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Exogenous glucose oxidation is reduced with carbohydrate feeding during exercise after starvation

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Abstract

Lean healthy individuals are characterized by the ability to rapidly adapt metabolism to acute changes in substrate availability and metabolic rate. However, in glucose-intolerance/insulin-resistant conditions, such as that induced by starvation, the flexibility of tissues to rapidly respond to change in substrate availability is diminished. We asked whether the conundrum of increased glucose demand by the contracting skeletal muscle during prolonged exercise and the glucose intolerance of starvation would result in the obstruction of oxidative disposal of ingested ^{13}C-labeled glucose during exercise. Seven lean, healthy, physically active individuals (2 women, 5 men) completed a randomized crossover study comparing the effects of the normal-fed condition vs a 67-hour water-only fast on the metabolic response to carbohydrate ingestion during 80 minutes of exercise at 56% of maximum oxygen uptake. Compared with the normal condition, fasting resulted in a large overall increase in the rate of fat oxidation (mean effect, 71%; 95% confidence limit, ±22%) and moderate reductions in both exogenous (−54%, ±10%) and endogenous (−40%, ±19%) glucose oxidation rates during exercise. Over the course of exercise, fat oxidation was impermeable to change in the fasting condition, but increased moderately (33%, ±19%) in the normal condition. These changes were associated with a large increase in plasma free fatty-acid concentration (120%, ±64%) and a moderate increase in blood lactate concentration (58%, ±50%). In contrast, large reductions in resting blood glucose (−21%, ±14%) and moderate reductions in plasma insulin concentrations (−47%, ±26%) were observed in the fast condition; but this effect was reversed for glucose (30%, ± 24%) and negated for insulin by the end of exercise. To conclude, a 67-hour fast leads to an impermeable increase in fat oxidation, suppression of both exogenous and endogenous carbohydrate oxidation, and a metabolic response consistent with resistance to contraction-induced exogenous glucose uptake and oxidation.

1. Introduction

The tissues of lean healthy individuals rapidly change substrate utilization in response to acute changes in substrate availability [1]. For example, if carbohydrate is suddenly presented/ingested in an amount exceeding that immediately required to maintain euglycemia, there is an increase in insulin-mediated carbohydrate disposal and oxidation [2], primarily within the skeletal muscle [3], and a compensatory reduction in lipid oxidation [2,3].

In contrast, when glucose availability has been scarce for a period of time, such as during starvation, and then ample glucose suddenly becomes available, a whole-body glucose intolerance exists [4], which is partly a function of resistance within the skeletal muscle to the action of secreted insulin on glucose uptake [5]. Furthermore, of that glucose transported into the muscle, storage is preferred over oxidation [6]; and whether by cause or effect, starvation is associated with higher levels of fat oxidation [7].

Furthermore, when carbohydrate availability is increased by glucose feeding or infusion during exercise, there is a shift toward higher carbohydrate oxidation by the muscle [8,9]. However, unlike the rested state where hepatic glucose uptake and release are quantitatively important, this metabolic response is dominated by skeletal muscle and driven

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more by contraction-mediated, and less by insulin-stimulated, glucose uptake, as insulin secretion during exercise is generally blunted [10].

The maintenance of euglycemia is important for normal central nervous system function, particularly in exercise where the contracting muscle competes with the brain for circulating glucose, the availability of which may limit physical work performance [8,13]. If the contraction-mediated stimulus for glucose uptake were to persist during starvation, this situation would present the body with a conundrum. That is, if the normal increase in carbohydrate metabolism observed during exercise were to also occur during exercise in the starved state, it would result in a rapid increase in glucose uptake and oxidation by skeletal muscle, restricting glucose availability to the brain. The situation of exercise during starvation is relevant given that, in hunter-gatherer societies, the requirement for physical activity was likely to be greatest when food availability was reduced and the ability to maintain neuromuscular function was important for survival [11]. Whereas the metabolic effects of the provision of carbohydrate during exercise in the normal postabsorptive state are well established, there is no information available regarding the effects of glucose provision during exercise in the starved state.

We hypothesized that the intolerance to glucose ingestion previously shown to exist during starvation would persistent during exercise in the starved state. If this were the case, then in contrast to the overnight-fasted state, labeled glucose ingested during exercise when starved should not be readily oxidized. To examine this hypothesis, we studied the oxidation of ingested glucose and endogenous substrates during exercise after either a normal 12-hour (overnight) fast or a 67-hour period of starvation. We have previously shown that 67 hours of starvation induces marked glucose intolerance/insulin resistance at rest in healthy lean individuals [4].

2. Methods

2.1. Subjects

Seven physically trained lean adults (2 women, 5 men) aged 30.3 ± 8.5 years (mean ± SD), standing 177.1 ± 8.7 cm tall, weighing 74.1 ± 12.4 kg, and comprising 15.0% ± 3.1% body fat, volunteered to complete the study. All participants had been engaged in 8 or more hours per week of endurance exercise (cycling, running, swimming, field hockey) for 6 months or longer before the study. Maximal oxygen uptake (VO_2max) during cycle ergometry was 56.9 ± 10 $mL \cdot kg^{-1} min^{-1}$. Participants were screened for cardiovascular, musculoskeletal, and reactionary contraindications to exercise and were fully informed of the purpose and risks associated with the procedures before beginning experimentation. The study protocol conformed to the standards set by the latest revision of the Declaration of Helsinki and was approved by the Massey University Human Ethics Committee.

