinder; Sigma, St. Louis, MO) were determined with colorimetric assays, and plasma glycerol was analyzed with a fluorometric assay (9). Plasma insulin concentrations were analyzed with a radioimmunoassay kit (catalog no. HI-11K; Linco Research, St. Charles, MO).

Isotope enrichment sample separation. Plasma samples (300 μl) were deproteinized with 450 μl of 0.3 N ZnSO4 and 450 μl of 0.3 N Ba(OH)2. Each tube was vortexed and incubated in an ice bath for 20 min. After centrifugation at 1,000 g for 15 min at 4°C, the supernatant was passed down an ion exchange column, which was then rinsed five times with 400 μl of deionized distilled water. The eluant was captured in glass 13 × 100-mm test tubes and then dried overnight with compressed air. Acetic anhydride (75 μl) and pyridine (75 μl) were added to dried samples and then incubated at 100°C for 1 h and subsequently dried with N2 gas. Samples were then resuspended in 4 ml of ethyl acetate and injected into a gas chromatograph-mass spectrometer with autosampler (Hewlett Packard 5890 Series II-5988A mass spectrometer), and the masses 145 and 148 of the triacetate derivative of glycerol and the masses 200 and 202 of the pentaacetate derivative of glucose were monitored with selective ion monitoring (23).

Glycerol and glucose kinetics. Glycerol and glucose kinetics during exercise were calculated with a modified one-pool non-steady-state model (35).

$$R_g = (F - V_d[(C_1 + C_2)/2\| (E_2 - E_1)/(t_2 - t_1))] / [E_2 + E_1]/2$$

$$R_d = (E_2 - C_2) / (t_2 - t_1)$$

$$\text{glycerol clearance rate} = R_g[(C_1 + C_2)/2]$$

where F is the isotope infusion rate, $V_d$ is the effective volume of distribution, C is the plasma concentration of the tracee, and $E_2 - E_1$ is the change in enrichment (i.e., $E = \text{tracer/tracee ratio}$) between two consecutive samples ($t_2 - t_1 = 10$ min). $R_d$ was estimated to be 230 ml/kg for glycerol (30) and 150 ml/kg for glucose (1). $R_g$ is a validated measurement of hepatic glucose production (1). $R_d$ is an index of muscle glucose uptake and oxidation (18). Glycerol clearance rate is an index of the glucose uptake relative to the prevailing plasma glucose concentration. The rate of appearance of glycerol in plasma ($R_d$ glycerol) was measured as an index of whole body lipolysis with the assumption that all glycerol released in the process of lipolysis appears in the plasma. $R_d$ glycerol could potentially underestimate whole body lipolysis if glycerol is utilized by tissues instead of being released into plasma (14). Glycerol kinase activity is low in mammalian skeletal muscle, although it appears sufficient to allow some glycerol to be used for muscle triglyceride resynthesis at rest (14). However, during exercise, it is unlikely that glycerol utilization takes place in human tissues to an extent that would meaningfully invalidate the measurement of lipolysis from plasma glycerol kinetics.

Measurements of gas exchange. During exercise, subjects inhaled a two-way valve while inspired air volume was measured with a pneumotachometer (Hans Rudolph, Kansas City, MO) and analyzed for oxygen (Applied Electrochemistry, SA3, Ametek, Pittsburgh, PA) and carbon dioxide (Beckman LB2, Schiller Park, IL). These instruments were interfaced to a computer for calculation of $V_{\text{O}2}$ and $V_{\text{CO}2}$.

Substrate oxidation. Fat and carbohydrate oxidation was calculated from $V_{\text{O}2}$ and $V_{\text{CO}2}$ measurements (12). Because at 50% $V_{\text{O}2\text{max}}$, it has been reported that 96–100% of $R_d$ glucose is oxidized, an estimate of whole body glycogen oxidation was calculated as total carbohydrate oxidation minus $R_d$ glucose (18).

Statistics. A two-way repeated-measures ANOVA (diet × time) with mean contrasts according to Bonferroni inequalities was used to analyze dependent variables at specific time points during exercise when there was a significant main effect or interaction. Statistical significance was defined as $P < 0.05$. The results are presented as means ± SE.

RESULTS

Glycerol $R_g$ (lipolysis). Throughout exercise, $R_d$ glycerol was significantly higher after the βB HF than after the βB CON diet (4.48 ± 0.14 vs. 2.63 ± 0.14 μmol·kg$^{-1}$·min$^{-1}$, mean of 15–55 min, $P < 0.05$; Fig. 1A). During exercise, βB HF significantly elevated $R_d$ glycerol above the resting value of 3.87 ± 0.74 μmol·kg$^{-1}$·min$^{-1}$ by 45 and 55 min (4.79 ± 0.35 and 4.89 ± 0.34 μmol·kg$^{-1}$·min$^{-1}$, respectively, vs. rest, $P < 0.05$), whereas during exercise in βB CON, $R_d$ glycerol did not significantly increase above the resting level.

