Effect of carbohydrate ingestion on adipose tissue lipolysis during long-lasting exercise in trained men

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De Glisezinski, I., I. Harant, F. Crampes, F. Trudeau, A. Felez, J. M. Cottet-Emard, M. Garrigues, and D. Riviere. Effect of carbohydrate ingestion on adipose tissue lipolysis during long-lasting exercise in trained men. J. Appl. Physiol. 84(5): 1627–1632, 1998.—To study whether sucrose administration acts on lipid mobilization during prolonged exercise, we used subcutaneous abdominal adipose tissue microdialysis in eight well-trained subjects submitted at random to two 100-min exercises (50% maximal aerobic power) on separate days. After 50 min of exercise, the subjects ingested either a sucrose solution (0.75 g/kg body wt) or water. By using a microdialysis probe, dialysate was obtained every 10 min from the subjects at rest, during exercise, and during a 30-min recovery period. During exercise without sucrose, plasma and dialysate glycerol increased significantly. With sucrose, the response was significantly lower for dialysate glycerol (P < 0.05). Plasma free fatty acid level was lower after sucrose than after water ingestion (P < 0.05). With water ingestion, plasma catecholamines increased significantly, whereas insulin fell (P < 0.05). With sucrose ingestion, the epinephrine response was blunted, whereas the insulin level was significantly increased. In conclusion, the use of adipose tissue microdialysis directly supports a lower lipid mobilization during exercise when sucrose is supplied, which confirms that the availability of carbohydrate influences lipid mobilization.

during muscular exercise, carbohydrates and lipids represent the major part of the substrates used for the production of energy. The contribution of one fuel or the other to muscle energy production depends on the level of mobilization and the level of oxidation of the substrate in the muscle; it is governed by exercise intensity (8). Recent observations made of subjects who were at rest suggested that the nature of substrate oxidation in human subjects is controlled more by the intracellular availability of glucose than of free fatty acids (FFAs) (30). It has also been suggested that availability of extracellular glucose acts on the level of mobilization of FFA during exercise indirectly indicated by lower plasma FFA and glycerol levels than in controls (2, 9). Nevertheless, a common practice among trained subjects is to drink beverages containing carbohydrate during prolonged exercise to supply energy and maintain performance (11, 13). Such a practice could then potentially alter FFA mobilization from their adipocytes. The purpose of our study was to determine the effect of carbohydrate ingestion during exercise on the mobilization of FFAs directly in the adipose tissue by using microdialysis.

METHODS

Subjects

Eight healthy well-trained male subjects [age, 42.9 ± 3.0 (SE) yr; body mass index, 22.5 ± 0.4; body fat, 10.5 ± 1.1%; maximal oxygen uptake, 64.9 ± 2.6 ml·kg<sup>-1</sup>·min<sup>-1</sup>] participated in the study. At the time of the study, they were logging on average 75 ± 5 km of running per week. The subjects were informed of the procedures of the experiment and gave their informed consent before the beginning of the study. The project was approved by the local ethics committee, i.e., Comité Consultatif de Protection des Personnes pour la Recherche Biomédicale, Toulouse 1.

Exercise Protocols

The subjects performed two exercise sessions, one with sucrose ingestion and the other with water ingestion, on two different days separated by at least 1 wk. They were not under medication; they were asked to avoid training 48 h before the test and to keep their normal dietary habits, except for the last evening meal, which was the same precompetitive dinner for the two experiments. The exercises were performed after an overnight fast. In both experiments, exercise was performed on a cycle ergometer (model HR 800, Jaeger) for 100 min at a heart rate (continuously monitored with a Baumann BHL 6000 cardiometer) corresponding to 50% of their maximal aerobic power (112 ± 4 beats/min). This corresponded to an average workload of 140 ± 4 W. For each subject, the workload was the same for the two exercises. Fifty minutes after the beginning of exercise, subjects had to rapidly drink either carbohydrate (sucrose: 0.75 g/kg body wt) dissolved in water to give isosmolality, i.e., 300 mosmol/kg H2O, or water. In the two experiments, the ingested volume was ~500 ml. The protocols were called sucrose experiment (SE) and water experiment (WE). The order of the two experiments was randomly determined.

Adipose Tissue Microdialysis

To evaluate the effect of carbohydrate ingestion on in situ lipid mobilization from adipose tissue, we used a microdialysis technique that has been described elsewhere (4). For both experiments, after insertion of a catheter into an antecubital vein for blood sampling, two microdialysis probes were inserted, under local anesthesia by using epinephrine-free
lidocaine, percutaneously into the abdominal adipose tissue, one on either side of and 5 cm from the navel.