2.2. Preliminary testing

One to 2 weeks before the start of the experimental trials, each participant performed an incremental exercise test on a cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) to determine maximum power output and VO_2max. After arriving at the laboratory, participants voided; and then body mass, height, and skin-fold thicknesses were recorded. After a 10-minute self-paced warm-up, the test started at a workload of 3.0 $W \cdot kg^{-1}$ body mass for men and 2.0 $W \cdot kg^{-1}$ body mass for women. The first-stage duration was 150 second, after which the load was increased by 50 W, and then by 25 W for every subsequent 150-second stage. *Exhaustion* was defined as the time at which the participant could no longer maintain a pedal cadence of 50 rpm after 3 warnings. Maximal oxygen uptake was measured online with a calibrated SensorMedics Vmax Spectra Series gas analyzer (SensorMedics, Yorba Linda, CA) and taken as the highest attained 20-second average oxygen uptake. Body composition was compartmentalized using standard methods and appropriate regressions [12] into fat and lean mass using extrapolation from body density estimated from the sum of 7 skin folds for men and the sum of 4 skin folds for women measured with Harpenden callipers.

2.3. Experimental design

2.3.1. Study design

The study was a balanced, randomized, crossover design of 3 experimental treatments:

(*a*) Background condition (Background)—67 hours of normal diet followed by exercise in which only water was ingested;
(*b*) Normal condition (Normal)—67 hours of normal diet followed by exercise in which a labeled carbohydrate solution was ingested;
(*c*) Fast condition (Fast)—a 67-hour water-only fast followed by exercise in which a labeled carbohydrate solution was ingested.

Each treatment was followed by an exercise trial consisting of 80 minutes of cycling on an electromagnetically braked cycle ergometer at a workload calculated to elicit 50% of VO_2max (determined from the power–oxygen consumption [VO_2] regression of the incremental test). During exercise in the Normal and Fast conditions, participants ingested a 12% maltodextrin solution (Glucidex-19; Roquette, Lestrem, France). During the Background trial, participants ingested plain water after an overnight fast to establish the background breath enrichment.

The Background trial was performed randomly 3 to 4 days before either the Fast or Normal trial. To quantify exogenous glucose oxidation, solutions were prepared from a corn-derived glucose polymer, which has a high natural abundance of ^{13}C (−10.55 δ per mill vs Pee Dee Bellemnitella).

The female participants were menstruating regularly. To control for possible small effects of the menstrual cycle on metabolism, we tested during the mid follicular phase of the cycle, which was determined as the seventh day after the onset of menstruation. Consequently, to match any random temporal effects, the men were tested a month apart.

2.4. Diet and physical activity before testing

To reduce the background breath ^{13}C enrichment, a prolonged intense training session of 2.5 to 4 hours (glycogen-depletion ride) was performed in the laboratory or field 7 days before each experimental trial. After this session and up to each testing day, participants were provided with guidelines and instructed not to consume foods with a high natural abundance of ^{13}C (carbohydrates derived from C4 plants such as maize and sugar cane). These procedures have been shown previously to reduce the background (change in ^{13}C) from endogenous substrate stores [13]. All training and meals ingested after the glycogen-depletion ride for the first trial were recorded and replicated during the proceeding conditions.

2.5. Protocols

2.5.1. Preconditioning

As a further standardized preconditioning measure before the initiation of the treatments to establish conditions for intramuscular substrate and insulin sensitivity determined previously [4], participants ingested standardized meals and completed a control exercise bout on the third day before the testing day. At approximately 1:00 PM, a mixed meal was ingested that provided 1.5 g of carbohydrate per kilogram of body weight with a macronutrient contribution of 50%, 35%, and 15% as carbohydrate, fat, and protein, respectively. At 5:00 PM, participants cycled for 60 minutes at a workload calculated to elicit 65% VO_2max. The exercise was performed at the same power output for each individual before all experimental conditions. Exercise was followed by the consumption of a standardized beverage and meal in the Normal condition and water only in the Fast condition. In the Normal condition, a commercial sports beverage containing 1 g of carbohydrate per kilogram body weight was provided immediately postexercise, which was followed by an evening meal (1.5 g carbohydrate per kilogram of body weight as 50% carbohydrate, 35% fat, and 15% of energy as protein) 2 hours later before sleep. Beginning the following morning and continuing for the remainder of the Normal dietary condition (48 hours), participants received a diet providing energy to match a daily expenditure of 1.5 times resting metabolic rate as used previously [4]. Participants performed only normal light daily activity during the Normal dietary condition and the Fast.

2.5.2. Exercise test

Participants reported to the laboratory between 7:00 and 9:00 AM after an overnight fast (Normal) or an approximately 67-hour water-only fast (Fast). For each individual, exercise on the second experimental block began within 15 minutes of the start time in the first block. After toileting, participants were weighed before a 20-gauge Teflon catheter (Becton Dickinson Medical, Singapore) was inserted into an antecubital vein of an arm and a 3-way stopcock was attached (Becton Dickinson Medical) for repeated blood sampling via syringe during exercise. The catheter was kept patent by flushing with 1 to 2 mL isotonic saline (0.9%, Becton Dickinson Medical).