Fat oxidation. Fat oxidation during exercise was significantly higher during βB HF by 8.46 ± 1.12 μmol·kg$^{-1}$·min$^{-1}$ compared with βB CON (18.46 ± 1.14 vs. 10.00 ± 0.74 μmol·kg$^{-1}$·min$^{-1}$, mean 10–55 min, $P < 0.05$; Fig. 1B).
Throughout exercise in both trials, there was not a significant effect of time on fat oxidation, as it remained steady.

Glycogen and total carbohydrate oxidation during exercise. Total carbohydrate oxidation during exercise was reduced 18% by βB HF compared with βB CON (132 ± 7 vs. 160 ± 9 μmol·kg⁻¹·min⁻¹, mean 10–55 min, P < 0.05; Fig. 2). Furthermore, calculated whole body glycogen oxidation was reduced 29% by βB HF diet (74 ± 7 vs. 104 ± 9 to μmol·kg⁻¹·min⁻¹, mean of 15–55 min of exercise, P < 0.05).

Ra glucose and Rd glucose. Resting glucose kinetics were not affected by diet. However, during exercise, Ra glucose (27.5 ± 2.1 vs. 24.3 ± 1.3 μmol·kg⁻¹·min⁻¹; Fig. 3) and Rd glucose (28.5 ± 2.8 vs. 24.7 ± 1.7 μmol·kg⁻¹·min⁻¹; Fig. 3) were significantly increased by 13 and 15% during βB HF vs. βB CON (βB HF > βB CON, mean 15–55 min, P < 0.05).

Glucose clearance rate during exercise was elevated by 26% in βB HF (6.74 ± 0.83 vs. 5.36 ± 0.40 ml·kg⁻¹·min⁻¹, mean 15–55 min, βB HF vs. βB CON, P < 0.05; Fig. 4).

Plasma glucose concentrations. At rest, plasma glucose concentrations were not significantly affected by the preceding diet (4.88 ± 0.16 vs. 4.89 ± 0.07 mM, βB HF and βB CON; Fig. 5). During exercise in βB CON, plasma glucose concentration was not significantly different from the resting value until 60 min of exercise when plasma glucose concentration decreased to 4.48 ± 0.22 mM (P < 0.05 vs. resting value). However, exercise during βB HF significantly lowered plasma glucose concentrations throughout 10–60 min of exercise compared with resting value (4.37 ± 0.18 mM, mean 10–60 min, vs. rest, P < 0.05); specifically, plasma glucose concentration was lowered to 4.07 ± 0.22 mM at 60 min of exercise (vs. rest, P < 0.05). Over the exercise period, mean plasma glucose concentrations tended to be lower during exercise in

Fig. 1. Glycerol rate of appearance (Ra) (A) and fat oxidation (B) during 60 min of exercise at 50% peak O₂ uptake (V˙O₂ peak) with propanolol ingestion (βB) 2 h before exercise after 2 days on either control (CON) or high-fat (HF) diet. †βB HF higher than resting value. ‡βB HF higher than βB CON, all P < 0.05.

Fig. 2. Total carbohydrate and whole body glycogen oxidation during 60 min of exercise at 50% V˙O₂ peak with propanolol ingestion 2 h before exercise after 2 days on either βB CON or βB HF diet. ‡βB HF lower than βB CON, P < 0.05.

Fig. 3. Plasma glucose Ra and uptake (Rd) during 60 min of exercise at 50% V˙O₂ peak with propanolol ingestion 2 h before exercise after 2 days on either βB CON or βB HF diet. ‡βB HF higher than βB CON, P < 0.05.
**Plasma FFA concentrations.** At rest, BB HF slightly but significantly elevated plasma FFA concentrations above BB CON (0.34 ± 0.06 vs. 0.27 ± 0.04 mM, *P* < 0.05; Fig. 6). Throughout exercise in both trials, plasma FFA concentrations were significantly lower than resting values (*P* < 0.05). Mean plasma FFA concentrations were similar with the two diets during exercise (0.21 ± 0.03 vs. 0.25 ± 0.02 mM, *P* = 0.28).

**Plasma insulin concentrations.** At rest, plasma insulin concentration was significantly lower during BB HF compared with BB CON (1.82 ± 0.29 vs. 2.86 ± 0.63 μIU/mL, *P* < 0.05). In both trials, plasma insulin concentrations were significantly lower than resting values throughout most of the exercise (i.e., 20–60 min), and there were no differences between the diets (Fig. 7).

