Glucose ingestion during endurance training does not alter adaptation

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Glucose ingestion during endurance training does not alter adaptation. J Appl Physiol 106: 1771–1779, 2009. First published February 19, 2009; doi:10.1152/japplphysiol.91534.2008.—Glucose ingestion during exercise attenuates activation of metabolic enzymes and expression of important transport proteins. In light of this, we hypothesized that glucose ingestion during training would result in 1) an attenuation of the increase in fatty acid uptake and oxidation during exercise, 2) lower citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity and glycogen content in skeletal muscle, and 3) attenuated endurance performance enhancement in the trained state. To investigate this we studied nine male subjects who performed 10 wk of one-legged knee extensor training. They trained one leg while ingesting a 6% glucose solution (Glc) and ingested a sweetened placebo while training the other leg (Plc). The subjects trained their respective legs 2 h at a time on alternate days 5 days a week. Endurance training increased peak power (Pmax) and time to fatigue at 70% of Pmax ~14% and ~30%, respectively. CS and β-HAD activity increased and glycogen content was greater after training, but there were no differences between Glc and Plc. After training the rate of oxidation of palmitate (Rox) and the % of rate of disappearance that was oxidized (%Rdox) changed. %Rdox was on average 16.4% greater during exercise after training whereas, after exercise %Rmax was 30.4% lower. Rox followed the same pattern. However, none of these parameters were different between Glc and Plc. We conclude that glucose ingestion during training does not alter training adaptation related to substrate metabolism, mitochondrial enzyme activity, glycogen content, or performance.

THE PLASTICITY of skeletal muscle is well documented (19, 22, 26, 29). Thus endurance training induces structural and functional changes, which ultimately result in increases in both endurance capacity and reliance on fat as an energy source during prolonged exercise at the same absolute intensity (26, 28). Although the precise mechanisms that initiate training adaptation remain unclear, the current knowledge favors a scenario in which recurrent changes in gene expression, which occur with every bout of exercise, lead to a change in phenotype (25) such as improvements in fatty acid (FA) transport and oxidation.

A single bout of exercise increases the muscle mRNA content of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) (40), transcriptional regulator of mitochondrial biogenesis (54). This event is thought to be an important initial step in training adaptation. Since exercise induces the expression of FA transport proteins fatty acid binding protein (FABPpm) (27), and CPT-1 (7).

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METHODS

Subjects. Nine physically active healthy male subjects, who were previously unaccustomed to one-legged knee extensor exercise, with mean age, height, weight, and body mass index (BMI) of 25.8 ± 1.8 yr, 179 ± 3 cm, 79 ± 3.8 kg, and 24.5 ± 1.0 kg/m², respectively, volunteered to participate in this study, which consisted of a 10-wk training intervention as described below. Before and after the training period subjects performed four exercise tests and were MR scanned as outlined in Fig. 1 and described below. For the duration of the study the subjects were instructed to refrain from any other form of exercise training.

The subjects were given both oral and written information about the experimental procedures before giving their written informed consent. All studies were approved by the Copenhagen and Frederiksborg-
berg Ethics Committee and performed according to the Declaration of Helsinki.

Training. With a randomized block design the subjects were assigned, in the order they were included in the study, to train one leg (Glc) while ingesting a 6% glucose solution (Glc drink), at a rate that resulted in a glucose intake of \(0.7 \, \text{g/kg/h} \), and ingesting a sweetened placebo (Plc drink; Sport light, Pebas, Køge, Denmark) at a corresponding rate while training the other leg (Plc). One-legged knee extensor exercise performed on a modified Krogh ergometer was the exercise mode of choice for all training and exercise tests in this study. Under supervision, the subjects trained one leg at a time on alternate days. The training frequency was 5 times per week, which resulted in an average weekly training frequency for each leg of 2.5 times. The subjects trained for 10 wk. The initial workload was set to 60% of maximal workload (P\(_{\text{max}}\)), and the duration was at 2 h throughout the study. The resistance on the flywheel was increased by 10% every other week. All training was performed between 6 and 12 AM after an overnight fast. Two extra training sessions were added before the posttraining 3-h test (Fig. 1) to reduce the risk of a diminished training response. The acute response to this training intervention was very well described in a previous study in which the same subjects were included (2).