Participants then mounted the cycle ergometer, and a resting breath sample was collected in duplicate from a mixing chamber into 10-mL Exetainer tubes (Labco, High Wycombe, United Kingdom) for determination of the $^{13}C/^{12}C$ ratio of the expired air. Resting blood samples were collected and transferred from syringe into EDTA- and LH-treated tubes (Becton Dickinson Medical) and stored on ice until further treatment. Next, the participants started the 80-minute exercise bout. Expiratory breath and blood samples were collected for a 4-minute period at 10-minute intervals until the cessation of exercise. The whole-body rates of VO_2 and carbon dioxide production (VCO_2) were then calculated using the final 2 minutes of those 4-minute data. All exercise tests were performed under normal and standard laboratory environmental conditions (18°C-22°C and 50%-60% relative humidity), and participants were cooled with fans at a standard wind speed to minimize thermal stress.

The total intake of 12% maltodextrin solution during the exercise bout was 900 mL, ingested as an initial bolus (300 mL) immediately after the first exercise (basal exercise response) gas and blood sampling point at 10 minutes, after which 150 mL was ingested every 15 minutes. The average carbohydrate ingestion rate during exercise was 1.2 $g \cdot min^{-1}$. This dose was provided with the intention of obtaining the normal average saturation oxidation rate for exogenous carbohydrate of 1.0 to 1.1 $g \cdot min^{-1}$, thought to be limited physiologically by intestinal transport processes [14].

3. Analyses

Blood collected in LH tubes was analyzed for glucose and lactate concentration 10 to 20 minutes after collection using an automated analyzer (Bayer Rapidlab 800; Bayer HealthCare, Tarrytown, NY). Remaining blood in EDTA was centrifuged at 2000*g* at 2°C for 10 minutes, and aliquots of the plasma were transferred into microtubes and stored at −80°C until further analysis. Plasma samples were analyzed for nonesterified free fatty acid (FFA) concentration enzymatically by acyl–coenzyme A (CoA) oxidase method using NEFA-C kit (Wako Pure Chemicals Industries, Osaka, Japan) on a Cobas-FARA (Roche Diagnostic Systems Inc., Branchburg, NJ). Plasma insulin concentration was determined by double antibody radioimmunoassay (catalog no. HI-14HK; Linco, Billerica, MA). Breath samples were analyzed for $^{13}C/^{12}C$ ratio by gas chromatography continuous-flow isotope ratio mass spectrometry (Finnigan Delta XP, Bremen, Germany).

4. Calculations

From nonprotein VO_2 and VCO_2, total fat and carbohydrate oxidation rates (in grams per minute) were calculated using the equation of Frayn [15]: carbohydrate oxidation = 4.55 VCO_2 − 3.21 VO_2; fat oxidation = 1.67 VO_2 − 1.67 VCO_2. The rate of exogenous carbohydrate oxidation was calculated from the VCO_2 and the stable isotope measurements (breath $^{13}C/^{12}C$ ratio). The isotope enrichment was expressed as δ per million difference between the $^{13}C/^{12}C$ ratio of the sample and the international standard Pee Dee Bellemnitella according to the formula of Craig [16]: $\delta^{13}C = [(^{13}C/^{12}C \text{ sample}/^{13}C/^{12}C \text{ standard}) - 1]10^3‰$. Exogenous carbohydrate oxidation (in grams per minute) was then calculated according to Pirnay et al [17]: $VCO_2 \cdot [(\delta Exp - \delta Exp_{bkg})/(\delta Ing - \delta Exp_{bkg})]1/k$, where δExp is the breath ^{13}C enrichment during the exercise trials with carbohydrate ingestion, δIng is the ^{13}C enrichment of the carbohydrate sources in solution, δExp_{bkg} is the Background ^{13}C enrichment of expired air, and k is the volume of CO_2 produced by the oxidation of 1 g of glucose (0.7467 L of CO_2 per gram glucose). The endogenous carbohydrate oxidation rate was calculated by subtracting the exogenous carbohydrate oxidation rate from the total carbohydrate oxidation rate. Energy equivalents (kilojoules per gram) for substrate oxidation rate calculations were 16.18 and 40.78 for carbohydrate and fat, respectively [15].

A methodological consideration when using $^{13}CO_2$ in expired air to calculate exogenous substrate oxidation is the delayed equilibration of $^{13}CO_2$ originating from the tissues with the large endogenous HCO_3^- pool. However, during exercise, CO_2 production increases several fold so that a physiologic steady-state condition occurs relatively rapidly; and $^{13}CO_2$ in the expired air will be confidently equilibrated with the $^{13}CO_2/H^{13}CO_2$ pool from 60 minutes of steady-state exercise [18,19]. The rate of tracer equilibration with the HCO_3^- pool is likely to be similar between the 2 experimental conditions to permit a qualitative comparison of the effect of treatment on temporal effects; confident quantitative measures for substrate oxidation rates are reported as the average of the 70th- and 80th-minute exercise samples.

5. Statistical analyses

The effects of the 67-hour fast on metabolism during exercise were estimated with mixed modeling (Proc Mixed, SAS Version 9.1; SAS Institute, Cary, NC). Visual inspection of the residuals for the main outcome measures revealed heteroscedasticity. Consequently, data were analyzed after natural log transformation to reduce effects of nonuniformity of error on the analysis and to express changes as percentages. Mixed linear models were applied to the exercise data sets, with 10 minutes being the first sample for the blood variables and substrate oxidation rates. In addition, the average effects at rest and 70 to 80 minutes (combined average) of exercise were derived for most data from a separate mixed model. For all data sets, treatment was the primary fixed effect. Time was interacted with treatment as a numeric effect in the linear models (as in linear regression) to provide temporal effects for appropriate data. The random effect variances included in the models were subject identity and the interaction between subject identity and exposure fasting.

