Meal Frequency of Pre-Exercise Carbohydrate Feedings

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Abstract
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This study compared the effect of single and multiple carbohydrate feedings before exercise on biochemical and physiological responses during exercise. Eight males performed 3 runs for 1 h at 70 % $\dot{V}O_{2max}$ after consuming a meal containing 2.5 g carbohydrate per kg body mass in a single dose 3 h before exercise (SF), the same meal in 5 equal doses at 3, 2.5, 2, 1.5, and 1 h before exercise (MF), or a liquid placebo 3 h before exercise (P). RER and carbohydrate oxidation rates were higher in SF and MF compared to P trials, but there was no difference between SF and MF trials.

Pre-exercise insulin was 2.0- and 3.4- fold higher in SF and MF, respectively, compared to P, and 1.7-fold higher in MF compared to SF. Glycerol and NEFA were higher in P compared to SF and MF trials before and at the end of exercise. In conclusion, a carbohydrate meal containing 2.5 g· kg^{-1} ingested in doses over 3 h before running produced higher hyperinsulinemia pre-exercise than that produced when the meal was consumed in a single dose. Nevertheless, estimated carbohydrate utilization and adipose tissue lipolysis during exercise after multiple feedings seemed to be as high as after a single feeding.

Introduction
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Carbohydrate (CHO) foods can be classified as high glycemic index (HGI), moderate glycemic index (MGI), or low glycemic index (LGI) according to the postprandial glycemic response they evoke [16]. Ingestion of HGI foods 3 – 4 h before exercise can increase liver [20] and muscle glycogen concentrations [7,27]. However, ingesting HGI foods results in hyperinsulinemia during the postprandial period [5,7,31], which may cause a transient reduction of blood glucose at the beginning (up to 15 – 30 min) of the subsequent exercise period, a condition often referred to as "rebound hypoglycemia" [17]. Additionally, there may be a suppression of lipolysis [13], a reduction in fat oxidation [5,7,31], and an elevation of muscle glycogen utilization [6,7,27].
In order to alleviate postprandial hyperinsulinemia and reduce the aforementioned metabolic perturbations, some investigators have used LGI foods before exercise [27,31,32]. The ingestion of LGI foods reduces postprandial glycemia and insulinemia and is accompanied by higher rates of fat oxidation [8,25 – 27,31,32].

An alternative approach to slowing down glucose absorption and blunting the postprandial insulin response is the increase of meal frequency. Ingesting a CHO load in small portions produces lower insulinemia in both healthy [15] and diabetic individuals [14], compared to a single bolus or to the traditional three-meals-a-day diet. Spreading the CHO load before exercise may prove useful to the endurance athlete, since, as mentioned earlier, the elevation of insulin before exercise is associated with unfavorable metabolic responses during exercise. To our knowledge, the effects of increasing meal frequency on the metabolic responses during exercise have not been compared to the responses produced by a single feeding of the same energy content.
Therefore, the main purpose of the present study was to investigate whether the hyperinsulinemia, hypoglycemia, reduced fat oxidation, and elevated CHO oxidation usually observed in endurance exercise after a CHO meal ingested in a single bolus are reduced when the meal is ingested in small doses.

Methods
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Subjects

Participants were eight healthy males, seven of which were physical education students and one was an amateur football player. Their age, height, body mass (BM), maximum oxygen uptake ($\dot{V}O_{2max}$), and maximum heart rate were 21.4 ± 1.3 years, 175 ± 2 cm, 73.9 ± 3.9 kg, 51.8 ± 0.7 ml·kg^{-1}·min^{-1}, and 199 ± 3·min^{-1} (mean ± SE), respectively. All subjects signed a formal consent statement after being fully informed, orally and in writing, about the nature of the experiment, any known risks, and their right to terminate participation at any time. A health history questionnaire was also completed. The study was approved by the University of Athens Ethics Committee.

Experimental design

Each subject was required to run for 60 min at 70% $\dot{V}O_{2max}$ on three different occasions separated by 5–7 days. On one occasion, a high carbohydrate meal was consumed in a single dose 3 h before exercise (single feeding, SF). On another occasion, the same meal was consumed in 5 equal doses at 3, 2.5, 2, 1.5, and 1 h before exercise (multiple feeding, MF). On a third occasion, a placebo was ingested in a single dose 3 h before exercise (P). Subjects performed the three trials at random. Blood samples and expired air were collected during the postprandial and exercise period.