Fatigue tests. The subjects performed an incremental exercise test (P\(_{\text{max}}\) test) to determine the maximal workload (P\(_{\text{max}}\)). The subjects were familiarized with one-legged knee extensor exercise on a modified Krogh ergometer on three separate occasions lasting 30 min per leg before performing the first P\(_{\text{max}}\) test.

On separate days, separated by 48 h, the subjects performed a P\(_{\text{max}}\) test with each leg to determine the maximal workload (Fig. 1) as has been described in detail elsewhere (2). Subjects with a P\(_{\text{max}}\) difference of >6 W between the right and left leg were excluded.

Subjects performed two endurance tests at 70% of P\(_{\text{max}}\) (End test 70%) with each leg before and after the training period (Fig. 1). The subjects reported to the laboratory in the morning after an overnight fast. After a 6 min warm-up at 30 W the load was increased to 70% of P\(_{\text{max}}\), the subject exercised until he could no longer maintain a kicking frequency of 60 extensions/min despite verbal encouragement, and time to fatigue (TTF) was recorded. Throughout the tests the subjects ingested either the Glc drink or the Plc drink. The Glc drink was ingested at a rate corresponding to an ingestion of \(0.7 \, \text{g glucose/kg/h} \), and the Plc drink was ingested at the same rate. A fourth of the hourly allotted drink was ingested every 15 min.

MR scanning. MR examinations to determine the size of the quadriceps muscle for both legs were performed with a 1.5-T Magnetom Vision clinical unit (Siemens, Erlanger, Germany) before and after training (Fig. 1). The imaging protocol consisted of a three-plane scout view and conventional axial longitudinal relaxation time (T1)-weighted spin-echo images [transverse relaxation time (TR) 420 ms, echo time (TE) 17 ms, averages = 3, slice thickness = 6 mm, distance factor 2.00, matrix 256 and field of view (FOV) = 40 cm]. All volunteers were imaged in the body coil, the imaging volume included acetabulum, femur, and knee.

Three-hour test. Before and after the training period (Fig. 1) the subjects performed one 3-h one-legged knee extensor exercise bout (3-h test) with each leg at the same relative exercise intensity, 55% of P\(_{\text{max}}\), as outlined in Fig. 2, one with each leg. Three days before the trials the subjects were given a 2-day pre-prepared food package. The

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**Table 1**

<table>
<thead>
<tr>
<th>Day</th>
<th>P(_{\text{max}})</th>
<th>P(_{\text{max}}) test</th>
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<th>MR-scanning</th>
<th>3H test</th>
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<td>MR</td>
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<tr>
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**Figure 2**

**Three-hour test.** Outline of 3-h one-legged knee extensor exercise test at 55% of P\(_{\text{max}}\) (3-h test) performed for both training with glucose (Glc) and training with placebo (Plc) before and after training. Sampling times and frequencies are represented by black arrows, and the time line is in hours.
food packages for the pre-Glc, pre-Plc, post-Glc, and post-Plc trials contained 3.84 ± 0.04, 3.84 ± 0.04, 4.08 ± 0.04, and 3.99 ± 0.044 g carbohydrate (CHO)/kg body wt and 2.213 ± 0.85, 2.213 ± 0.85, 2.309 ± 0.98, and 2.256 ± 0.96 kcal/day, respectively. The macronutrient energy distribution in the diet was 56.2 ± 0.1% of total energy intake (%E) from CHO, 15.1 ± 0.1%E from protein, and 28.7 ± 0.1%E from fat. The food packages were not different with regard to total energy content or percentage of energy derived from CHO, protein, or fat between any of the trials. The subjects were instructed only to eat the food that was provided to them and to drink only water. Subjects were required to keep a daily food diary and report all food intake over the 2 days. The subjects only drank water on the experimental day.