Microdialysis was performed with probes having a dialysis membrane of 31.4 mm² (length, 20 mm; diameter, 0.5 mm) and a 20,000-molecular weight cutoff (CMA/20 microdialysis probe, Carnegie, Stockholm, Sweden). The input tubing of the probe was connected to a microinjection pump (model 22, Harvard Apparatus, South Natick, MA) and continuously perfused with a flow of 2.5 µl/min. As an indicator of adipose tissue lipolysis, the glycerol concentration was measured in the outgoing dialysate. The in vivo recovery rate at 2.5 µl/min was evaluated at rest (calibration period) for each probe by using a method first used in brain microdialysis (23) and now used for adipose tissue microdialysis (6). To do this, dialysate glycerol concentrations were measured at four different perfusion rates (i.e., 5, 1.5, 0.9, and 2.5 µl/min). These concentrations were plotted against the perfusion rates. The glycerol concentration at zero flow was then calculated by regression analysis. The in vivo recovery rate of the probe is given by the ratio between the dialysate glycerol concentration at 2.5 µl/min and the calculated interstitial glycerol concentration. During the two experiments, ethanol (0.1 g/l) was added to the perfusate to estimate changes in the adipose tissue blood flow, as previously described (16).

Dialysate was continuously recovered after the calibration period in microcentrifuge tubes that were removed every 10 min. The rest period lasted 20 min (two samples), and the 100-min exercise was followed by a 30-min recovery period. All fractions were kept on ice during the experiment. After the end of the recovery period, an aliquot of dialysate was removed from each tube for an immediate assay of ethanol. The remaining dialysate in each tube was kept frozen at −80°C until glycerol assay.

Blood Samples

During the preexercise period, a blood sample was taken 10 min and immediately before the beginning of exercise while the subject was seated on the bicycle ergometer. Then, blood was sampled at 25, 50, 75, and 100 min of exercise. For the recovery period, blood was taken at 10 and 30 min after the end of exercise. Each blood sample was centrifuged immediately after collection and frozen at −80°C until analysis. Plasma concentrations of glycerol, FFAs, glucose, lactate, insulin, and catecholamines were then determined in each sample.

Analytic Method

Dialysate glycerol and ethanol levels were measured by using a bioluminescence technique (14, 24). The bioluminometer (LKB 1251, LKB Wallac, Stockholm, Sweden) was coupled with distributor (LKB 1291) for luciferase distribution (luciferase from Photobacterium fisheri and incubation enzymes, Boehringer Mannheim, Meylan, France).

Plasma glycerol was also analyzed by bioluminescence by using the same method. Plasma FFA concentration was measured with a commercial colorimetric method (NEFA C, Wako Unipath, Dardilly, France). Plasma glucose and lactate levels were assayed with an automated analyzer (YSI 27, Bioblock Scientific, Illkirch, France). Plasma insulin concentration was measured with a commercially available radioimmunoassay (Trousses Bi-Insulin immunoradiometric assay, ERIA Diagnostics Pasteur, Marnes-la-Coquette, France). The catecholamine (epinephrine and norepinephrine) levels were determined by high-performance liquid chromatography as previously described (25).

Statistical Analysis

Values are expressed as means ± SE. Analysis of variance for repeated measures and Wilcoxon’s paired test were used for comparisons when appropriate. P < 0.05 was considered as being statistically significant.

All statistical comparisons were performed by means of a statistical software package (Statview II, Abacus Concepts, Berkeley, CA).

RESULTS

Dialysate Samples

Glycerol concentrations (Fig. 1). With the subjects at rest, by using the different perfusion flows, we esti-
mated the interstitial glycerol level to be 172.82 ± 13.79 μM in WE and 111.90 ± 25.46 μM in SE. At a perfusion rate of 2.5 μl/min, the glycerol level in the dialysate was 71.05 ± 6.23 μM in WE and 46.75 ± 8.87 μM in SE. Thus the recovery rate was 41.5 ± 3.4% in WE and 41.4 ± 3.5% in SE. The differences in these parameters between the two experiments were not significant.

During exercise and recovery, glycerol level was expressed as the level measured at a given time minus the resting glycerol level for each subject. The glycerol concentration in the dialysate increased significantly starting from 10 min of exercise for both conditions (P < 0.01). At the end of exercise, the increase in concentration was 210.37 ± 39.48 μM in WE and 130.41 ± 12.18 μM in SE. The glycerol level was significantly higher in WE at 100 min of exercise (P < 0.05) and at 20 min (P < 0.05) and 30 min (P < 0.01) of recovery. During recovery, the glycerol concentrations in the dialysate did not return to basal values in either of the experimental conditions.