Preliminary testing

Subjects performed two preliminary tests: (a) a 16-min incremental submaximal running test on a motorized level treadmill (Runrace Technogym, Gambettola, Italy) to establish a relationship between running speed and oxygen uptake ($\dot{V}O_2$) for use in the main trials, and (b) an incremental running test to exhaustion to determine $\dot{V}O_{2max}$. The tests were conducted as previously described [28]. Subjects then undertook a 30-min treadmill run at a speed corresponding to 70% $\dot{V}O_{2max}$ 5–7 days before the first experimental trial in order to fully familiarize themselves with the drinking pattern and the measurements used during the main trials.

Experimental meals

The CHO meals consumed in the SF and MF trials were designed to provide 2.5 g of CHO per kg BM. They consisted of white bread, jam, cornflakes, skimmed milk, orange juice, and tap water and provided about 87% of energy from CHO, about 11% from protein and less than 2% from fat. The energy content of the meals was about 800 kcal for a 70-kg person. The meals were considered to have a MGI since their estimated glycemic index was 70 [10,29]. In SF, subjects were instructed to consume the meal within 15 min, whereas in MF, each of the five portions of the meal was consumed within 3 min.

The placebo consisted of concentrated sugar-free orange juice diluted with water (0.5 ml/kg BM juice and 9.5 ml/kg BM water), with aspartame added for sweetness. The energy content of this drink was about 3 kcal per 100 ml. In the P trial, subjects ingested 10 ml per kg BM within about 5 min. The energy content of the drink was about 22 kcal for a 70-kg person.

Experimental protocol

Subjects arrived at the laboratory at 8:00 a.m., after a 10-h overnight fast. After standing for about 20 min, subjects sat on a chair and, within the next minute, a 10-ml blood sample from an ante-cubital vein was obtained. Also, duplicate 20-μl capillary blood samples were obtained from the thumb using a finger prick needle after having the subjects place their hand in warm water for about 3 min. Then a 5-min expired air sample was obtained while the participants were sitting. In addition, subjects assessed abdominal discomfort (AD) and sensation of gut fullness (GF) on two scales ranging from 0 (AD: completely comfortable; GF: empty) to 10 (AD: unbearable pain; GF: bloated). Afterwards, subjects ingested either the whole meal (in SF) or one-fifth of it (in MF) or placebo. During the subsequent 3-h postprandial period, capillary blood samples, 5-min average expired air samples, and ratings of AD and GF were obtained every 30 min as described above. Immediately afterwards the remaining portions of the meal were ingested in MF.

Twenty-five minutes before the completion of the postprandial period, the subjects' nude BM was recorded and then they changed into their running clothes. Subjects stood during the next 20 min of the postprandial period before sitting on a chair to provide venous blood samples, capillary blood samples, expired air samples, and ratings of AD and GF as before. Afterwards, the participants warmed up for 5 min on the treadmill at a speed equivalent to 60% $\dot{V}O_{2max}$. Thereafter, the speed of the treadmill was increased to correspond to 70% $\dot{V}O_{2max}$, and the subjects continued running for 60 min.

Every 15 min of exercise, 3-min average expired air samples, capillary blood samples, rating of perceived exertion (RPE) using the Borg scale [3], and ratings of AD and GF were obtained. After taking these and in order to maintain euhydration during exercise, subjects ingested 2 ml of tap water per kg BM through plastic syringes to prevent spillage at about 15, 30, and 45 min. Heart rate was continuously monitored telemetrically (Polar a$_1$, Kempele, Finland). Within 10 s of completion of exercise, subjects sat and provided the last venous blood sample. Five minutes afterwards the subjects undressed, wiped themselves with a towel, and postexercise nude BM was recorded. Fluid balance was estimated as the difference between post- and pre-exercise BM.

All trials were conducted under laboratory conditions that were not significantly different between trials (temperature: SF, 21 ± 1°C; MF, 21 ± 1°C; P, 22 ± 1°C; relative humidity: SF, 48 ± 2%; MF, 54 ± 4%; P, 51 ± 2%).

Finally, on a day after completion of the three trials, a subcutaneous fat sample was obtained by needle biopsy from the buttock area of the participants as described [2]. The purpose of biopsy was to compare the fatty acid profile of serum non-esterified fatty acids (NEFA) to that of their major source during exercise, adipose tissue triacylglycerols (TG).

Previous nutrition and exercise

Subjects weighed and recorded their normal food intake for 2 days before the first main trial, and were asked to replicate this diet for the same period of time before the subsequent two trials. Dietary records were analyzed in Microsoft Access by the use of a food database developed in our laboratory based on published data [12] and food labels. Furthermore, subjects followed the same training schedule two days before each trial and did not train the day before each trial.