On the experimental day, subjects arrived at the lab between 7 and 7:30 AM after a 12-h overnight fast. After the subject rested for 10 min on a reclined modified Krogh ergometer two catheters were placed in antecubital veins, one in each arm. One catheter was used for blood sampling, and the other was used for tracer infusion. The trial started (t = −2 h) with the subject resting for 2 h in the supine position. Infusion of tracers was started at t = −2 h (Fig. 2). Before the infusion started, expired air was collected (as explained below) and a blood sample was taken, followed by administration of two priming doses. The bicarbonate pool was primed with a bolus injection of NaH13CO3 (6 μmol/kg) followed by administration of a [6, 6-H2]glucose prime (17.6 μmol/kg body wt). A continuous infusion of [6,6-H2]glucose dissolved in 150 ml of isotonic saline (Sygehus Apotekerne, Copenhagen, Denmark) and [U-13C]palmitate dissolved in 100 ml of 20% human albumin (ZLB Behring, Bern, Switzerland) was started (t = −2 h) and continued for 7 h (Fig. 2). All stable isotope-labeled tracers were purchased from Cambridge Isotope Laboratories (Cambridge, MA).

At t = 0 h the subjects started 3 h of one-legged knee extensor exercise in the upright position on a modified Krogh ergometer at 55% of Pmax (Fig. 2). After exercise the subjects rested in the supine position for 2 h. Oxygen uptake (VO2) and carbon dioxide production (VCO2) were measured with an open-circuit indirect calorimeter system (Moxus Modular VO2 System, AEI Technologies, Pittsburgh, PA). Indirect calorimetry measurements were performed for 5 min at each time point before collection of air for analysis of carbon dioxide enrichment. Expired air was collected in a 15-liter nondiffusing gas collection bag (Hans Rudolph, Kansas City, MO) and transferred to 10-ml vacutainer tubes (BD, Franklin Lakes, NJ) with a winged infusion set (Terumo, Leuven, Belgium), where it was stored until analysis. Expired air for indirect calorimetry measurements was sampled and expired air collected at −2, −1, 0, 1, 2, 3, 3.5, 4, and 5 h (Fig. 2). Indirect calorimetry measurements were used to calculate the rate of fat (g/min) and CHO (g/min) oxidation for each trial with nonprotein formulas (36): CHO oxidation = (4.585 × VCO2) − (3.226 × VO2) and fat oxidation = (1.695 × VCO2) − (1.701 × VO2).

At time points −2, 0, 1, 2, 3, 3.5, 4, and 5 h 11 ml of blood was collected (Fig. 2). Additionally, 1.5-ml blood samples were collected at 60 min, −20 min, −10 min, and every 15 min from the onset of exercise for analysis of plasma enrichment and plasma glucose and palmitate concentration. Plasma was immediately separated from blood cells by centrifugation at 2,150 × g at +4°C for 15 min and stored at −80°C until analysis. Plasma concentrations of glucose, lactate, glyceral, and FFA were analyzed on an automatic analyzer (Cobas Fara, Roche, Basel, Switzerland). Glucagon plasma concentration was measured with radioimmunoassay kits (Linco Research, St. Charles, MO and CIS bio international, GIF-Sur-Yvette, France, respectively). ELISA kits were used to measure insulin (DakoCytomation, Glotech, Denmark) and cortisol (DSL, Webster, TX) in plasma. Plasma concentration of epinephrine was measured by HPLC (Hewlett-Packard, Waldbronn, Germany) with electrochemical detection. Changes in plasma volume were calculated as described by Dill and Costill (14). Hormone and metabolite concentrations were corrected for changes in plasma volume.

Stable isotope-labeled metabolites: analysis and calculations. Plasma palmitate concentration and enrichment were measured by gas chromatography and gas chromatography-isotope ratio mass spectrometry, respectively, as has been described in detail previously (49). The enrichment of 13C in expired air was determined by gas chromato-

graphy-isotope ratio mass spectrometry (49). Plasma glucose enrichment was measured by using liquid chromatography-mass spectrometry as described previously (41).

