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Effect of training status on fuel selection during submaximal exercise with glucose ingestion

LUC J. C. VAN LOON, ASKER E. JEUKENDRUP,
WIM H. M. SARIS, AND ANTON J. M. WAGENMAKERS

Department of Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands

Van Loon, Luc J. C., Asker E. Jeukendrup, Wim H. M. Saris, and Anton J. M. Wagenmakers. Effect of training status on fuel selection during submaximal exercise with glucose ingestion. *J. Appl. Physiol.* 87(4): 1413–1420, 1999.—In this study, an oral glucose load was enriched with a [^{13}C]glucose tracer to determine differences in substrate utilization between endurance-trained (T) and untrained (UT) subjects during submaximal exercise at the same relative and absolute workload when glucose is ingested. Six highly trained cyclists/triathletes [maximal workload (W_{\max}), 400 ± 9 W] and seven UT subjects (W_{\max} , 296 ± 8 W) were studied during 120 min of cycling exercise at 50% W_{\max} (~55% maximal O_2 consumption). The T subjects performed a second trial at the mean workload of the UT group (148 ± 4 W). Before exercise, 8.0 ml/kg of a ^{13}C -enriched glucose solution (80 g/l) was ingested. During exercise, boluses of 2.0 ml/kg of the same solution were administered every 15 min. Measurements were made in the 90- to 120-min period when a steady state was present in breath $^{13}\text{CO}_2$ and plasma glucose ^{13}C enrichment. Energy expenditure was higher in T than in UT subjects (58 vs. 47 kJ/min, respectively; $P < 0.001$) at the same relative intensity. This was completely accounted for by an increased fat oxidation (0.57 vs. 0.40 g/min; $P < 0.01$). At the same absolute intensity, fat oxidation contributed more to energy expenditure in the T compared with the UT group (44 vs. 33%, respectively; $P < 0.01$). The reduction in carbohydrate oxidation in the T group was explained by a diminished oxidation rate of muscle glycogen (indirectly assessed by using tracer methodology at 0.72 ± 0.1 and 1.03 ± 0.1 g/min, respectively; $P < 0.01$) and liver-derived glucose (0.15 ± 0.03 and 0.22 ± 0.02 g/min, respectively; $P < 0.05$). Exogenous glucose oxidation rates were similar during all trials (± 0.70 g/min).

exogenous glucose; endurance training; carbohydrate metabolism; stable isotopes; substrate metabolism

CARBOHYDRATE (CHO) and fatty acids are the main fuels oxidized by skeletal muscle to provide energy during prolonged exercise at intensities between 30 and 80% maximal O_2 uptake ($\dot{V}\text{O}_{2\max}$) (29). The main CHO sources are muscle and liver glycogen, liver gluconeogenesis, and ingested CHOs (29). The relative contribution of these fuel sources varies with exercise intensity (1, 3, 5, 37) and training status (2, 7, 9, 18–20, 22).

Whole body calorimetry measurements have clearly shown that endurance training leads to an increase in total fat oxidation and a decrease in total CHO oxidation during exercise in subjects investigated in the overnight-fasted state (4) and also in subjects investi-

gated between 1 and 6 h after ingestion of a standardized CHO-rich meal (7, 15, 20). Stable isotope tracers have been used to show that endurance training decreases the flux and oxidation rate of blood glucose at the same absolute intensity, whereas no change in glucose flux was seen at the same relative intensity in subjects investigated 1–2 h after ingestion of a standardized breakfast (15).

CHO ingestion during prolonged exercise at moderate intensities can increase time to exhaustion (6, 10, 11) and, therefore, CHO ingestion has become widespread among athletes, both professional and amateur, in a wide variety of sports. CHO ingestion leads to a better maintenance of blood glucose concentration and increases the ability to maintain high CHO oxidation rates during more prolonged exercise periods (10, 11, 17). It has been shown previously that ingested CHOs are oxidized with a maximal rate of ~1 g/min (17, 22, 39) and may account for >50% of the total CHO oxidation rate (39). Fat oxidation is reduced by CHO ingestion (12).

However, little information is available on the effects of training status on the relative use of CHO and fat as well as on the relative use of the different CHO sources in subjects who ingest CHOs during exercise. By using a [^{13}C]glucose tracer, Krzentowski et al. (26) measured the oxidation rate of orally ingested glucose in subjects before and after a training period during exercise at 40% of pretraining $\text{VO}_{2\max}$. They reported a 17% increase after training. They failed to find a decrease in total CHO oxidation, nor did they observe an increase in total fat oxidation. Jeukendrup et al. (22) compared a group of trained and untrained subjects exercising at the same relative intensity. In contrast with Krzentowski et al. (26), they found similar oxidation rates of the ingested glucose, a decrease in total CHO oxidation, and an increase in total fat oxidation. Blood glucose enrichment was not measured in any of these studies, so it was not possible to quantitate the relative contributions of muscle glycogen and of glucose released by the liver to the total energy expenditure.