Analysis of biological samples

Expired air was analyzed using a MedGraphics CPX/D metabolic cart (MedGraphics, St. Paul, MN, USA) calibrated just before the ingestion of the CHO meals and P, and every hour thereafter.

From the analysis, the average $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and respiratory exchange ratio (RER) over each 5- or 3-min collection period were calculated. From $\dot{V}O_2$ and $\dot{V}CO_2$, the CHO and fat oxidation rates were estimated assuming no contribution from proteins [21].

Capillary blood samples were dispensed into Eppendorf vials containing 200 µl of 2% perchloric acid, mixed well, and centrifuged at 1500 g for 4 min. The supernatant was removed and stored at – 40 °C for subsequent analysis. The two deproteinized supernatants obtained at each time point from each participant were analyzed through photometric methods for glucose using a kit from Randox (Crumlin, UK) and for lactate using chemicals (nicotinamide adenine dinucleotide, lactate dehydrogenase, and glycine buffer) from Sigma Diagnostics (St. Louis, MO, USA) and lactate standards from Trinity Biotech (Wicklom, Ireland), according to the method described in the kit provided in the past by Sigma Diagnostics (no. 826-UV).

Venous blood samples were left to clot for 30 min and then centrifuged at 1500 g for 10 min. Serum was separated, divided into aliquots of 1 ml, and stored at – 40 °C for subsequent analysis. Insulin was determined by ^{125}I radioimmuno assay, using a Bio-Source INS-IRMA kit (Nivelles, Belgium) and a gamma counter (B-Stratec Riamat, Pforzheim, Germany). Glycerol was determined photometrically using a kit from Randox. NEFA and the fatty acid composition of adipose tissue TG were determined by a combination of thin-layer chromatography and gas chromatography as described [18].

The incremental area under the blood glucose-time curve (IAUC) was calculated using the trapezoidal rule with fasting values taken as the baseline and negative areas being ignored [29].

Statistical analysis

Data were analyzed using the SPSS (SPSS Inc., Chicago, IL, USA, version 11.0). A two-way (treatment × time) analysis of variance (ANOVA) with repeated measures on both factors was used to compare respiratory, cardiovascular, and metabolic responses to feeding and exercise. The postprandial responses were assessed separately from the responses during exercise in $\dot{V}O_2$, RER, CHO oxidation, fat oxidation, AD, GF, blood glucose, and blood lactate. Dietary, fluid loss, IAUC over the entire 3-h postprandial period, and laboratory environment data were compared using one-way ANOVA for repeated measures. To identify differences between means in the event of a significant interaction (in two-way ANOVA) or a significant main effect (in one-way ANOVA), simple main effects were used with Bonferroni adjustment for multiple comparisons. Data are reported as means ± SE. The level of significance was set at $p < 0.05$.

Results

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Dietary, discomfort, and fluid loss data

There were no significant differences among trials in the daily intake of energy (SF, 2787 ± 108 kcal; MF, 2721 ± 112 kcal; P, 2608 ± 157 kcal), CHO (SF, 303 ± 24 g; MF, 308 ± 21 g; P, 298 ± 21 g), fat (SF, 127 ± 13 g; MF, 118 ± 13 g; P, 110 ± 13 g) or protein (SF, 115 ± 8 g; MF, 99 ± 6 g; P, 107 ± 9 g) during the 2 days prior to each trial.

Rates of perceived exertion were similar among conditions and averaged 10.8 ± 0.5 (range 7 – 14), 11.0 ± 0.6 (range 7 – 16), and 11.2 ± 0.4 (range 8 – 14) in SF, MF, and P trials, respectively. The mean GF during the 3-h resting period was significantly higher

($p < 0.05$) only in SF compared to P (SF, 3.1 ± 0.2; MF, 2.7 ± 0.3; P, 1.5 ± 0.4). During exercise, GF was higher ($p < 0.01$) in MF compared to P at 15 min (MF, 3.1 ± 0.4; P, 0.5 ± 0.4) and 30 min (MF, 3.0 ± 0.6; P, 0.6 ± 0.2). During the 60-min exercise period, GF averaged 1.6 ± 0.2, 2.8 ± 0.5, and 0.7 ± 0.2 in SF, MF, and P, respectively. These means were different ($p < 0.01$) only between MF and P trials. The sensation of AD, on the other hand, was low (ranging from 0 to 3) and not different among trials at rest or during exercise.