5.1. Presentation of data

Subject descriptive and outcome data in line graphs are raw means and standard deviations. Unless otherwise noted, mean effects derived from the analysis of log-transformed

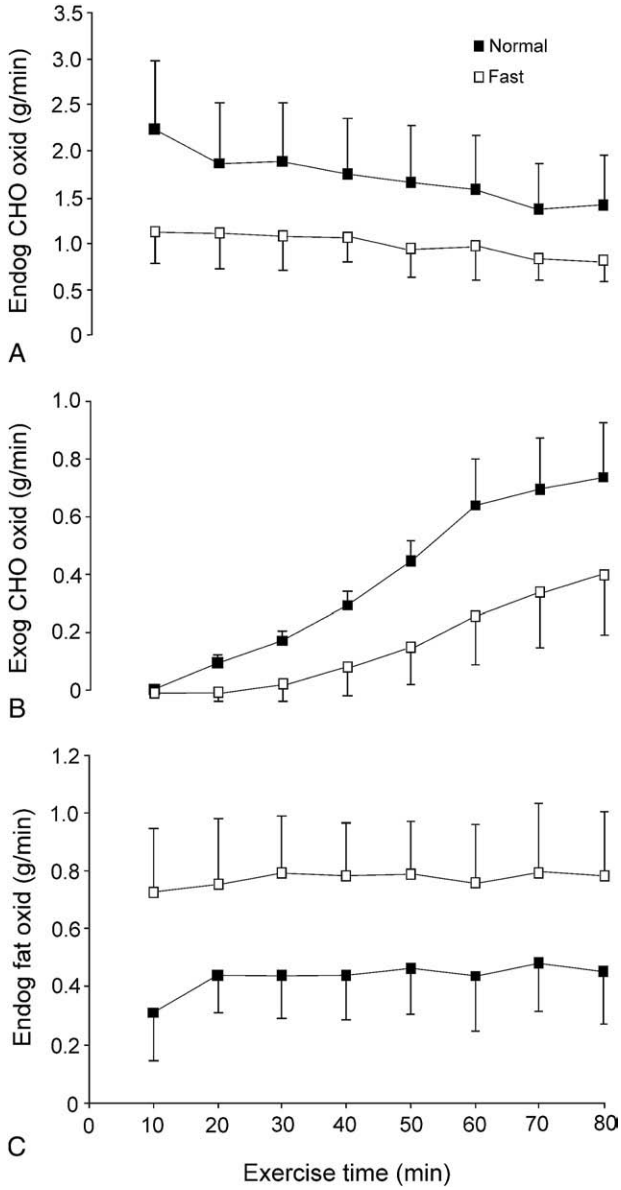

Fig. 1. The effect of Fast on exogenous glucose, and endogenous carbohydrate and fat oxidation rates. Data are raw means and standard deviation.

variables are back log-transformed least squares means, with the associated spread represented appropriately by factor (geometric) standard deviations (×/÷). Conversion of factor standard deviation to a unit value may be achieved by multiplication or division by the mean. All data are rounded to 2 significant figures.

5.2. Estimate uncertainty and statistical inference

Most outcomes are expressed as percentage changes. Statistical uncertainty is presented as 95% confidence limits (CLs) or interval. After standardization, magnitude-based inferences about the true (large sample) values of outcomes were qualified using a modification of the Cohen effect size classification system (trivial, 0.0-0.2; small, 0.2-0.6; moderate, 0.6-1.2; large, 1.2-2.0; very large, >2.0) [20,21]; under this scheme, the threshold for a *substantial* effect is 0.2. Effects were described as *unclear* if the confidence interval spanned both substantial positive *and* negative values.

6. Results

6.1. Body weight

The 67-hour fast reduced body mass by 3.1% (95% CL, ±0.3%; $P < .0001$).

6.2. VO_2, respiratory exchange ratio, and total carbohydrate and fat oxidation rates

Mean overall (10-80 minutes) VO_2 during exercise was similar between trials at 2.37 $L \cdot min^{-1}$ (×/÷ 1.27), corresponding with 56% VO_2max. Mean overall respiratory exchange ratio (RER) was 0.086 lower (95% CL, ±0.006; $P < .0001$) in Fast relative to Normal. The RER did not change over the course of exercise in Fast (increase of 0.004, ± 0.012, $P = .54$), but declined by 0.015 (±0.012, $P = .02$) in Normal, with a moderate-sized difference of 0.018 (±0.012, $P = .03$). The corresponding overall effect of Fast relative to Normal was a moderate reduction in total carbohydrate oxidation of 46% (±7%, $P < .0001$) and a moderate increase in endogenous fat oxidation rate of 85% (±12%, $P < .0001$) (Fig. 1). Substrate oxidation rates and statistical outcomes during the tracer steady state (70-80 minutes) are shown in Table 1.