Therefore, the first aim of the present study was to investigate whether a group of trained subjects has a higher rate of fat oxidation than do untrained subjects during moderate-intensity exercise while ingesting CHO, as has been previously observed in subjects who were investigated after an overnight fast. The second aim was to investigate whether trained subjects, who are accustomed to ingest larger amounts of CHO in their regular diets and who ingest oral CHO solutions during training sessions, oxidize orally ingested CHO at a similar or possibly at a higher rate than do untrained subjects. The third aim was to quantitate the

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oxidation rate and relative contribution of the other main CHO sources (muscle glycogen and glucose released from the liver). Therefore, six healthy trained cyclists/triathletes and seven healthy untrained subjects were studied during 120 min of cycling exercise at 50% maximal workload (W_{max}) while they ingested a glucose solution enriched with a [$U-^{13}C$]glucose tracer. The trained subjects also performed a second exercise trial at the same absolute workload as the untrained group, so that comparisons can be made both at the same absolute and relative workload. Total fat oxidation and total CHO oxidation were measured by using indirect calorimetry and the oxidation rates of the CHOs that originated from the different sources were indirectly quantitated by using tracer methodology, as described in detail in the following section.

METHODS

Subjects. Two groups of subjects participated in this study. One group consisted of seven well-trained cyclists or triathletes. The other group included eight healthy, fit, but untrained, subjects not active in any sport and without any history of endurance training. Subjects' characteristics are listed in Table 1. Subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. This study was approved by the local Ethical Committee.

Pretesting. $\dot{V}O_{2max}$ and W_{max} were measured on an electronically braked cycle ergometer (Lode Excalibur, Groningen, the Netherlands) during an incremental exhaustive exercise test (27) 1 wk before the first experimental trial (Table 1) to determine the 50% W_{max} (~55% $\dot{V}O_{2max}$) workload used in the following experimental trials (148 ± 4 and 199 ± 5 W in the untrained and trained groups, respectively).

Experimental trials. All subjects performed an exercise trial that consisted of 120-min of cycling at 50% W_{max} . Subjects in the trained group performed a second identical trial, with the workload set at the mean absolute workload performed by the untrained subjects (148 W). This was done to study differences in fuel selection between the two groups when performing exercise at the same absolute workload. The order of the trials performed by the trained subjects was randomized, and trials were separated by at least 7 days. During the tests, subjects ingested a glucose solution that was enriched with a [$U-^{13}C$]glucose tracer.

Protocol. The subjects arrived at the laboratory at 8:00 AM after an overnight fast. A Teflon catheter (Baxter, Utrecht, The Netherlands) was inserted into an antecubital vein, and a resting blood sample was drawn at 8:30 AM. Resting breath gases were collected (Oxycon β , Mijnhardt, Mannheim, Germany), and Vacutainer tubes (Becton-Dickinson, Meylan,

France) were filled directly from a mixing chamber in duplicate to determine the $^{13}C/^{12}C$ ratio in expired CO_2 . At 8:50 AM, the subjects started a warm-up of 5 min at 100 W; this was followed by 5 min at 40% W_{max} . At 9:00 AM, workload was set at 50% W_{max} for 120 min. During the first minute, subjects drank an initial bolus (8 ml/kg) of an 8% (80 g/l) glucose solution. Thereafter, a beverage volume of 2 ml/kg was provided every 15 min. This feeding schedule was chosen because it reflects fluid intake observed in cyclists during competition and has proven in earlier studies (22, 23) to result in tracer steady states after 60–90 min. On average, a total of 1.07 ± 0.03 and 1.07 ± 0.02 g/min glucose was ingested in the untrained and trained groups, respectively. Blood samples were drawn at 15-min intervals, and expiratory gases were collected every 15 min until the end of exercise.

Glucose. The 8% (80 g/l) glucose solution provided was prepared from corn-derived glucose (Amylum, Belgium), which has a high natural abundance of ^{13}C [$-11.2 \delta_{\text{‰}}$ vs. Pee Dee Bellemitella (PDB)]. Glucose solutions were further enriched by adding 0.0526 g [$U-^{13}C$]glucose/l (Cambridge Isotope Laboratories). The glucose solutions provided to the subjects had a ^{13}C -enrichment of 67.1 $\delta_{\text{‰}}$ vs. PDB [determined by elemental analyzer-isotope ratio mass spectrometry (IRMS; Carlo Erba-Finnigan MAT 252, Bremen, Germany)]. To minimize possible shifts in background enrichment as a result of a change in endogenous substrate utilization and differences in background enrichment of the different fuel stores, subjects were instructed not to consume any products with a high natural abundance of ^{13}C during the entire experimental period. The magnitude of shifts in background ^{13}C enrichment reported in subjects on a European diet (40) is $<1\%$, compared with the enrichment of the glucose solution used here, which obviates the need for background correction. Subjects were further instructed to refrain from any sort of heavy physical labor and to keep their diet as constant as possible during the days before the trial(s).