Before exercise, at 0 h, the glucose and palmitate rate of appearance (Ra) and rate of disappearance (Rd) were calculated with steady-state equations: Rd(t) = F/E and Ra(t) = R, where F is the infusion rate of the tracer (μmol·kg−1·min−1) and E is the plasma enrichment corrected for background enrichment of glucose or palmitate expressed in tracer-tracer ratios (TTR). During and after exercise the glucose and palmitate Ra and Rd were calculated with the modified non-steady-state Steele equation (46), adapted for stable isotopes (53):

$\begin{align*}
R_d(t) &= F - pV(C_2 + C_1)/2[(E_2 - E_1)/(t_2 - t_1)][(E_1 + E_2)/2] \\
R_a(t) &= pV(C_2 - C_1)/(t_2 - t_1)
\end{align*}$

E1 and E2 are the plasma enrichments of glucose or palmitate at sample times t1 and t2 (min), respectively, and C1 and C2 are the plasma concentrations (mmol/mol) of glucose or palmitate at t1 and t2, respectively. pV is the volume of distribution, which has been set to 0.04 and 0.16 l/kg body wt for palmitate and glucose, respectively.

The volume of expired CO2 (VCO2) labeled with 13C was calculated as Exp13CO2 = ECO2 × VCO2/A, where ECO2 is the enrichment (TTR) of the expired air calculated as the ratio of 13C to 12C in breath and A is the acetate correction factor determined by continuous infusion of [1,2-13C]lactate under very similar conditions with the same exercise mode in the same laboratory and with similar subjects (48). The palmitate rate of oxidation (palmitate Ra) was calculated as palmitate Ra = Ra × Exp13CO2/F × 16, where the factor 16 represents the number of 13C atoms in [U-13C]palmitate. The fraction of palmitate Ra oxidized to CO2 (%RaCO2) can subsequently be calculated as %RaCO2 = Ra0/Ra × 100.

Biopsies: glycogen concentration and enzyme activities. Muscle biopsies were obtained from the vastus lateralis by the Bergstrom percutaneous needle biopsy technique with suction (6) at rest (0 h), immediately after exercise (3 h), and after 2 h of recovery (5 h) during the 3-h test (Fig. 2). Biopsy samples (100–200 mg) were immediately placed in liquid nitrogen and stored at −80°C until analysis. For enzyme activities and glycogen content measurements 5- to 10- to 20- to 20-mg sections of the biopsy samples were used, respectively. The sections were freeze-dried and dissected free from visible blood and connective tissue. Glycogen concentration was determined by boiling the muscle section in 1 M HCl for 2 h at 100°C and analyzing the supernatants for glucose concentration (Roche UniKit, Paris, France) on an automatic analyzer (Cobas Fara, Roche, Basel, Switzerland). The maximal activities of CS, β-HAD, lactate dehydrogenase (LDH), and phosphofructokinase (PFK) were determined with enzymatic fluorometric assays according to Lowry and Passonneau (32).

Statistical analysis. For all data, the distribution was evaluated with probability plots and Kolmogorov-Smirnov tests. Accordingly, plasma concentrations of glucagon, insulin, cortisol, epinephrine, glyceral, and palmitate, skeletal muscle CS activity, HAD activity, LDH activity, as well as glucose Ra, glucose Rd, palmitate Ra oxidized, CHO oxidation, and fat oxidation were logarithmically transformed in order to fit a normal distribution. Untransformed data are presented as means ± SE or 95% confidence intervals (CI), while log-transformed data are presented as geometric means with geometric SE. Overall effects of training, glucose supplementation, and time were evaluated with two- or three-way repeated-measures mixed model analyses (PROC MIXED, SAS version 9.1, SAS Institute, Cary, NC) as appropriate. Furthermore, for Ra, palmitate P%Raco, the two variables for which there was a time by training interaction, area under the curve (AUC) was calculated for the exercise and recovery phases of the 3-h test with the trapezium rule and analyzed with a two-way mixed model. To further evaluate the
effects of training and supplementation, post hoc analysis was done with Tukey-adjusted paired t-tests. To further evaluate the effect of time, pairwise comparisons between time points were made with least square means (LSMEANS, SAS) embedded in a one-way repeated-measures mixed model. The P values obtained via this procedure were subsequently used in a multiple testing procedure (PROC MULTTEST, SAS) in order to obtain a set of comparisons that met an approximate 0.05 false discovery rate adjustment (4). Distribution and variance homogeneity of the residuals derived from the variance analyses were evaluated with probability plots and scatterplots. Significance for all tests was set at P < 0.05.

RESULTS

All subjects completed the full study protocol. Adherence to the training was 100%. After the 10 wk of training, there was a small and nonsignificant reduction in body weight (posttraining weight 77.5 ± 3.7 kg, P = 0.067).