The decreases in body mass after 60 min of running were not different in the SF (0.91 ± 0.14 kg), MF (0.91 ± 0.10 kg), and P conditions (0.99 ± 0.18 kg). During exercise, subjects ingested 0.44 ± 0.03 l of tap water in all trials to maintain euhydration. Therefore, when body fluid loss was corrected for fluid intake, the average decreases in body mass were 1.35 ± 0.17 kg, 1.35 ± 0.12 kg, and 1.45 ± 0.19 kg in SF, MF, and P, respectively (n. s.). These represented body mass changes of 1.8 ± 0.1 % for SF and MF trials and 2.0 ± 0.2 % for P (n. s.).

Respiratory responses

Oxygen uptake during the 3-h postprandial period was 318 ± 17 ml·min⁻¹, 295 ± 21 ml·min⁻¹, and 283 ± 18 ml·min⁻¹ in SF, MF and P, respectively, and was higher in SF compared to P ($p < 0.05$). The consumption of the meal in a single dose (SF) increased $\dot{V}O_2$ 30 min after ingestion compared to the other two conditions (SF: 333 ± 19 ml·min⁻¹ vs. MF: 274 ± 20 ml·min⁻¹ vs. P: 285 ± 17 ml·min⁻¹; $p < 0.05$). Furthermore, $\dot{V}O_2$ was higher 2 hours postprandially (– 60 min) in both meal trials compared to control (SF: 328 ± 23 ml·min⁻¹ vs. MF: 315 ± 24 ml·min⁻¹ vs. P: 269 ± 15 ml·min⁻¹; $p < 0.05$). During exercise, $\dot{V}O_2$ was not different among trials and averaged 2.61 ± 0.18 l·min⁻¹, 2.59 ± 0.18 l·min⁻¹, and 2.66 ± 0.20 l·min⁻¹ in the SF, MF, and P trials, respectively. The mean relative exercise intensities were 68.5 ± 1.3 %, 68.0 ± 1.4 %, and 69.7 ± 1.8 % $\dot{V}O_{2max}$ in SF, MF, and P, respectively (n. s.).

The RER data are presented in ○ **Fig. 1**. RER was higher during the postprandial and exercise periods (– 150 min up to 60 min) only in SF compared to P ($p < 0.05$), whereas in MF, RER values were higher compared to P ($p < 0.05$) after 90 min of ingestion (– 90 min) till the end of exercise. No difference was observed in RER at any time between the two meal trials.
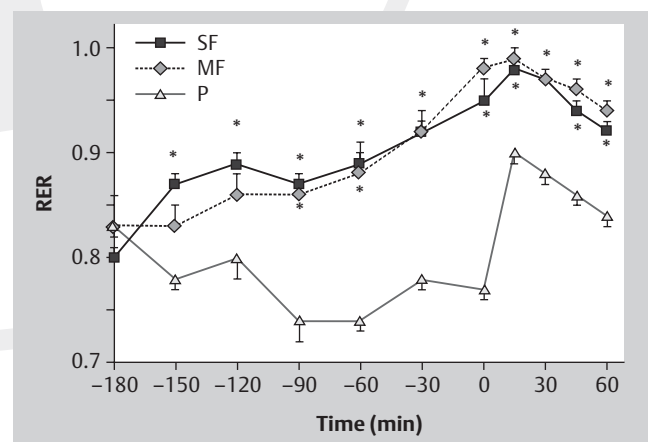
Fig. 1 Respiratory exchange ratio (mean and SE) during the postprandial (– 180 to 0 min) and exercise periods (0 to 60 min) in single feeding (SF), multiple feeding (MF), and placebo (P). * Significantly different from P ($p < 0.05$).

The estimated mean CHO oxidation rates during the 3-h resting period were 0.23 ± 0.02, 0.21 ± 0.03, and 0.08 ± 0.01 g·min⁻¹ in SF, MF, and P, respectively, and were higher in both SF and MF compared to P ($p < 0.01$). During exercise, the CHO oxidation rate was also higher in both SF and MF, compared to P and averaged 2.6 ± 0.1, 2.7 ± 0.2, and 1.7 ± 0.1 g·min⁻¹, respectively ($p < 0.01$). Subjects oxidized an additional 54 – 60 g of CHO during exercise in the SF and MF trials compared to the P trial. This amount was equivalent to about 29 – 32% of the total CHO load consumed in the pre-exercise meals (185 g).

On the other hand, the estimated mean fat oxidation rates during the 3-h resting period were 0.06 ± 0.01, 0.06 ± 0.01, and 0.11 ± 0.01 g·min⁻¹ in SF, MF and P, respectively, and were higher in P compared to both meal trials ($p < 0.01$). Similarly, fat oxidation rates during exercise were lower ($p < 0.01$) in SF and MF compared to P and averaged 0.23 ± 0.05, 0.17 ± 0.03, and 0.63 ± 0.09 g·min⁻¹, respectively.