Ethanol concentrations (Fig. 1). The ethanol level was expressed as the percentage of perfused concentration, i.e., dialysate ethanol-to-perfusate ethanol ratio, as previously described (20). We found no significant variation during exercise in either condition (water or sucrose).

Plasma Samples

Plasma glycerol (Fig. 2). At rest, the plasma glycerol concentration was not significantly different in the two conditions (68.9 ± 17.9 μM in WE and 57.9 ± 10.3 μM in SE). Plasma glycerol increased similarly in both conditions during exercise (P < 0.01). However, we measured a trend toward a higher plasma glycerol level immediately at the end of exercise (100 min) in WE. The values reached 317.8 ± 47.5 and 225.7 ± 28.4 μM, respectively, in the water and the sucrose conditions.

After 30 min of recovery, the plasma glycerol concentration was not significantly different from rest in either condition.

Plasma FFAs (Fig. 2). At rest, there was no significant difference in plasma FFA concentrations between the two experiments. In the WE, there was no significant variation of FFAs during exercise, whereas in SE the plasma FFA concentration decreased significantly (563 ± 104.9 μM at rest vs. 436.5 ± 124.9 μM at 100 min of exercise; P < 0.05). At the end of exercise, plasma FFA concentrations were significantly different between the two experiments (P < 0.05). After 10 min of recovery, the FFA level increased significantly (P < 0.05) in both conditions.

Plasma glucose (Fig. 3). At rest, plasma glucose was not significantly different in the two experiments (4.75 ± 0.11 mM in WE and 4.88 ± 0.16 mM in SE). During exercise in SE, we measured a significantly higher level of glucose 25 min after the ingestion of sucrose (6.01 ± 0.27 mM vs. 4.59 ± 0.08 mM; P < 0.01). In WE, no significant variation of plasma glucose was observed.

Ten minutes after the end of exercise, the glucose concentration increased significantly in SE. Thus, there was a significant difference between the two conditions (P < 0.01).

Plasma insulin (Fig. 3). From rest until sucrose ingestion, plasma insulin levels were similar in both conditions and decreased during exercise. In WE, the concentration was 3.47 ± 0.57 mIU/l at rest and 1.37 ± 0.24 mIU/l at the end of exercise (P < 0.05).

Twenty-five minutes after sucrose ingestion, plasma insulin followed the variations of plasma glucose and was higher than before sucrose ingestion (P < 0.01). The concentration reached 8.16 ± 1.63 mIU/l. The difference with WE was significant (P < 0.05).

Ten minutes after the end of exercise, the plasma insulin level increased in both conditions, but it was higher in SE than in WE (P < 0.05).
Plasma catecholamines (Fig. 4). During exercise, plasma catecholamine levels increased significantly. In WE, for epinephrine, the values were 46.62 ± 5.01 and 146.87 ± 28.05 pg/ml, respectively, at rest and at 100 min of exercise (P < 0.05). For norepinephrine, the values were 313.37 ± 20.99 and 1,087.37 ± 140.02 pg/ml, respectively (P < 0.001).

In SE, for epinephrine the values were 45.81 ± 2.84 pg/ml at rest and 96.62 ± 12.61 pg/ml at 100 min of exercise; for norepinephrine the values were 384.37 ± 45.62 and 960.50 ± 93.2 pg/ml, respectively. Plasma epinephrine concentrations 25 and 50 min after sucrose ingestion were significantly lower than in WE (P < 0.05). There was no significant difference in plasma norepinephrine concentrations between the two experiments.

Plasma lactate (Fig. 5). In WE, the plasma lactate concentration increased significantly during exercise but remained at a low level (1.56 ± 0.13 mM at the end of exercise vs. 1.08 ± 0.08 mM at rest; P < 0.05).

In SE, the plasma lactate concentration increased more than in WE (1.77 ± 0.10 mM at the end of exercise vs. 1.00 ± 0.07 mM at rest; P < 0.001). After 10 min of recovery, the values were significantly different between the two conditions, i.e., 1.25 ± 0.10 mM in WE and 1.85 ± 0.12 mM in SE (P < 0.001).
DISCUSSION

We found an increase in the glycerol level in the dialysate during exercise in both conditions (SE and WE), indicating a higher lipid mobilization from the adipocytes during exercise. However, the main result of our study was a lower glycerol level in the dialysate of subjects receiving sucrose during exercise.