Effect of training and glucose supplementation on performance and muscle volume. In response to the training, maximal power output during incremental one-legged knee extensor exercise increased markedly (Fig. 3, Plc: mean increase 13%, CI 8–18%, P < 0.05; Glc: mean increase 14%, CI 6–22%, P < 0.05). Training also increased time to fatigue (P < 0.05) and total work (P < 0.05) during End test 70% (Table 1). Supplementation with glucose during training had no effect on either time to fatigue or total work during End test 70%.

In contrast, glucose supplementation during End test 70% (Table 1) tended to increase time to fatigue and total work but was only significantly different after training (P = 0.015).

In response to training, quadriceps muscle volume increased from 2,609 ± 105 and 2,635 ± 108 ml to 2,702 ± 120 and 2,726 ± 114 ml for Plc and Glc, respectively, but the differences were not significant. Quadriceps muscle volume was not affected by the supplementation (P = 0.890).

Effect of training and glucose supplementation on resting skeletal muscle metabolic enzyme activity. The enzymatic activity of both CS (Fig. 4A) and β-HAD (Fig. 4B) increased in response to training (P < 0.05 for both enzymes), while glucose supplementation had no effect (CS: P = 0.414; β-HAD: P = 0.214). Post hoc analysis revealed no difference between Glc and Plc before (CS: P = 0.657; β-HAD: P = 0.999) or after (CS: P = 0.534; β-HAD: P = 0.397) training.

The activities of PFK and LDH (Fig. 4, C and D, respectively) were affected neither by training (PFK: P = 0.304; LDH: P = 0.177) nor by glucose supplementation (PFK: P = 0.413; LDH: P = 0.571).

Effect of training and glucose supplementation on hormone levels during acute exercise. In response to acute exercise circulating levels of epinephrine and glucagon rose, and after exercise they fell to resting levels (data not shown). Cortisol remained unchanged throughout exercise and fell in the recovery period to levels below resting, whereas insulin fell during exercise and remained suppressed until the end of the trial (data not shown). None of the hormone levels was significantly affected by training (epinephrine: P = 0.179; cortisol: P = 0.239; insulin: P = 0.739; glucagon: P = 0.786) or glucose supplementation during exercise (epinephrine: P = 0.917; cortisol: P = 0.364; insulin: P = 0.446; glucagon: P = 0.937).

Effect of training and glucose supplementation on carbohydrate metabolism during acute exercise. After the training period plasma glucose concentration (Fig. 5A) was significantly lower during the acute exercise trial (P = 0.037). In addition, plasma glucose decreased over time during the acute exercise (P < 0.001). In contrast, supplementation with glucose during training had no effect on plasma glucose levels during acute exercise (P = 0.518). Glucose Rg and R4 both increased markedly in response to acute exercise (Fig. 5, C and D, P < 0.001). Training had no effect on either glucose Rg (Fig. 5C, P = 0.838) or glucose R4 (Fig. 5D, P = 0.532). Supplementation with glucose during training did not have any effect on glucose Rg (P = 0.979) or glucose R4 (P = 0.933). However, a reduction in plasma lactate (Fig. 5B) during the acute exercise was observed after training (P < 0.001), while supplementation with glucose had no effect on plasma lactate levels during acute exercise (P = 0.158). Furthermore, the glycogen content (Fig. 5E) was higher at all time points (P < 0.005) after training. Post hoc analysis detected no overall difference for glycogen concentration between Plc and Glc (P = 0.849). Thus training but not glucose supplementation increased muscle glycogen content. Glycogen utilization during exercise was independent of the supplementation (Plc: mean decrease 53%, CI 41–64%; Glc: mean decrease 59%, CI 49–69%; effect of supplementation: P = 0.410). Total CHO

Table 1. Endurance test at 70% of \( P_{max} \)

<table>
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<td>Placebo</td>
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Results from endurance tests at 70% of peak power (\( P_{max} \)) (End test 70%) are presented as means and 95% confidence intervals (CI). *P < 0.01, effect of training; †P < 0.05, effect of drink.