The mean HR during exercise was not different between SF (161 ± 4 b·min⁻¹; range 144 – 184 b·min⁻¹), MF (164 ± 5 b·min⁻¹; range 143 – 189 b·min⁻¹), and P (165 ± 4 b·min⁻¹; range 144 – 188 b·min⁻¹).

Metabolic responses

Ingestion of the meal in a single dose (SF) increased blood lactate concentration 30 min postprandially compared to P (1.3 ± 0.2 vs. 0.6 ± 0.1 mmol·l⁻¹; $p < 0.05$). The mean blood lactate concentration during the 3-h postprandial period in SF, MF, and P was 0.8 ± 0.1, 0.9 ± 0.1, and 0.6 ± 0.1 mmol·l⁻¹, respectively, being different only between MF and P ($p < 0.05$). On the other hand, blood lactate concentration was similar during exercise in all three trials and averaged 1.8 ± 0.3, 2.0 ± 0.2, and 1.9 ± 0.2 mmol·l⁻¹, respectively.

The blood glucose concentration peaked 30 min postprandially in both meal trials (○ **Fig. 2**) and was higher compared to P during the whole postprandial period except at the end (0 min). In MF, blood glucose was higher compared to SF 1 h postprandially. The IAUC for blood glucose over the 3-hour postprandial period was higher ($p < 0.01$) in SF (167 ± 19 mmol·l⁻¹·180 min) and MF (227 ± 26 mmol·l⁻¹·180 min) trials compared to P trial (12 ± 4 mmol·l⁻¹·180 min), but no difference was observed between SF and MF trials. At 15 min of exercise, blood glucose was higher in P compared to SF and MF ($p < 0.05$). However, blood glucose was not different among conditions during the remainder of the exercise period.

Serum insulin was 1.7-fold higher in MF compared to SF ($p < 0.05$) and 3.4-fold higher in MF compared to P ($p < 0.01$) before the initiation of exercise (○ **Fig. 3**). Serum insulin was also 2.0-fold higher in SF than in P ($p = 0.058$). At the end of the post-prandial period, serum insulin was elevated compared to pre-feeding levels in the MF trial ($p < 0.01$). At the end of exercise, serum insulin decreased in all trials compared to pre-exercise levels ($p < 0.01$).

The serum glycerol concentration was lower in the SF and MF trials compared to P before and after exercise (○ **Fig. 4**). In addition, serum glycerol was higher after exercise compared to pre-exercise in all trials ($p < 0.01$). The serum total NEFA concentrations were lower in both meal trials compared to control pre- and postexercise (○ **Fig. 5**). In both SF and MF trials, total NEFA decreased as a result of meal ingestion ($p < 0.01$), but increased as a result of exercise ($p < 0.01$).

Gas chromatography revealed the presence of 12 NEFA, namely, myristate (14:0), palmitate (16:0), palmitoleate (16:1ω7), stea-
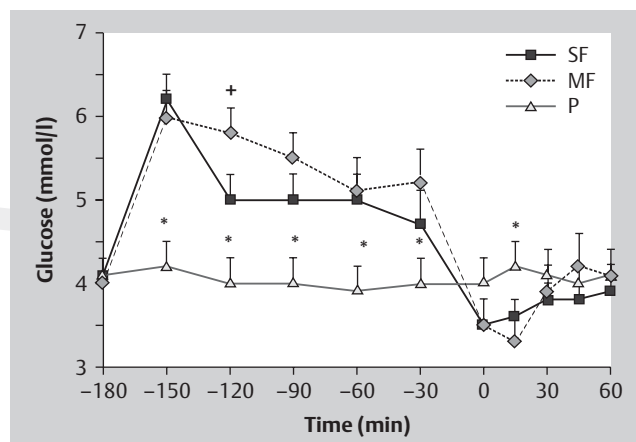
Fig. 2 Blood glucose concentration (mean and SE) during the postprandial (– 180 to 0 min) and exercise (0 to 60 min) periods in single feeding (SF), multiple feeding (MF), and placebo (P). * Significantly different from SF and MF ($p < 0.05$). + Significantly different from SF ($p < 0.05$).
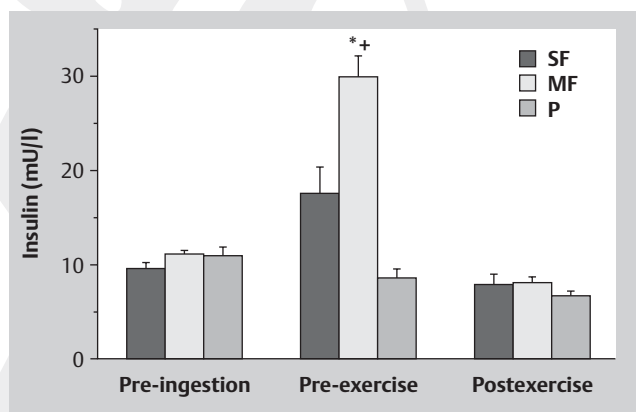