The increase in glycerol level measured in the dialysate during exercise in both conditions is consistent with other observations in exercised humans by using glycerol measured in the plasma as the index of lipolysis (18) or more directly in the dialysate (5). The increase in glycerol level in the dialysate could be the result of a higher rate of adipose tissue lipolysis, a decrease in adipose tissue blood flow (10), or interstitial contamination by plasma glycerol. This third point is not to be considered in our study because interstitial glycerol concentrations were higher than plasma glycerol concentrations.

The use of ethanol to estimate the adipose tissue blood flow gives an idea of the change in vasomotricity in adipose tissue. In our study, there was no difference in the percentage of perfused ethanol between the two experiments. In both conditions this ratio tended to be lower at the beginning of exercise, suggesting higher adipose tissue blood flow (3). The ratio increased while the exercise continued and then returned to the basal value. In both conditions, these variations were not significant, suggesting that in this type of exercise there was little change in adipose tissue blood flow. Thus we can hypothesize that the changes in interstitial glycerol concentration observed during the two experiments are due more to changes in lipolysis itself than to large changes in adipose tissue blood flow.

With microdialysis, we were able to measure an effect of carbohydrate ingestion on lipid mobilization in the adipose tissue. These effects were due to sucrose ingestion, and not to exercise, because in both conditions exercise intensity was the same.

Our results confirm the inhibiting effect of carbohydrate ingestion on adipose tissue lipolysis during exercise (2, 11, 13). In studies suggesting such an effect, a higher dosage of carbohydrate was used, i.e., from 100 to 200 g of pure glucose. Such a load of glucose resulted in a decrease of plasma glycerol, which was not observed in the present study. An explanation could be the difference in load of glucose and/or the type of carbohydrate. We used 50 g of sucrose because sucrose is an easily available carbohydrate for homemade preparations intended for energy supply during exercise. The duration of the exercise after carbohydrate ingestion [50 min in our study vs. 100 min in the study of Ahlborg and Felig (2)] could be another explanation for this discrepancy in plasma glycerol level.

The decrease in the level of lipid mobilization after sucrose ingestion could be attributed to several mechanisms. The first possible mechanism is a higher insulin level in the SE subjects (30). Insulin is known to inhibit adipose tissue lipolysis and adipocyte membrane transport of FFAs (1, 26). Usually, insulin decreases during prolonged exercise (18). When this decrease is prevented by experimental manipulations, FFA mobilization is lowered (21, 31). Our results are consistent with such observations. Another mechanism that could explain the decrease of lipid mobilization in the abdominal adipose tissue is inhibition through the higher lactate level we measured after sucrose ingestion. Lactate is known to inhibit FFA mobilization from the adipose tissue in exercised dogs (17) and humans (7). Even if we did not have these data, we could hypothesize that this higher level of lactate could be a consequence of glucose ingestion because it is known that an acceleration of the glycolytic pathway may be sufficient to increase lactate production despite an adequate supply of oxygen (12). Another mechanism that could be involved in the lowered FFA mobilization is the lower plasma epinephrine response we found after sucrose ingestion, which was previously observed in experiments that used carbohydrate supplementation during exercise (27, 29). Epinephrine is known to be a lipolytic agent, particularly active in trained subjects (14). The decreased epinephrine response could be the result of mechanisms acting on the adrenal medulla via the central nervous system (15). It could also be related to the experimental conditions. Our subjects had been fasted for 12–14 h on their arrival in our laboratory. Such short-term starvation stimulates catecholamine secretion in humans (19, 28). Part of the increase in epinephrine observed during exercise was probably caused by a lower liver glycogen level (25). When we supplemented the subjects with sucrose, that part of the epinephrine response caused by the lowered liver glycogen was probably abolished.

During the 30-min recovery phase, we observed a large increase in FFA levels that was similar in both experiments. This increase and that of glucose could be explained by the sudden decrease that has already been observed in glucose and FFA utilization by skeletal muscle after exercise (22). This rebound of glucose associated with the decrease in catecholamine levels...
could also explain the increase in insulin level during recovery (18).

In conclusion, by using microdialysis, we measured an increase in subcutaneous adipose tissue lipid mobilization during prolonged exercise. However, when sucrose was supplied to the same subjects performing the same exercise, we observed lower subcutaneous adipose tissue lipid mobilization, as indicated by a lower glycerol level in the dialysate. Increased levels of insulin and lactate and decrease in catecholamine level were probably responsible for the effects observed in sucrose-fed subjects. Thus our results bring direct evidence supporting the effect of the availability of carbohydrates on the lipid mobilization from adipose tissue during exercise.

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