Fig. 3. \( P_{max} \). Shown is maximal power output during the \( P_{max} \) test before and after 10 wk of training. Open bars, Glc before and after training; filled bars, Plc before and after training. Data are presented as means ± SE. Overall effect of training.
oxidation (Fig. 5F) increased during exercise ($P < 0.0001$) and was higher during exercise after training ($P = 0.005$) but was not affected by glucose supplementation during training ($P = 0.897$).

**Effect of training and glucose supplementation on fat metabolism during acute exercise.** Plasma concentrations of palmitate, FFA, and glycerol (Fig. 6, A–C) rose during exercise ($P < 0.0001$ for all 3 metabolites) and remained elevated above baseline levels during recovery after exercise (0 vs. 5 h, $P < 0.0001$ for all 3 metabolites). However, palmitate and FFA plasma concentrations were on average 23.6% (CI 14.0–33.1%) and 23.0% (CI 9.5–36.5%) lower during exercise after the training period ($P < 0.0001$ and $P = 0.009$ for FFA and palmitate, respectively), but there was no effect of supplementation with glucose during training ($P = 0.475$ and $P = 0.414$ for FFA and palmitate, respectively). Palmitate $R_a$ and $R_d$ both increased markedly in response to acute exercise (Fig. 6, E and F, $P < 0.001$). Training did not have any effect on either palmitate $R_a$ (Fig. 6E, $P = 0.600$) or palmitate $R_d$ (Fig. 6F, $P = 0.969$), and neither did supplementation with glucose during training ($P = 0.528$ and $P = 0.949$ for palmitate $R_a$ and $R_d$, respectively). Total fat oxidation (Fig. 6D) increased during exercise ($P < 0.0001$) but was not affected by either training ($P = 0.178$) or glucose supplementation during training ($P = 0.145$). On the other hand, there was a significant interaction between training and time for palmitate $R_{ox}$ and $\%R_{dox}$ (Fig. 6, H and G; $P = 0.008$ and $P < 0.0001$ for palmitate $R_{ox}$ and $\%R_{dox}$, respectively). After the 10 wk of training, a greater proportion of the palmitate $R_d$ was oxidized during exercise (Fig. 6G), $\%R_{dox}$ was on average 16.4% (CI 7.3–25.6%) greater during exercise ($P = 0.016$), whereas after exercise $\%R_{dox}$ was 30.4% (CI 6.8–53.9%) lower ($P = 0.020$). Palmitate $R_{ox}$ followed the same pattern (Fig. 6H), but the difference between before and after training during exercise was only borderline ($P = 0.052$). After the training period palmitate $R_{ox}$ was 9.0% (CI 0.5–18.5%) lower ($P = 0.030$) during recovery after exercise.

**DISCUSSION**

This is the first study to investigate whether ingestion of glucose during 10 wk of endurance training has an effect on training adaptation. We hypothesized that glucose ingestion during exercise would blunt skeletal muscle endurance training adaptation, primarily by reducing FA uptake and oxidation ability. After the 10 wk of training there was no apparent difference in substrate utilization, performance, glycogen content, or metabolic enzyme activity between Glc and Plc. It appears that glucose ingestion, despite the acute effects on substrate utilization, enzyme activation, and gene expression, does not alter the adaptation to 10 wk of one-legged knee extensor endurance training for the measured parameters.

In the present study time to fatigue during the End test 70% increased after the training period by $-30\%$ (Table 1), but this was not affected by whether glucose was ingested during training or not. Interestingly, in another study performed in our lab (21) subjects trained one leg twice per day every other day, and the other leg was trained once per day. This meant that the leg that trained twice in one day started the second training sessions with low muscle glycogen whereas the other leg started every training session with high muscle glycogen. The leg that was trained twice per day performed better in a time to fatigue test after the training period. It is difficult to discern why there is no coherence in these two different intervention models, but there are some clear differences between the interventions. Training twice daily induces a larger hormonal
response during the second exercise bout (43), and exercising with a low muscle glycogen content augments gene transcription of certain genes (38), which might imply that there is a larger training stimulus. Glucose ingestion, on the other hand, dampens gene expression of certain metabolic genes (8, 9) and dampens the hormonal and metabolic response to exercise (2), which suggests that the training stimuli might be smaller. In light of this, the interventions are in fact quite different and probably would elicit different responses.