**DISCUSSION**

The primary finding of this study was that the uptake of blood glucose during exercise (i.e., **R_d glucose**) was significantly increased by low muscle glycogen associated with a high-fat diet, when lipolysis and fat oxidation were impaired by β-adrenergic blockade. Previously, using the same dietary and exercise protocol (38), we reported that, in the overnight-fasted state without BB, the decrease in glycogen concentration and utilization common to a high-fat diet is compensated by a marked increase (i.e., 71%) in fat oxidation associated with a
similar increase in whole body lipolysis (i.e., 78%). This compensatory increase in fat oxidation was apparently very well matched to the reduction in glycogen utilization, because the other major fuel source, plasma glucose as measured from $R_g$ glucose, was unaffected by the high-fat diet (17.3 ± 1.0 vs. 18.4 ± 1.5 $\mu$mol·kg$^{-1}$·min$^{-1}$, high-fat vs. control diet) (38). In the present study, the increase in lipolysis and fat oxidation caused by the high-fat diet was attenuated by $\beta$-adrenergic blockade and thus may be responsible for the 15, 26, and 13% caused by the high-fat diet was attenuated by $\beta$-adrenergic blockade and thus may be responsible for the 15, 26, and 13% compensatory increase in $R_g$ glucose, glucose clearance rate, and $R_a$ glucose, respectively.

Previously, we (38) reported that the same high-fat diet, in similarly trained subjects without $\beta$B, significantly lowered muscle glycogen concentration by 51% and decreased glycogen oxidation during exercise by 42%. This reduction in muscle glycogen previously did not increase $R_a$ glucose, glucose clearance rate, or $R_g$ glucose; yet it caused an $\sim$14 $\mu$mol·kg$^{-1}$·min$^{-1}$ increase in fat oxidation and an $\sim$6 $\mu$mol·kg$^{-1}$·min$^{-1}$ increase in triglyceride lipolysis during exercise in the normal overnight-fasted state (38). In the present study, under conditions of $\beta$-adrenergic blockade, the increases in fat oxidation and lipolysis were attenuated, as the increase in fat oxidation was only $\sim$8.5 $\mu$mol·kg$^{-1}$·min$^{-1}$ and the increase in triglyceride lipolysis was $\sim$2 $\mu$mol·kg$^{-1}$·min$^{-1}$ (vs. Ref. 36, both $P < 0.05$). Interestingly, $R_g$ glucose during exercise, an index of muscle glucose uptake, increased by 15% following the high-fat diet, and, because mean exercise glucose concentration also tended to be lower (4.37 vs. 4.66 mM, $P = 0.16$), the glucose clearance rate was increased by 26% above control. Changes in plasma insulin concentrations were not responsible for this elevated $R_a$ glucose or glucose clearance rate, as insulin concentrations were similar during exercise with both diets. As has been reported before, the reduction in fat oxidation caused by nonselective $\beta$-adrenergic receptor blockade increases $R_a$ glucose and clearance rate (20, 28), and we report here that this effect is further augmented by low muscle glycogen.

Whether low muscle glycogen concentration from a high-fat, low-carbohydrate diet affects glucose uptake has been controversial. Previous studies have reported that 2 days (15, 37, 38) and 7 wk (16) of a high-fat diet and exercise regimen and associated low muscle glycogen concentration did not increase plasma glucose utilization, whereas others have reported elevated muscle glucose uptake after $\sim$16–24 h on a high-fat diet and glycogen-depleting exercise regimen (4, 13). In light of our present findings, the elevated muscle glucose uptake previously reported by others (4, 13) may have been due to an inadequate compensation from fat oxidation, considering that adaptation to a high-fat diet may require 24 h or more, even in those who engage in exercise training (33). In addition, reports of the inverse relationship between muscle glycogen concentration and glucose uptake in the rat hindlimb perfusion model (7, 17, 27) may have been due to the inability of the muscle to increase fat oxidation to compensate for low glycogen because muscle glycogen was typically lowered fewer than 24 h before measurement of muscle glucose uptake. Moreover, because the sources for the elevated fat oxidation following a high-fat diet have been reported to be due to elevated muscle triglyceride concentration and breakdown (19, 38) and increased VLDL-triglyceride uptake (16), the absence of catecholamines (i.e., to facilitate IMTG breakdown) and VLDL-triglyceride in the perfusion medium of rat muscle would have likely compromised the ability of fat oxidation to increase and, according to the present results, would have increased dependence on glucose uptake. Therefore, the hindlimb perfusion model for assessing the effect of low glycogen on glucose uptake is distinct from the in vivo human studies wherein IMTG breakdown is facilitated by catecholamines (5) and plasma triglycerides are available to skeletal muscle (16).