Analysis. Blood (7 ml) was collected in EDTA-containing tubes and was centrifuged at 1,000 g and 4°C for 5 min. Aliquots of plasma were frozen immediately in liquid nitrogen and were stored at $-40^{\circ}C$. Glucose (Uni Kit III, 07367204; La Roche, Basel, Switzerland), lactate (16), free fatty acid (FFA; Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany), and glycerol concentrations (GPO trinder method, 337, Sigma Diagnostics, St. Louis, MO) were analyzed with a COBAS FARA semiautomatic analyzer (Roche, Basel, Switzerland). Insulin concentrations were analyzed by RIA (Linco ultrasensitive human insulin RIA kit). Breath samples were analyzed for $^{13}C/^{12}C$ ratio by gas chromatography (GC)-IRMS (Finnigan MAT 252). To determine the $^{13}C/^{12}C$ ratio in plasma glucose, glucose was first extracted with chloroform-methanol-water, and derivatization was performed with butyl-boronic acid and acetic anhydride as described before (33). The measured $^{13}C/^{12}C$ ratios in the derivative (GC combustion-IRMS) were corrected for the isotopic carbon dilution. This was done by measuring a series of glucose standards both in the derivatized form (GC combustion-IRMS) and by direct combustion of underivatized glucose (elemental analyzer-IRMS). The standard curve thus constructed was linear over a range from 0 to 500 $\delta_{\text{‰}}$ vs. PDB. From CO_2 production ($\dot{V}CO_2$) and O_2 uptake ($\dot{V}O_2$) (Oxycon- β , Mijnhardt, Mannheim, Germany), total energy expenditure, CHO oxidation, and fat oxidation values were computed by indirect calorimetry. From $\dot{V}CO_2$ and stable isotope measurements ($^{13}C/^{12}C$) (GC combustion-IRMS, Finnigan MAT 252), the oxidation rates of exogenous glucose, muscle glycogen, and glucose released

Table 1. *Subjects' characteristics*

	Untrained Group	Trained Group
<i>n</i>	7	6
Age, yr	20.4 \pm 0.7	25.0 \pm 4.6
Height, cm	185 \pm 2	181 \pm 9
Body mass, kg	73.0 \pm 2.1	72.5 \pm 1.5
BMI, kg/m ²	21.3 \pm 0.5	22.1 \pm 0.4
$\dot{V}O_{2max}$, l/min	4.0 \pm 0.1	5.2 \pm 0.1*
W_{max} , W	296 \pm 8	400 \pm 9*

Values are means \pm SE. BMI, body mass index; $\dot{V}O_{2max}$, maximal O_2 uptake; W_{max} , maximal workload. *Significant difference between untrained and trained subjects, $P < 0.001$.

from the liver were calculated, as suggested by Derman et al. (14).

Calculations. From the recorded $\dot{V}O_2$ and $\dot{V}CO_2$, total CHO and fat oxidation and energy expenditure were calculated (32)

$$CHO = 4.585\dot{V}CO_2 - 3.226\dot{V}O_2 \quad (1)$$

$$\text{Fat oxidation} = 1.695\dot{V}O_2 - 1.701\dot{V}CO_2 \quad (2)$$

Isotopic enrichment is expressed here as $\delta\text{‰}$ difference between the $^{13}C/^{12}C$ ratio of the sample and a known laboratory reference standard, according to the formula of Craig (13)

$$\delta^{13}C = \left[\left(\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}} \right) - 1 \right] \times 10^3\text{‰} \quad (3)$$

The $\delta^{13}C$ is then related to an international standard (PDB).

Exogenous glucose oxidation (EGO) was calculated by using the formula

$$EGO = \dot{V}CO_2 \cdot \left(\frac{\delta\text{Exp} - \delta\text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \delta\text{Exp}_{\text{bkg}}} \right) \left(\frac{1}{k} \right) \quad (4)$$

in which δExp is the ^{13}C enrichment of expired breath during exercise at the relevant time point, δIng is the ^{13}C enrichment of the ingested glucose, $\delta\text{Exp}_{\text{bkg}}$ is the ^{13}C enrichment of expired breath before exercise (background), and k (0.7467) is the amount of CO_2 (in liters) produced by the oxidation of 1 g glucose.

Blood glucose enrichment was measured, and the following formula was used to calculate plasma glucose oxidation (PGO)

$$PGO = \dot{V}CO_2 \cdot \left(\frac{\delta\text{Exp} - \delta\text{Exp}_{\text{bkg}}}{\delta\text{PG} - \delta\text{PG}_{\text{bkg}}} \right) \left(\frac{1}{k} \right) \quad (5)$$

in which δPG is the plasma glucose ^{13}C enrichment, $\delta\text{PG}_{\text{bkg}}$ is the plasma glucose ^{13}C enrichment before exercise (background), and constant k is the same as in Eq. 4.