Transport of LCFA into the mitochondrion is a potential rate-limiting step in FA oxidation during exercise (44), and a reduction in the transport capacity of LCFA could therefore change the substrate selection of the muscle during exercise. The uptake of FFA from the bloodstream during exercise at the same absolute workload increases with endurance exercise training (27). The expression of FABPm (27), which is an important FA transporter in mammalian cells (1), is increased with endurance training. Interestingly, endurance training in the fed state compared with the fasted state attenuates the expression of FABPm (12), and the expression of genes coding for the LCFA transport proteins CD36 and CPT-1 (8) is attenuated if glucose is ingested during exercise. This suggests that glucose ingestion during endurance training might attenuate the increased reliance on fat as a fuel during exercise typically seen with endurance training. Although gene expression of CD36 and CPT-1 is attenuated if glucose is ingested during an acute bout of exercise (8), this does not necessarily mean that protein expression will be affected. The concentration of FA transporters was not measured in this study, and we cannot rule out that the concentration of CPT-1 and CD36 is as
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Training in this study resulted in increased CHO oxidation during exercise (Fig. 5F). In contrast, others have reported unchanged (5) or lower (28) rates of CHO oxidation after training. This is probably an exercise mode-related phenomenon. During cycling at an intensity of ~60% of maximum \( \dot{V}O_2 \) (\( \dot{V}O_{2\text{max}} \)) the respiratory quotient (RQ) across the leg is typically ~0.92 and respiratory exchange ratio (RER) measured at the mouth is ~0.90 (20). During one-legged exercise at an intensity of ~60% of \( P_{\text{max}} \) the difference between RQ, which is ~0.93–0.92, and RER, which is ~0.90–0.87, is greater (49). As the absolute exercise intensity increased (by ~14% in this study) after the training period, the leg RQ will represent a larger fraction of the RER and thus elevate the RER, resulting in the total CHO oxidation being higher.

Training induced no changes in \( R_e \) or \( R_d \) of glucose (Fig. 5, D and C) during exercise at the same relative intensity, which is in line with other training studies (5, 16), and ingestion of glucose during the training period did not influence the Rd of glucose (Fig. 5F). In a recent study by De Bock and coworkers (12) training in the fasted state compared with the fed state resulted in a greater concentration of the glucose transporter Glut4 in the vastus lateralis muscle, but this is not necessarily associated with a higher glucose uptake in trained muscle (42).

Endurance training increases glycogen concentration within the muscle (26, 42), as was the case in the present study (Fig. 5E). AMPK activation is thought to play a role in the elevated

Fig. 6. Fat metabolism during acute exercise. Shown are plasma palmitate concentration (A), plasma free fatty acid (FFA) concentration (B), plasma glycerol concentration (C), total fat oxidation (D), palmitate \( R_d \) (E), palmitate \( R_a \) (F), % of palmitate \( R_d \) oxidized (G), and palmitate \( R_{\text{ox}} \) (H) during the 3-h test before and after 10 wk of training. ■ Glc before training; ● Plc before training; ▲ Plc after training. Horizontal arrow indicates the exercise period. Data are presented as means ± SE except for palmitate concentration, palmitate \( R_d \) oxidized, and total fat oxidation, which are presented as geometric means ± geometric SE. *Interaction between the effect of training and time; †overall effect of training; ‡overall effect of time. NS, no overall effect of training or supplementation.