Cellular mechanisms that may be responsible for elevated muscle glucose uptake with low muscle glycogen may involve changes in AMP-activated protein kinase (AMPK) activity and glucose 6-phosphate concentrations. AMPK activation may be responsible for glucose uptake in type II muscle fibers, as its activity and glucose uptake have been reported to be potentiated by low muscle glycogen in rat skeletal muscle (7). In addition, low muscle glycogen may decrease glucose 6-phosphate concentrations (27) and thus dishibit hexokinase and thereby increase glucose phosphorylation and uptake. Elevated fatty acid availability and oxidation in the normal overnight-fasted state following a high-fat diet (38) may retard the effects of low glycogen on AMPK and/or glucose 6-phosphate concentrations, as fatty acid elevation and oxidation (i.e., via Intralipid and heparin infusion) have been reported to elevate glucose 6-phosphate concentrations (e.g., Randle effect) and decrease AMP concentration (8).

The elevated $R_a$ glucose following the high-fat diet in the present study may have been derived from increased hepatic glycogenolysis and/or elevated gluconeogenesis. Because a high-fat diet reportedly lowers liver glycogen concentrations (21) and increases gluconeogenesis (26), the latter seems to be a more plausible source of the increase in $R_a$ glucose with the high-fat, low-carbohydrate diet. Enhanced stimulation of $R_a$ glucose may also have resulted from the tendency for lower plasma glucose concentration to stimulate hepatic $R_a$ glucose independently of adrenergic stimulation (6).

Together, our present and recent (38) findings that low muscle glycogen concentration associated with a high-fat, low-carbohydrate diet increases glucose uptake only when lipolysis and fat oxidation are limited suggest that there is a hierarchy of substrate compensation, with fat being the primary compensatory substrate and plasma glucose secondary for reduced muscle glycogen availability. This apparent hierarchy is not surprising in light of the limited supply of liver glycogen ($\sim$200 kcal after overnight fast) relative to the large amount of energy available from fatty acids stored as triglycerides in muscle and adipose tissue (>$30,000$ kcal). An increased dependence on glucose uptake could certainly challenge already-limited liver glycogen stores and thus threaten the maintenance of euglycemia. As suggested by the tendency for a decrease in mean exercise plasma glucose concentration and the statistically significant ($P < 0.05$) lower glucose concentrations at the 30- and 60-min time points after the high-fat diet in the $\beta$B condition (4.37 ± 0.18 vs. 4.66 ± 0.18, $\beta$B HF vs. $\beta$B CON, $P = 0.16$) and the well-maintained euglycemia after the high-fat diet in control conditions in Ref. 38 (4.86 vs. 4.92 mM, CON vs. HF, $P = 0.5$), the elevated lipolysis and fat oxidation caused by a high-fat diet defend the body from systemic hypoglycemia. Similarly, the exercise-trained state is another condition whereby the oxidation of IMTG is increased and plasma glucose turnover is slowed, resulting in better maintenance of euglycemia during 90 min of exercise compared with...
exercise in the untrained state performed at the same absolute work rate (24, 25).

We recently reported that IMTG breakdown during exercise could account for the increase in fat oxidation and lipolysis caused by this high-fat diet (38). The present results also support this in that β-adrenergic receptor blockade impairs IMTG breakdown (5) and concomitantly attenuates the increase in fat oxidation caused by the high-fat diet. We have also reported that suppression of plasma FFA concentration with the adipose lipolysis inhibitor acipimox does not impair the marked increase in fat oxidation caused by a high-fat diet (38). Despite the β-adrenergic receptor blockade, fat oxidation and lipolysis were still increased by the high-fat compared with the control diet, and this may have been due to elevated VLDL-triglyceride uptake (16) and/or an incomplete β-adrenergic receptor blockade of IMTG breakdown as well as the possibility that IMTG breakdown is still elevated by the high-fat diet via mechanisms independent of β-adrenergic stimulation, such as changes in intracellular fatty acid-binding proteins (2) and elevated IMTG concentration (10, 34, 36).

In conclusion, when lipolysis and fat oxidation are impaired during exercise, in this case by βB, increases in fat oxidation derived from a high-fat diet cannot completely compensate for a reduction in muscle glycogen utilization, and consequently plasma glucose turnover increases. These findings suggest that there is a hierarchy of substrate compensation during exercise in response to reduced muscle glycogen availability following a high-fat, low-carbohydrate diet, with fat being the primary compensatory substrate and plasma glucose the secondary substrate. This apparent hierarchy likely serves to protect against hypoglycemia when endogenous glucose availability is low.

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REFERENCES