Because PGO represents the oxidation of both glucose coming from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis/gluconeogenesis), the oxidation rate of glucose released from the liver could be calculated by the following formula

$$\text{Liver-derived glucose oxidation} = PGO - EGO \quad (6)$$

Liver-derived glucose equals the sum of glucose that originates from liver glycogen breakdown and from gluconeogenesis. In a previous study, we have shown that 90–95% of the glucose that is released by the liver is oxidized during cycling exercise at 50% W_{max} (24).

Muscle glycogen oxidation could be estimated by using the formula

$$\text{Muscle glycogen oxidation} = TCO - PGO \quad (7)$$

in which TCO is total CHO oxidation.

Recovery of $^{13}CO_2$ from oxidation will approach 100% after 60 min of exercise when dilution in the bicarbonate pool becomes negligible (30, 36). Therefore, calculations on substrate oxidation were done between 90 and 120 min of exercise.

Statistics. All data are expressed as means \pm SE. Unpaired t -tests were used to compare the differences in substrate utilization and blood parameters between the trained and the untrained group (at the same relative and absolute workloads). Analysis of variance for repeated measures was per-

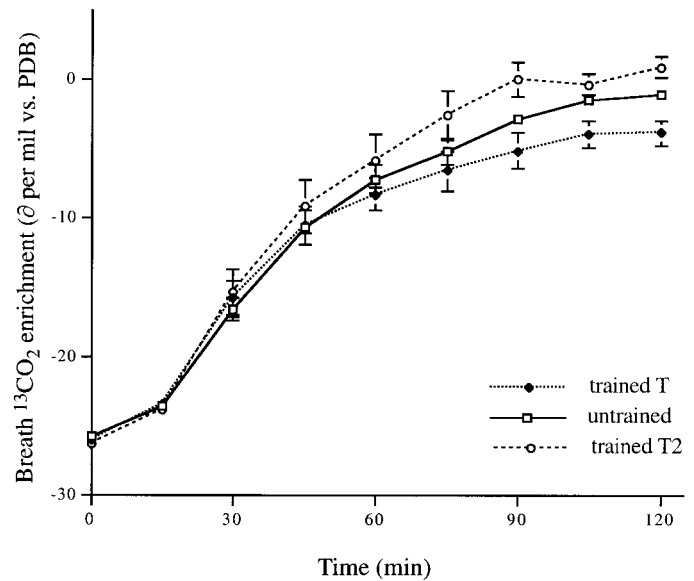


Fig. 1. Increase in breath $^{13}CO_2$ enrichment during exercise; values are means \pm SE. Untrained group at 50% maximal O_2 consumption ($\dot{V}O_{2\text{max}}$); T, trained group at 50% $\dot{V}O_{2\text{max}}$; T2, trained group at same absolute workload as untrained group. Enrichment of breath CO_2 before tracer ingestion averaged -26.04 ± 0.10 $\delta\text{‰}$ vs. Pee Dee Bellemmitella (PBD).

formed to study differences over time between groups. In case of a significant F -ratio, a Scheffé post hoc test was applied to locate the differences. Statistical significance was set at $P < 0.05$.

RESULTS

Stable-isotope measurements. Both breath $^{13}CO_2$ enrichment and plasma glucose ^{13}C enrichment reached a steady state after 75 min of exercise (Figs. 1 and 2).

Blood parameters. No differences between the trained and untrained subjects were found in plasma glucose, insulin, and glycerol levels (Fig. 3 A, C, and E, respec-

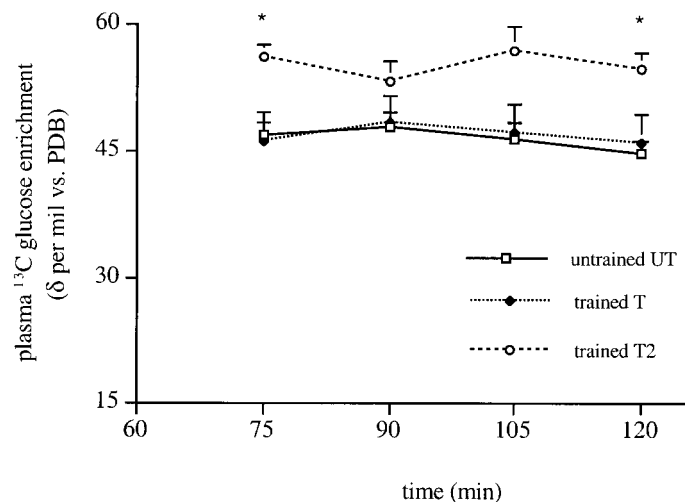


Fig. 2. Plasma $[U-^{13}C]$ glucose enrichment during exercise; values are means \pm SE. Plasma $[U-^{13}C]$ glucose enrichment at $t = 0$ was -24.54 ± 0.38 for untrained group (UT), -24.57 ± 0.50 for trained group (T), and -23.01 ± 0.84 for T2 group, $\delta\text{‰}$ vs. PBD, respectively. *Significant difference compared with UT group, $P < 0.05$.