high in the Glc leg as it is in the Plc leg, despite the expected difference in expression. In line with this, total fat oxidation remained unchanged (Fig. 6H) in the present study and there was no change in \( R_d \) or \( R_e \) of palmitate due to the training or glucose ingestion. On the other hand, palmitate \( R_{\text{ox}} \) and consequently \%\( R_{\text{ox}} \) were greater after the training period (Fig. 6,
glycogen concentration seen after a period of endurance training (52). Chronic AMPK activation increases the concentration of glycogen in skeletal muscle (23). We had therefore expected to find lower glycogen concentration in Glc because AMPK activation was lower during exercise with glucose ingestion (2), but there were no differences between Plc and Glc (Fig. 5E). It is possible that there is redundancy in the signaling cascade and that AMPK activation is not an essential step for an increase in glycogen concentration within the muscle to occur as a response to training. On the other hand, we have only measured the AMPK response during a single exercise bout where glucose was ingested (2). It is therefore uncertain whether AMPK activation was attenuated throughout the whole 10-wk training period. In a study comparing trained and untrained subjects AMPK (Thr\(^{\text{172}}\)) phosphorylation, which is a surrogate for AMPK activation, was blunted at the end of a 20-min exercise bout at 80% of \(\dot{V}_{\text{O}_2\text{max}}\) (35). In line with this, 3 wk of endurance training lowered the activation of AMPK during moderate-intensity exercise at the same absolute workload (34). Although no studies have investigated the effect of training on the AMPK response to exercise at the same relative intensity, it is possible that the activation of AMPK during exercise at the same relative intensity could be reduced after endurance training and thereby reduce the potential effect that glucose ingestion could have on AMPK activity during exercise. On the other hand, a recent study found that AMPK activity was greater at rest after a 3-wk training period, suggesting that trained subjects would have a higher AMPK activity at the onset of every training bout (18). How this would affect training adaptation and the potential role of glucose ingestion on AMPK activation is at present unclear. Furthermore, there is some controversy as to whether glucose ingestion during exercise attenuates AMPK activation (31). Lee-Young and coworkers (31) had their subjects ingest glucose during 2 h of cycle ergometer exercise and were not able to detect any difference in AMPK activation compared with the placebo trial. This is in contrast to previous findings from our lab (2). It could be that different exercise protocols have different effects on AMPK activity when glucose is ingested. Interestingly, the change in \(\alpha_2\)-AMPK activity was larger in our previous study (2), fourfold compared with threefold in the study by Lee-Young and coworkers (31), and this might have made it easier for us to detect a difference between glucose ingestion and placebo. Others have also investigated the effect of glucose ingestion on AMPK during exercise and found that the increase in AMPK Thr\(^{\text{172}}\) phosphorylation was unaffected by glucose ingestion (13). This would suggest that glucose ingestion during exercise does not affect AMPK activity, but AMPK (Thr\(^{\text{172}}\)) phosphorylation reflects phosphorylation of both catalytic AMPK isoforms (\(\alpha_1\) and \(\alpha_2\)) and might therefore be a less sensitive measurement for detecting changes in \(\alpha_2\)-AMPK activity. The potential role of AMPK activation in training adaptation and how glucose ingestion might affect it is therefore difficult to discern based on data from this study.

On the basis of the present study it does not appear that glucose ingestion has a detectable effect on training adaptation for the parameters measured. However, there is still the possibility that there were small differences between Glc and Plc that were not detected in the present study because of type II errors. The randomized double-blind placebo design in this study, where there is no genetic difference between Glc and Plc, reduces the variability between the two groups considerably and strengthens the design. To address the issue of possible type II errors we calculated the minimal detectable difference that could be detected with a power of 0.8, using the method proposed by Altman (3). A difference between Glc and Plc for \(P_{\text{max}}\), CS activity, glycogen concentration, and palmitate \(R_\text{ox}\) and \(R_\text{d}\) of more than 3 W, 5 \(\mu\text{mol}\cdot\text{g dry wt muscle}^{-1}\cdot\text{min}^{-1}\), 46 \(\mu\text{mol}/\text{kg}^{-1}\cdot\text{min}^{-1}\), 0.4 \(\mu\text{mol}/\text{kg}^{-1}\cdot\text{min}^{-1}\), and 0.5 \(\mu\text{mol}/\text{kg}^{-1}\cdot\text{min}^{-1}\), respectively, could have been detected. Thus the possible differences between Plc and Glc that could have been overlooked because of type II errors are most likely very small, and the physiological importance would therefore be questionable.

In summary, we have investigated the effect of glucose ingestion during exercise on the adaptation to endurance training. The results from this study suggest that a shift toward a larger dependence on fat from the bloodstream for oxidation during exercise occurred with one-legged knee extensor endurance training, whereas glucose ingestion during one-legged knee extensor endurance training does not seem to alter substrate utilization, performance, glycogen content, or metabolic enzyme activity of the trained leg.

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