Fig. 3 Serum insulin concentration (mean and SE) during the postprandial (– 180 to 0 min) and exercise periods (0 to 60 min) in single feeding (SF), multiple feeding (MF), and placebo (P). *Significantly different from P ($p < 0.01$). + Significantly different from SF ($p < 0.05$).
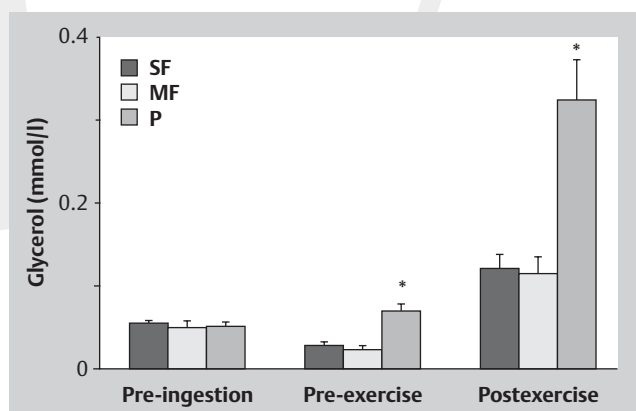
Fig. 4 Serum glycerol concentration (mean and SE) before meal ingestion (pre-ingestion), before exercise (pre-exercise), and after exercise (postexercise) in single feeding (SF), multiple feeding (MF), and placebo (P). * Significantly different from SF and MF ($p < 0.05$).

rate (18:0), oleate (18:1ω9), *cis*-vaccenate (18:1ω7), linoleate (18:2ω6), γ-linolenate (18:3ω6), α-linolenate (18:3ω3), gon-
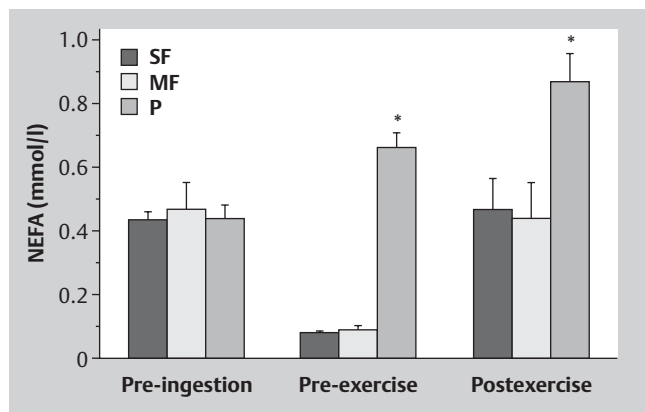
Fig. 5 Serum total NEFA concentration (mean and SE) before meal ingestion (pre-ingestion), before exercise (pre-exercise), and after exercise (postexercise) in single feeding (SF), multiple feeding (MF), and placebo (P). * Significantly different from SF and MF (p < 0.01).

doate (20:1ω9), dihomo-γ-linolenate (20:3ω6), and arachidonate (20:4ω6). The percentages of most of these fatty acids (16:0, 16:1ω7, 18:0, 18:1ω9, 18:3ω3, 18:3ω6, and 20:4ω6) exhibited significant interactions of treatment and time, suggesting different responses to the three feedings over time. To simplify the presentation of such multitude of data, we have calculated the following indices of the fatty acid profile: monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and unsaturated-to-saturated ratio (U/S). Again, there was a significant interaction of treatment and time in these indices. The most remarkable differences were observed in MUFA and U/S (○ **Fig. 6**). Both indices were significantly lower (p < 0.05) in SF and MF compared to P pre-exercise. As a response to exercise, MUFA increased significantly in SF and MF (p < 0.001), whereas U/S increased significantly in all three feedings (p < 0.05).

As mentioned under Methods, we performed adipose tissue biopsy to compare the fatty acid profile of serum NEFA to adipose tissue TG, which are their major source during exercise. ○ **Table 1** presents the fatty acid profile of adipose tissue TG. Half of the TG acyl groups belonged to 18:1ω9. Consequently, MUFA were the major lipid class of adipose tissue TG.