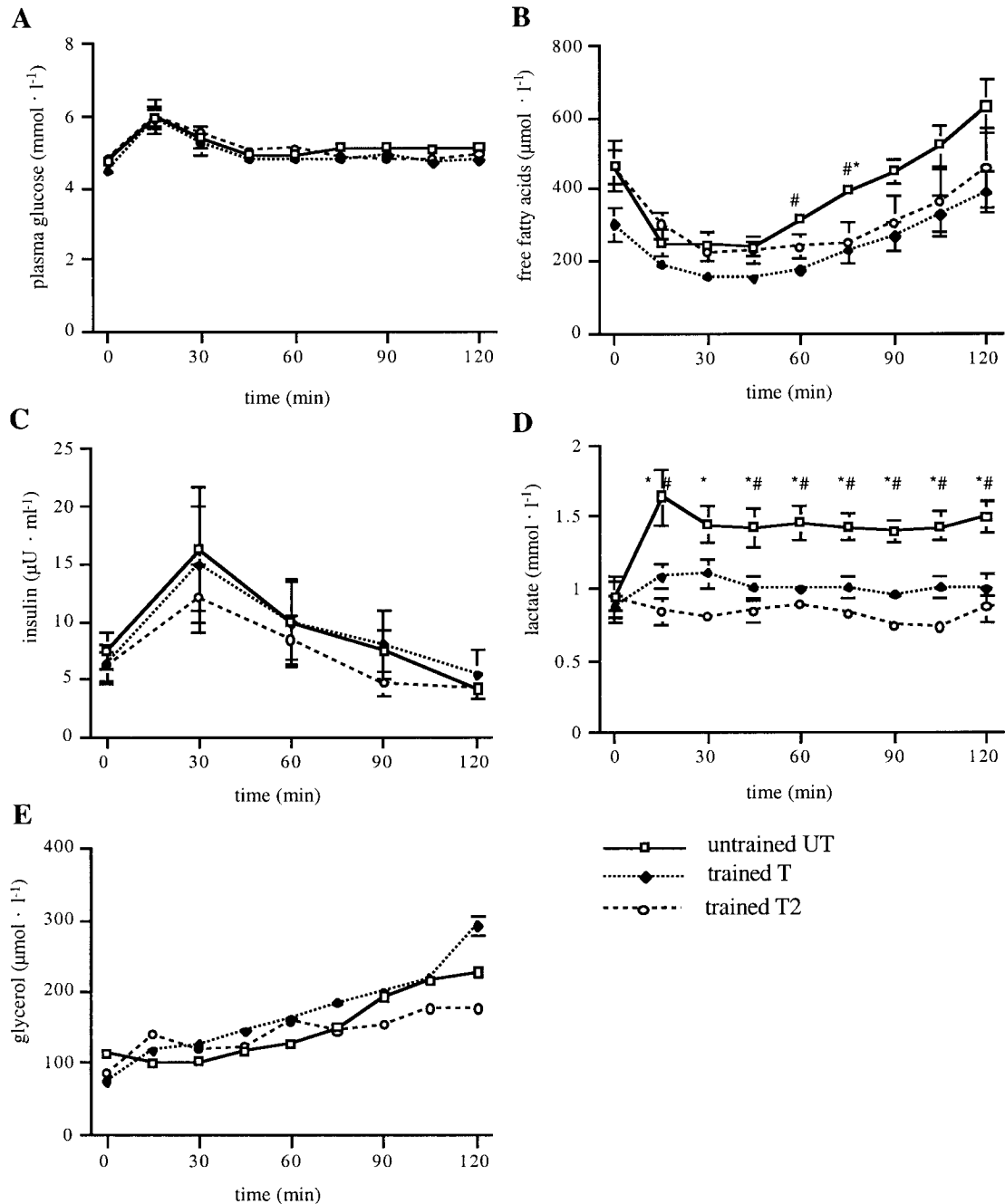


Fig. 3. Blood parameters for each group; values are means \pm SE. A: plasma glucose, B: free fatty acids, C: insulin, D: lactate, E: glycerol. *Significant difference between T2 and UT at (same absolute workload) $P < 0.05$. #Significant difference between T and UT at (same relative workload) $P < 0.05$.

tively). Plasma lactate levels were significantly higher in the untrained subjects than in the trained subjects both at the same relative and absolute workloads ($P < 0.05$) almost during the entire exercise period (Fig. 3D). A slightly higher increase in FFA levels was found in the untrained subjects. Differences were statistically different compared with the trained group at 60 and 75 min (Fig. 3B).