With respect to temporal changes over the exercise period, after Fast, total carbohydrate oxidation rate increased 13% (±9%); but there was no clear effect in Normal (2%, ±8%). The difference was only small in magnitude at 10% (±12%, $P = .078$). Change in endogenous fat oxidation rate was not affected by Fast (6%, ±15%), but increased moderately by 33% (±19%) in Normal; however, the difference was also small at 20% (±16%, $P = .026$).

6.3. Exogenous glucose and endogenous carbohydrate oxidation rates

Outcomes for exogenous glucose and endogenous carbohydrate oxidation rates during the tracer steady state are shown in Table 1. Fast led to moderate- and small-to-moderate–sized reductions in exogenous glucose and endogenous carbohydrate oxidation rates, respectively.

6.4. Blood parameters

Plasma nonesterified FFA and insulin concentrations, and blood glucose and lactate concentrations at rest and during exercise are illustrated in Figs. 2 and 3; and the statistical outcomes at 70 to 80 minutes are shown in Table 1.

At rest before exercise, Fast raised the concentration of plasma FFA by 230% (95% CL, ±280%; $P = .009$),

Table 1
Substrate oxidation rates and blood parameters during the 70- to 80-minute period of exercise

	Condition		Outcome		
	Fast[a] (factor SD)	Normal[a] (factor SD)	Mean effect of Fast (%); ± 95% CL[b]	*P* value	Qualitative inference[c]
	Substrate oxidation rates				
Exogenous glucose ($g \cdot min^{-1}$)	0.32 (1.56)	0.69 (1.56)	−54, ±10	<.001	Moderate decrease
Endogenous carbohydrate ($g \cdot min^{-1}$)	0.79 (1.63)	1.30 (1.49)	−40, ±19	.008	Small to moderate decrease
Total carbohydrate ($g \cdot min^{-1}$)	1.1 (1.45)	2.0 (1.39)	−43, ±11	<.001	Moderate to large decrease
Endogenous fat ($g \cdot min^{-1}$)	0.76 (1.36)	0.44 (1.36)	71, ±22	<.001	Large to very large increase
	Blood parameters				
Plasma FFA ($mmol \cdot L^{-1}$)	0.58 (1.88)	0.26 (1.80)	120, ±64	.001	Moderate to very large increase
Plasma insulin ($pmol \cdot L^{-1}$)	62 (1.65)	57 (1.46)	10, ±50	.68	Unclear
Blood lactate ($mmol \cdot L^{-1}$)	2.8 (1.75)	1.8 (1.56)	58, ±50	.025	Trivial to large increase
Blood glucose ($mmol \cdot L^{-1}$)	6.6 (1.26)	5.1 (1.10)	30, ±24	.018	Moderate to very large increase

[a] Data are the back-transformed least squares mean of the combined 70- and 80-minute samples derived from the statistical analysis.
[b] Add and subtract this number to the mean effect to obtain the 95% CLs for the true difference.
[c] The likely range of the true magnitude of the effect as defined by the effect size descriptors: small, 0.2 to 0.6; moderate, 0.6 to 1.2; large, 1.2 to 2.0; very large, greater than 2.0.

decreased blood glucose by 21% (±14%, $P = .016$), lowered plasma insulin by 47% (±26%, $P = .015$), and increased blood lactate by 32% (±33%, $P = .017$) relative to Normal.

During exercise, Fast resulted in a very large increase in overall (10-80 minutes) FFA concentration of 164% (±101%, $P = .0007$) relative to Normal. The FFA concentrations did not clearly change from 10 to 80 minutes in Normal (decrease 10%, ±17%) but declined 22% (±15%) in Fast; however, the difference in the change was unclear (13%, ±24%, $P = .31$). Overall, Fast resulted in a moderate reduction in plasma insulin of 33% (±25%, $P = .034$) relative to Normal. From 10 to 80 minutes, insulin concentration increased 306% (±164%) in Fast but only 57% (±63%) in Normal, resulting in a very large Fast-induced increase of 160% (±153%, $P = .001$).

Blood glucose concentration increased in both Fast and Normal conditions during the 80 minutes of exercise up to 40 to 50 minutes, then declined to near resting concentrations in Normal while remaining elevated in Fast; however, there was no clear overall difference in blood glucose concentration (8%, ±18% higher in Fast, $P = .27$). Over the course of exercise after glucose ingestion (10-80 minutes), there was no clear change in blood glucose concentration in Normal (1.4%, ±12%), but a net increase of 66% (±19%) in Fast; the increase in the change in response to Fast was very large at 64% (±27%, $P < 001$). Blood lactate concentration was overall 38% (±49%, $P = .067$) higher in Fast compared with Normal. During the course of exercise, lactate increased 32% (±12%) in Fast, but declined 9% (±8%) in Normal; the increase was moderate at 45% (±18%, $P < .0001$). The magnitude of the effect of Fast on plasma insulin and blood glucose responses during the tracer steady state (70-80 minutes) was different to that observed during the entire 10 to 80 minutes (Table 1); that is, the difference in insulin became unclear and glucose was substantially higher in the Fast condition.