Discussion
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The main finding of the present study was that the ingestion of a high glycemic CHO meal in small doses (5 × 37 g CHO) over a 3-h period resulted in about 70% higher serum insulin concentration before exercise than the one produced when the same amount of CHO was ingested in a single dose (○ **Fig. 3**). This finding was contrary to the aim of reducing pre-exercise insulin levels by increasing meal frequency. Nevertheless, CHO oxidation rates, as estimated by indirect calorimetry, were similar in the two meal trials and were also higher throughout exercise compared to control.

In several studies it has been found that, when the daily food is taken in many (> 13) small feedings, the postprandial insulin response is reduced compared to the usual 3–4 meals in both healthy [4,30] and diabetic individuals [14]. Postprandial insulin was also reduced when only 50 g of glucose were ingested in a continuous sipping fashion over a 4-h period compared to a sin-
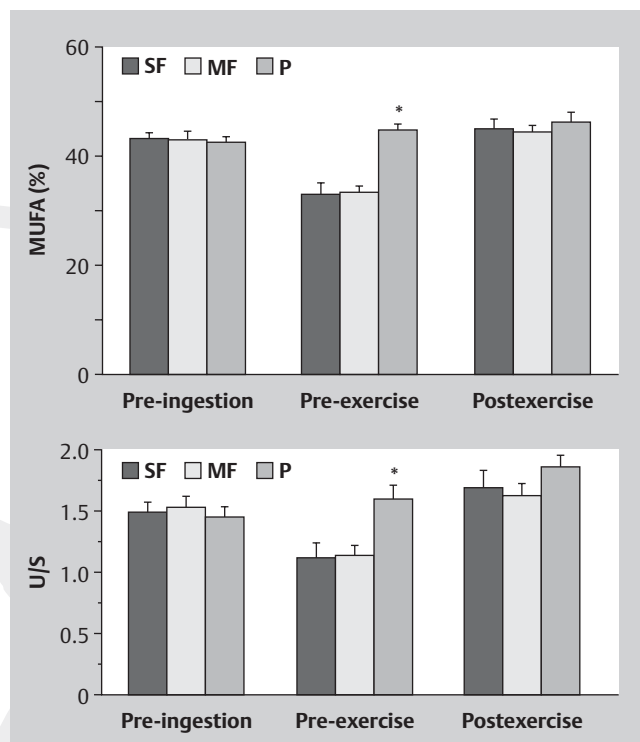
Fig. 6 Monounsaturated fatty acids (MUFA) and unsaturated to saturated ratio (U/S) in serum NEFA (mean and SE) before meal ingestion (pre-ingestion), before exercise (pre-exercise), and after exercise (postexercise) in single feeding (SF), multiple feeding (MF), and placebo (P). * Significantly different from SF and MF (p < 0.05).

Table 1 Fatty acid profile of adipose tissue TG (mean ± SE)

Fatty acid	Molar percentage
14:0	2.77 ± 0.22
16:0	21.50 ± 2.82
16:1ω7	4.62 ± 0.30
18:0	3.97 ± 0.27
18:1ω9	50.00 ± 1.03
18:1ω7	2.06 ± 0.11
18:2ω6	13.86 ± 1.21
18:3ω6	0.03 ± 0.00
18:3ω3	0.34 ± 0.03
20:1ω9	0.54 ± 0.02
20:3ω6	0.14 ± 0.02
20:4ω6	0.16 ± 0.02
Index	
MUFA (%)	57.24 ± 1.36
PUFA (%)	14.52 ± 1.26
U/S	2.58 ± 0.15

MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; U/S: unsaturated-to-saturated ratio

gle bolus [15]. However, when the amount of ingested CHO is increased and/or the postprandial period is reduced, the insulin concentrations are elevated compared to the single bolus approach. Siu and co-workers [24] provided their subjects with 1.5 g CHO per kg BM, which were ingested in three equal portions during the recovery period after running (about 32 g CHO at 20 min, 1, and 2 hours after exercise). This multiple feeding resulted in higher insulin levels 3 h after ingestion compared to a single bolus of about 96 g CHO. Furthermore, in a different study,

45 g and 75 g CHO were consumed in four equal doses within one hour before cycling. Even such small amounts of CHO produced serum insulin concentrations which were respectively 2.5-fold and 3.5-fold higher than the corresponding pre-ingestion values [23]. In that study, however, only multiple feeding trials were compared and no direct comparison with a single bolus of CHO was made.