Energy expenditure. Raw data for calorimetry are illustrated in Fig. 4. Fuel oxidation is summarized in Table 2 and illustrated in Fig. 5. At a workload of 50% W_{max} , $\dot{V}O_2$ in the untrained and trained group was

2.23 ± 0.08 and 2.76 ± 0.09 l/min, respectively (56 ± 2 and $53 \pm 2\%$ $\dot{V}O_{2max}$). Energy expenditure was significantly higher in the trained than in the untrained group (58 ± 1.8 vs. 47 ± 1.6 kJ/min, respectively) when compared at the same relative workload. Mean heart rates were different in the untrained and trained group (160 ± 4 and 144 ± 3 beats/min, respectively; $P < 0.05$). In the trained group at the same absolute workload (148 ± 0 W), $\dot{V}O_2$ [2.19 ± 0.05 l/min ($42 \pm 1.2\%$ $\dot{V}O_{2max}$)] and energy expenditure (46 ± 0.9 kJ/min) were similar to the untrained group. At the same absolute workload, mean heart rates were significantly higher in the

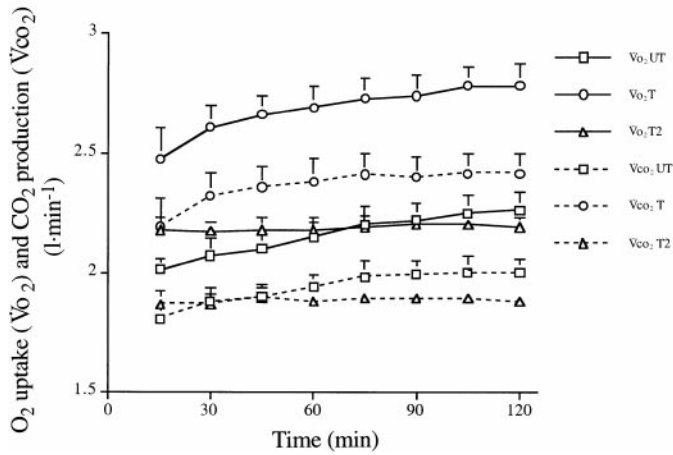


Fig. 4. Raw data for calorimetry; values are means \pm SE. $\dot{V}O_2$, O_2 uptake; $\dot{V}CO_2$, CO_2 output.

untrained than in the trained group (160 ± 4 vs. 121 ± 4 beats/min, respectively; $P < 0.05$).

Fat and CHO oxidation. When compared at the same relative intensity, fat oxidation contributed significantly more to energy expenditure in the trained (23.2 ± 1.5 kJ/min or 0.57 ± 0.03 g/min; 40% of total energy expenditure) than in the untrained group (15.9 ± 1.2 kJ/min or 0.38 ± 0.03 g/min; 33% of total energy expenditure; $P < 0.05$). No differences were found in CHO oxidation (with percentages of total energy expenditure in parentheses) [trained, 35.1 ± 2.4 kJ/min or 2.17 ± 0.15 g/min (60%); and untrained, 31.5 ± 1.2 kJ/min or 1.95 ± 0.07 g/min (67%), respectively]. Consequently, the higher energy expenditure in the trained group was completely accounted for by an increased fat oxidation. When compared at the same absolute intensity, fat oxidation also contributed significantly more to total energy expenditure in the trained subjects [20.6 ± 1.6 kJ/min or 0.50 ± 0.03 g/min (44%); $P < 0.05$]. Concomitantly, TCO was lower in the trained compared with the untrained group [25.6 ± 0.9 kJ/min or 1.58 ± 0.05 g/min (56%) vs. 31.5 ± 1.2 kJ/min or 1.95 ± 0.07 g/min (67%), respectively; $P < 0.05$].

CHO sources. No differences were found in TCO as well as in total endogenous CHO oxidation between the trained and untrained groups at the same relative intensity. PGO rates were similar in both groups [0.91 ± 0.04 g/min ($69.3 \pm 2.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and 0.99 ± 0.07 g/min ($76.1 \pm 5.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in the

untrained and trained groups, respectively]. Muscle glycogen oxidation was not different at the same relative workload between the untrained and trained groups [1.03 ± 0.02 g/min (36%) vs. 1.20 ± 0.13 g/min (32%) respectively]. Oxidation rates of liver-derived glucose were also similar in the untrained and trained subjects when compared during exercise at the same relative workload [0.22 ± 0.02 g/min (7%) vs. 0.24 ± 0.05 g/min (7%), respectively]. PGO rates in the trained group at the same absolute workload were similar (0.87 ± 0.04 g/min; $66.5 \pm 4.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) when compared with the untrained group. However, at the same absolute workload, endogenous CHO oxidation was significantly lower in the trained compared with the untrained subjects [0.87 ± 0.06 g/min (31%) vs. 1.25 ± 0.07 g/min (43%) respectively; $P < 0.005$]. This could be attributed to decreased oxidation rates of both muscle glycogen [0.72 ± 0.08 g/min (26%) vs. 1.03 ± 0.06 g/min (36%) respectively; $P < 0.01$] and liver-derived glucose [0.15 ± 0.03 g/min (5%) vs. 0.22 ± 0.02 g/min (7%) respectively; $P < 0.05$].