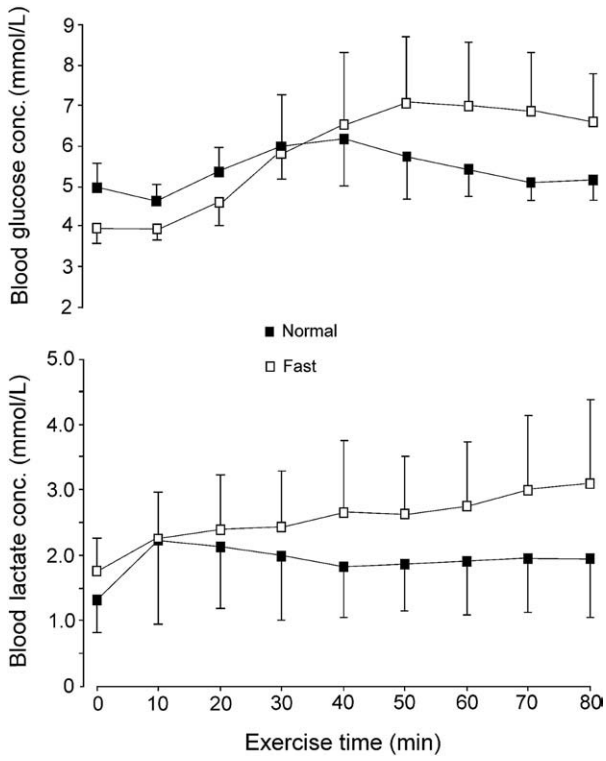

Fig. 3. The effect of Fast on plasma glucose and lactate concentrations. Data are raw means and standard deviation.

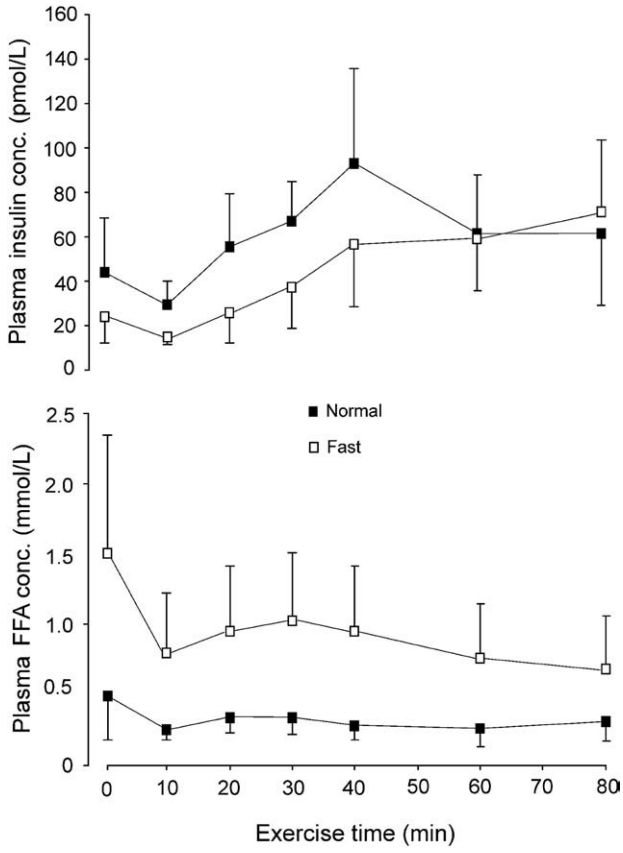

Fig. 2. The effect of Fast on plasma insulin and nonesterified FFA concentrations. Data are raw means and standard deviation.

7. Discussion

The purpose of this study was to determine if starvation-induced intolerance to ingested carbohydrate persists during the metabolic state induced by prolonged moderate-intensity exercise (elevated muscle and blood-borne substrate oxidation and contraction-mediated glucose disposal). We found that starvation substantially suppressed both exogenous and endogenous glucose oxidation during exercise; this was accompanied by a relative hyperglycemia implicating tissue-level glucose intolerance relative to the normal overnight-fasted condition. This finding supports the finding of Knapik et al [7]

who showed that the uptake and oxidation of endogenously formed circulating glucose were attenuated during exercise after starvation. Furthermore, during exercise in the Fast condition, fat oxidation rates began and remained moderately higher than those in the Normal condition, but changed little during exercise even after glucose ingestion; this contrasted to the Normal response where there was greater change in substrate utilization during exercise (increased fat oxidation) and to a high glucose load (increased exogenous carbohydrate oxidation at the expense of the oxidation of endogenous store). Together, the down-regulated glucose metabolism and the absence of the metabolic shift from fat toward carbohydrate oxidation are consistent with the retention of high rates of fat metabolism and suppression of glucose uptake and oxidative disposal during exercise after starvation. As the primary consumer of glucose during exercise is the contracting skeletal muscle, these data suggest that, akin to insulin-mediated glucose uptake at rest [4], contraction-induced exogenous glucose uptake may also be reduced during exercise after short-term starvation.