It seems that the amount of CHO consumed by the subjects in the present study placed a considerable burden on the gastrointestinal tract, which possibly was unable to digest and absorb all this nutrient load within 3 h. It may be speculated that this was more pronounced in MF where 37 g CHO every 30 min was probably too much or/and too frequent. The fact that in both meal trials insulin concentrations before the initiation of exercise were above baseline is an indication that the digestion/absorption process was still in progress. However, no measurements of gastric emptying or intestinal absorption were performed in the present study.

The estimated CHO oxidation rates during exercise and the serum glycerol and NEFA concentrations were similar in the two meal conditions despite the difference in the pre-exercise serum insulin concentrations. It can be speculated that this difference in insulin concentration did not produce a different response in CHO oxidation and that blood glucose uptake was elevated in both meal trials, thus reducing fat oxidation in muscle. Furthermore, it seems that the total insulinemic response during the postprandial period and the residual effect of insulin on exercise metabolism, rather than the insulin concentration before exercise, affected the serum glycerol and NEFA concentrations [7].

The similar postprandial and exercise responses of serum glycerol and NEFA to the two CHO feedings were paralleled by similar responses of the MUFA content and the U/S ratio of serum NEFA (Fig. 6). These can also be attributed to the suppression of lipolysis in adipose tissue by insulin in the postprandial period and to the stimulation of lipolysis by exercise, since the MUFA and U/S of adipose tissue TG, the main source of plasma NEFA (57.24 and 2.58%, respectively, Table 1) were higher than in serum NEFA (ranging from 33.21 to 46.47% and from 1.12 to 1.85%, respectively, Fig. 6). Thus, it is reasonable that a stimulation of lipolysis will bring the profile of serum NEFA closer to that of adipose tissue TG, as has indeed been shown [19], whereas a suppression of lipolysis will enhance the difference of the two profiles.

The blood glucose concentration was lower at 15 min of exercise after ingestion of both meals compared to P (Fig. 2). This response is often observed when CHOs are ingested from 30 min up to 4 h before exercise [5,8,17,22,27,31]. Even the ingestion of as little as 22 g CHO in four doses one hour before exercise produces this transient drop in blood glucose [23]. Although in the present study five out of the eight subjects had blood glucose concentrations below 3.5 mmol·l^{-1} in both meal trials at 15 min of exercise, no subject reported any symptoms or presented any signs of hypoglycemia or discomfort. The low blood glucose concentrations in meal trials may be the consequence of an increased glucose uptake by muscle in the presence of an increased plasma insulin concentration. The inability of the liver to replenish blood glucose at the same rate as muscle takes it up may lead to hypoglycemia [1]. Nevertheless, the blood glucose concentration returned to baseline later during exercise, a response similar to the findings in other studies [5,8,22,27,31]. In both meal trials CHO feeding produced similar metabolic perturbations. It has been suggested that high insulin concentra-

tions at the start of exercise decrease the availability of NEFA to the working muscle. Thus, there is an increase in muscle glycogen utilization [6] that may lead to premature onset of fatigue [9]. Although performance was not assessed in the present study, subjects showed similar degrees of exertion in all trials, as reflected by the RPE values, indicating a similar degree of subjective fatigue. Actually, an improvement in endurance performance has been observed despite metabolic perturbations as a consequence of pre-exercise CHO feedings [5,22]. Possibly, the large amounts of CHO used in some studies (> 2 g per kg BM) were enough to offset any detrimental effects produced by the accelerated CHO oxidation. In this as well as in other investigations, these CHO loads are usually far higher than the excess CHO oxidized as a consequence of pre-exercise hyperinsulinemia [5]. The fact that multiple feedings before exercise did not produce any further elevation of CHO oxidation or a more pronounced reduction of fat oxidation compared to single feeding may be of practical use to the endurance athlete or the sports nutritionist. Many runners refuse to eat a considerable amount of food before training or competition because of associated gastrointestinal discomfort during exercise [11]. Therefore, the multiple feeding approach may help them to avoid such discomfort and optimize body CHO stores before an endurance activity.

In conclusion, the ingestion of a meal containing 2.5 g CHO per kg BM in small doses over 3 h before endurance running produced pre-exercise hyperinsulinemia that was higher than the one produced when the meal was consumed in a single dose 3 h pre-exercise. Nevertheless, carbohydrate use and blood glucose concentrations during exercise, as well as indices of adipose tissue lipolysis before and after exercise, were not different between multiple and single feeding. Thus, dividing a pre-exercise CHO load into multiple doses does not seem to affect substrate utilization during exercise compared with ingesting a single meal containing the same total amount of CHO.

Acknowledgements
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