No differences were found in exogenous CHO oxidation between the two groups. At the same relative workload, oral glucose oxidation rates were 0.69 ± 0.03 and 0.75 ± 0.03 g/min in the untrained and trained group, respectively. At the 148-W workload, an oral glucose oxidation rate of 0.72 ± 0.02 g/min was found in the trained subjects. These oxidation rates represented, respectively, 23.6 ± 0.6 , 21.0 ± 0.9 , and $25.1 \pm 0.7\%$ of total energy expenditure in each trial.

DISCUSSION

In this study, we investigated the effect of training status on fuel selection and oxidation in subjects exercising at the same absolute (148 W) and relative workload (50% W_{max}) while ingesting glucose. By addition of a [$U\text{-}^{13}\text{C}$]glucose tracer to the glucose solution taken repeatedly in boluses, a plateau was obtained in blood glucose ^{13}C enrichment and breath $^{13}\text{CO}_2$ enrichment after 75 min of exercise. This enabled us to quantitate the following components of CHO oxidation: TCO, oxidation of the ingested glucose, oxidation of glucose derived from the liver, and muscle glycogen oxidation.

We observed that endurance-trained subjects have a substantially increased fat oxidation during prolonged moderate-intensity exercise (37–50% W_{max}) with glu-

Table 2. Contribution of substrates to total energy expenditure

	Energy Expenditure, kJ/min		
	Untrained group (UT)	Trained group (T)	Trained group (T2)
Fat	15.9 ± 1.2 (33%)	23.2 ± 1.5 (40%)*	20.6 ± 1.6 (44%)*
Muscle glycogen	16.7 ± 0.9 (36%)	19.0 ± 2.0 (32%)	11.6 ± 1.2 (26%)*
Liver-derived glucose	3.5 ± 0.3 (7%)	3.9 ± 0.7 (7%)	2.4 ± 0.4 (5%)*
Exogenous CHO	11.2 ± 0.5 (24%)	12.2 ± 0.6 (21%)	11.6 ± 0.3 (25%)
Total energy expenditure	47.4 ± 1.6	58.3 ± 1.8 *	46.2 ± 0.9

Values are means \pm SE. UT, untrained group at 50% W_{max} ; T, trained group at 50% W_{max} ; T2, trained group at 50% W_{max} of UT group (148 W). CHO, carbohydrate; nos. in parentheses, relative contribution of substrate source to total energy expenditure. *Significant difference between trained and untrained group, $P < 0.05$.

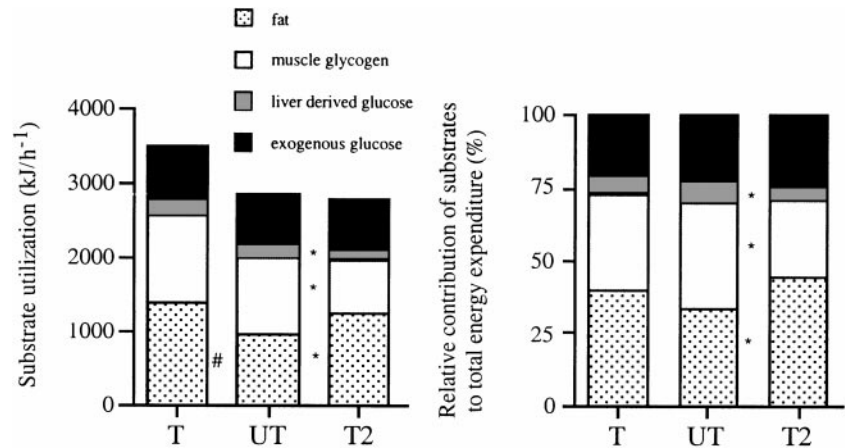


Fig. 5. Contribution of substrate sources to total energy expenditure; values are means \pm SE. *Significant difference in T2 compared with UT, $P < 0.05$. #Significant difference in T compared with UT, $P < 0.05$.

cose ingestion, as has been previously observed by others both in the presence (22) and absence (7, 8, 18, 20, 21) of glucose ingestion. When we made comparisons at the same relative workload (50% W_{max}), we found a similar rate of TCO. The higher energy expenditure (as absolute workload is higher) recorded in the trained subjects was completely accounted for by the increased fat oxidation. When we compared both groups during exercise at the same absolute workload, fat contributed significantly more to total energy expenditure and, concomitantly, TCO was decreased in the trained group.