Based on an evaluation of metabolic research, we propose physiologic control points to help explain starvation-induced suppression of carbohydrate metabolism during exercise. The first and commonly accepted metabolic control point is described by the glucose–fatty acid cycle of Randle et al [22], whereby the reduction in acetyl CoA/CoA ratio inhibits earlier reactions in glycolysis, resulting in product level inhibition of the hexokinase reaction, which then acts to inhibit plasma glucose uptake. Moreover, short-term starvation in lean healthy subjects is associated with up-regulation of the skeletal muscle pyruvate dehydrogenase kinase–4 isoform, which in turn acts to decrease pyruvate dehydrogenase activity [23]. Pyruvate dehydrogenase inhibition would help explain our observations of substantially lower glucose oxidation rates and elevated exercising blood lactate concentrations during exercise in Fasting vs Normal; the latter has been previously observed in fasted subjects during exercise [24]. Blood lactate efflux by muscle and the Cori cycling permits indirect access to skeletal muscle carbohydrate stores, which are reasonably well preserved during starvation provided no contraction occurs [7,25]. Secondly, Norton et al [26] have recently shown a reduction in the skeletal muscle glucose transporter–4 (GLUT-4) protein during starvation, and this was associated with impaired insulin-mediated glucose uptake. Because translocation of GLUT-4 to the cell surface underpins insulin- and contraction-mediated glucose uptake, albeit via different signaling pathways [27,28], it is not surprising that glucose uptake by muscle becomes attenuated from the fed to starved state. The lower rates of both exogenous and endogenous carbohydrate oxidation during exercise in Fasting vs Normal suggest one or a combination of causal factors: inhibition of exogenous glucose intestinal absorption, suppressed muscle contraction or insulin-mediated glucose transport, lower intramyocellular glycogen concentration, or intramyocellular biochemical up-regulation of fat oxidation and corresponding suppression of carbohydrate metabolism. Irrespective of the precise causation, together, the data show how there is a coordinated metabolic response favoring fat oxidation while restricting the skeletal muscle metabolism of both ingested and endogenous carbohydrate.

Paradoxically, there are remarkable metabolic similarities between starvation in healthy lean individuals and obese sedentary individuals that include the following: elevated circulating lipids [29,30], glucose intolerance [4,29], whole-body and skeletal muscle resistance to insulin-mediated glucose uptake [6,26,31,32], and intramyocellular lipid (IMCL) accumulation [30,32]. However, whereas skeletal muscle of insulin-resistant obese individuals is able to respond to contraction by increasing glucose uptake [33], it is clear from the present study that this effect is modulated in starvation in healthy individuals. This difference may be partly explained by the theory that exercise-stimulated glucose transport is impaired only when total skeletal muscle GLUT-4 protein is decreased [34]. Alternatively, or perhaps additionally, it has been suggested that, because the inability of metabolism to readily adapt to changes in substrate availability in the insulin-resistant condition is closely associated with IMCL accumulation, this lipid depot dominates fuel selection [1]. Certainly, this appears to be the case in the present study, as conditions in which IMCL is elevated result in greater lipid oxidation, in lean subjects at least, and preferential use of this substrate during exercise even when exogenous carbohydrate is available [35,36].

A limitation of our study method is that we are unable to differentiate between oxidation of endogenously produced glucose (ie, that released by the liver) and muscle glycogen. However, short-term starvation does not appear to alter hepatic sensitivity to insulin; hepatic glucose output is similarly suppressed after 12- and 48-hour fasts [37], meaning that the fasting-associated changes in glycemia we observe are more likely to be peripheral in nature. Furthermore, a 4-day period of starvation has previously been shown to retard gastric emptying; and this delay may have accounted for a slower systemic appearance of glucose [38]. By delivering glucose to the intestine at a supersaturation rate, however, we expected to minimize gastric emptying as a covariate. Indeed, plasma glucose concentrations were substantially higher in Fast from the 40th minute of exercise, suggesting that delivery to the circulation was not compromised as a result of fasting during the 70th to 80th minute of exercise where the exogenous glucose oxidation calculations were made. If the argument for high exogenous glucose delivery to the circulation holds, then the rate of endogenous carbohydrate oxidation is likely representative of the rate of oxidation of previously stored glycogen within the skeletal muscle. Our data, therefore, point to a starvation-induced suppression of both glycogen

and exogenous glucose metabolism within the skeletal muscle during exercise.

8. Conclusions

Exercise undertaken after 67 hours of starvation results in a large but inflexible increase in fat oxidation but suppressed exogenous and endogenous carbohydrate oxidation, a response consistent with tissue resistance to glucose uptake and oxidation. Together with the known starvation-induced suppression of peripheral insulin-mediated glucose uptake at rest, these metabolic responses appear to be part of a coordinated physiologic mechanism to ensure preferential utilization of fatty acids available in abundance during exercise, which is supported by a relative blockade of the metabolism of endogenous and ingested carbohydrate sources.

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References

[1] Frayn KN. The glucose–fatty acid cycle: a physiological perspective. Biochem SocTtrans 2003;31:1115-9.
[2] Pehleman TL, Peters SJ, Heigenhauser GJ, Spriet LL. Enzymatic regulation of glucose disposal in human skeletal muscle after a high-fat, low-carbohydrate diet. J Appl Physiol 2005;98:100-7.
[3] DeFronzo RA, Gunnearsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependant (type II) diabetes mellitus. J Clin Invest 1985;76: 149-55.
[4] Johnson NA, Stannard SR, Rowlands DS, Chapman PG, Thompson CH, O'Connor H, et al. Effect of short-term starvation vs. high-fat diet on intramyocellular triglyceride accumulation and insulin resistance in physically fit humans. Exp Physiol 2006;91:693-703.
[5] Webber J, Taylor J, Greathead H, Dawson J, Buttery PJ, Macdonald IA. Effects of fasting on fatty acid kinetics and on the cardiovascular, thermogenic and metabolic responses to the glucose clamp. Clin Sci 1994;87:697-706.
[6] Mansell PI, Macdonald IA. The effect of starvation on insulin-induced glucose disposal and thermogenesis in humans. Metabolism 1990;39: 502-10.
[7] Knapik JJ, Meredith CN, Jones BH, Suek L, Young VR, Evans WJ. Influence of fasting on carbohydrate and fat metabolism during rest and exercise in men. J Appl Physiol 1988;64:1923-9.
[8] Coggan AR, Coyle EF. Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. J Appl Physiol 1987;63: 2388-95.
[9] Coyle EF, Coggan AR, Hemmert MK, Ivy JL. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. J Appl Physiol 1986;61:165-72.
[10] Galbo H. Hormonal and metabolic adaptations to exercise. Stuttgart: Georg Thieme Verlag; 1983.
[11] Stannard SR, Johnson NA. Insulin resistance and elevated triglyceride in muscle: more important for survival than "thrifty" genes? J Physiol 2004;554:595-607.
[12] Norton K, Olds T. Anthropometrica—a textbook of body measurement for sports and health courses. Sydney: University of New South Wales Press; 1996.
[13] Wagenmakers A, Brouns F, Saris W, Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. J Appl Physiol 1993;75:2774-80.
[14] Jeukendrup AE. Carbohydrate intake during exercise and performance. Nutrition 2004;20(7-8):669-77.
[15] Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983;55:628-34.
[16] Craig H. Isotopic standards for carbon and oxygen and correction factors. Geochem Cosmochim Acta 1957;12:133-49.
[17] Pirnay F, Lacroix M, Mosora F, Luyckx A, Lefebvre PJ. Glucose oxidation during prolonged exercise evaluated with naturally labelled [13C] glucose. J Appl Physiol 1977;43:258-61.
[18] Robert JJ, Koziet J, Chauvet D, Darmaun D, Desjeux JF, Young VR. Use of 13C labelled glucose for estimating glucose oxidation: some design considerations. J Appl Physiol 1987;63:1725-32.
[19] Pallikarakis N, Sphiris N, Lefebvre P. Influence of the bicarbonate pool of the occurrence of 13CO2 in exhaled air. Eur J Appl Physiol 1991;63: 179-83.
[20] Cohen J. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum; 1988.
[21] Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. Int J Sport Physiol Perf 2006;1:50-7.
[22] Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1:785-9.
[23] Spriet LL, Tunstall RJ, Watt M, Mehan KA, Hargreaves M, Cameron-Smith D. Pyruvate dehydrogenase activation and kinase expression in human skeletal muscle during fasting. J Appl Physiol 2004;96: 2082-7.
[24] Elia M, Lammert O, Zed C, Neale G. Energy metabolism during exercise in normal subjects undergoing total starvation. Human Nutr: Clin Nutr 1984;38C:355-62.
[25] Hultman E, Bergstrom J. Muscle glycogen synthesis in relation to diet studied in normal subjects. Acta Med Scand 1967;182:109-17.
[26] Norton L, Parr T, Bardsley RG, Ye H, Tsintzas K. Characterization of GLUT4 and calpain expression in healthy human skeletal muscle during fasting and refeeding. Acta Physiologica 2007;189: 233-40.
[27] Krook A, Wallberg-Henriksson H, Zierath JR. Sending the signal: molecular mechanisms regulating glucose uptake. Med Sci Sports Exerc 2004;36:1212-7.
[28] Zorzano A, Palacin M, Guma A. Mechanisms regulating GLUT4 glucose transporter expression and glucose transport in skeletal muscle. Acta Physiol Scand 2005;183:43-58.
[29] Coon PJ, Bleecker ER, Drinkwater DT, Meyers DA, Goldberg AP. Effects of body composition and exercise capacity on glucose tolerance, insulin, and lipoprotein lipids in healthy older men: a cross-sectional and longitudinal intervention study. Metabolism 1989; 38:1201-9.
[30] Stannard SR, Thompson MW, Fairbairn K, Huard B, Sachinwalla T, Thompson CH. 72 hours of fasting increases intramyocellular lipid content in non-diabetic, physically-fit men. Am J Physiol 2002;283: E1185-91.
[31] Greco AV, Mingrone G, Giancaterini A, Manco M, Morroni M, Cinti S, et al. Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. Diabetes 2002;51:144-51.
[32] Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 1997;46:983-8.
[33] Minuk HL, Vranic M, Marliss EB, Hanna AK, Albisser AM, Zinman B. Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. Am J Physiol 1981;240:E458-64.
[34] Hayashi T, Wojtaszewski JFP, Goodyear LG. Exercise regulation of glucose transport. Am J Physiol 1997;273:E1039-51.

[35] Goodpaster BH, Wolfe RR, Kelley DE. Effects of obesity on substrate utilization during exercise. Obes Res 2002;10:575-84.
[36] Johnson NA, Stannard SR, Mehalski K, Trenell MI, Sachinwalla T, Thompson CH, et al. Intramyocellular triacylglycerol in prolonged cycling with high and low carbohydrate availability. J Appl Physiol 2003;94:1365-72.
[37] Bergman BC, Cornier MA, Horton TJ, Bessesen DH. Effects of fasting on insulin action and glucose kinetics in lean and obese men and women. Am J Physiol 2007;293:E1103-11.
[38] Corvilain B, Abramowicz M, Fery F, Schoutens A, Verlinden M, Balasse E, et al. Effect of short-term starvation on gastric emptying in humans: relationship to oral glucose tolerance. Am J Physiol 1995;269:G512-7.