In this study, we found that training status had a significant effect on total muscle glycogen utilization during prolonged exercise of moderate intensity with glucose ingestion only when comparisons were made at the same absolute workload. Previously, a glycogen-sparing effect of endurance training has been reported in overnight-fasted subjects at both the same relative (21) and the same absolute workload (20). When compared at the same relative workload, oxidation of glucose produced by the liver (coming from gluconeogenesis and glycogenolysis) was not influenced by training status. However, oxidation rates of liver-derived glucose were significantly lower in the trained compared with the untrained subjects when both were exercising at the same absolute workload. These findings have also been observed in a study by Friedlander et al. (15) 1–2 h after a standardized CHO-containing breakfast. Coggan et al. (7, 9) studied subjects before and after an endurance training period at 60% of their pretraining peak $\dot{V}O_{2max}$. Coggan et al. observed that endurance training reduces both hepatic glycogenolysis and gluconeogenesis during prolonged exercise at the same absolute workload in subjects after an overnight fast (9) and 6 h after a standardized light breakfast (7).

Combined data obtained from experiments in humans indicate that splanchnic glucose output rises linearly with exercise intensity up to 50–60% $\dot{V}O_{2max}$ (25). This would explain the similar oxidation rates of glucose derived from the liver in the trained and untrained subjects in our study during exercise at the same relative intensity. One possible explanation for the observed reduction in liver-derived glucose oxidation in trained subjects at the same absolute intensity

is that the hormonal changes drive liver glucose output and are decreased after endurance training. Both decreases in plasma insulin and increases in epinephrine, norepinephrine, and glucagon have been implicated in the control of glucose output during exercise (7, 9, 25). Coggan et al. (7, 9) observed smaller decreases in insulin levels and a diminished increase in epinephrine, norepinephrine, and glucagon levels during exercise after endurance training.

In this study, insulin concentrations did not decrease during exercise, most probably as a consequence of the glucose ingestion. This explains the similar insulin concentrations recorded during all three trials. Glucagon and catecholamines were not measured. Another factor that could contribute to a reduced hepatic glucose production is the reduced availability of gluconeogenic precursors during exercise (9). We observed lower plasma lactate concentrations (Fig. 3D) in the trained subjects at the same absolute intensity. However, when compared at the same relative intensity, oxidation rates of glucose released from the liver were similar, although blood lactate was lower in the trained subjects. The fact that gluconeogenesis is low in subjects ingesting glucose (25) also seems to argue against a major role for gluconeogenic precursor availability in the control of liver-derived glucose oxidation.

It is concluded that both oxidation of liver-derived glucose and muscle glycogen are affected by training status when compared at the same absolute exercise intensity. Glucose ingestion during exercise seems to have no effect on the decrease in reliance on endogenous CHO oxidation in trained subjects when compared at the same absolute intensity.

Training status had no effect on EGO rates during moderate-intensity exercise at the same absolute and relative exercise intensity. Jeukendrup et al. (22) also did not find differences in oral glucose oxidation rates during moderate-intensity exercise between trained and untrained individuals at the same relative exercise intensity. EGO previously has been suggested to be directly related to workload or $\dot{V}O_2$ (28, 31, 34, 35). However, no significant differences were found between the trials, although EGO rates tended to be higher in the trained subjects at the higher workload (Table 2). Recently it was suggested that exogenous CHO oxida-

tion (up to a maximum of 1.0–1.1 g/min) is only limited by the rate of absorption from the intestine (24, 39). The observed EGO rates were below maximal values because only low- to moderate-intensity exercise was performed. Furthermore, it has been suggested that endurance training, because of concomitant high dietary CHO intake and regular CHO supplementation during and after exercise, could lead to an adaptation in the gut that results in increased glucose absorption rates during exercise (38). Nonetheless, training status did not seem to affect EGO rates, at least not at this glucose-ingestion rate and workload.

In conclusion, this study shows that endurance training increases fat oxidation during submaximal exercise when glucose is ingested. When compared during exercise at the same relative intensity (55% $\dot{V}O_{2\max}$), endurance-trained subjects oxidize more fat to meet the higher energy demand, and they oxidize the same amount of CHOs. When they are compared during exercise at the same (absolute) workload, trained subjects have both an increased rate of fat oxidation and reduced oxidation rates of muscle glycogen and liver-derived glucose. These findings are similar to previous observations in studies of endurance training performed in the absence of glucose ingestion. Exogenous glucose oxidation rate is not affected by training status during moderate-intensity exercise.

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Address for reprint requests and other correspondence: L. J. C. van Loon, Dept. of Human Biology, Maastricht Univ., PO Box 616, 6200 MD Maastricht, The Netherlands (E-mail: L.vanLoon@HB.Unimaas.nl).

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