Higher dietary carbohydrate content during intensified running training results in better maintenance of performance and mood state


The aim of this study was to determine whether consumption of a diet containing 8.5 g carbohydrate (CHO)·kg\(^{-1}\)·day\(^{-1}\) (high CHO; HCHO) compared with 5.4 g CHO·kg\(^{-1}\)·day\(^{-1}\) (control; Con) during a period of intensified training (IT) would result in better maintenance of physical performance and mood state. In a randomized cross-over design, seven trained runners [maximal O\(_2\) uptake (V\(_{\text{O}_2}\)\(_{\text{max}}\)) 64.7 ± 2.6 ml·kg\(^{-1}\)·min\(^{-1}\)] performed two 11-day trials consuming either the Con or the HCHO diet. The last week of both trials consisted of IT. Performance was measured with a preloaded 8-km all-out run on the treadmill and 16-km all-out runs outdoors. Substrate utilization was measured using indirect calorimetry and continuous [U-\(^{13}\)C]glucose infusion during 30 min of running at 58 and 77% V\(_{\text{O}_2}\)\(_{\text{max}}\). Time to complete 8 km was negatively affected by the IT: time significantly increased by 61 ± 23 and 155 ± 38 s in the HCHO and Con trials, respectively. The 16-km times were significantly increased (by 8.2 ± 2.1%) during the Con trial only. The Daily Analysis of Life Demands of Athletes questionnaire showed significant deterioration in mood states in both trials, whereas deterioration in global mood scores, as assessed with the Profile of Mood States, was more pronounced in the Con trial. Scores for fatigue were significantly higher in the Con compared with the HCHO trial. CHO oxidation decreased significantly from 1.7 ± 0.2 to 1.2 ± 0.2 g/min over the course of the Con trial, which was completely accounted for by a decrease in muscle glycogen oxidation. These findings indicate that an increase in dietary CHO content from 5.4 to 8.5 g CHO·kg\(^{-1}\)·day\(^{-1}\) (41 vs. 65% total energy intake, respectively) allowed better maintenance of physical performance and mood state over the course of training, thereby reducing the symptoms of overtraining.

Overload training, i.e., creating a level of exercise stress that is slightly greater than those previously encountered by the athlete, is necessary to induce performance improvements and is therefore incorporated into most training programs (11). With inadequate recovery from this overload training, athletes will start to show symptoms of short-term overtraining or overreaching (10). The main symptoms associated with overreaching are a decreased performance, high fatigue rating at rest and during exercise, and altered mood state, but a large number of additional symptoms have been reported (for reviews, see Refs. 10, 45). In previous studies (13, 20), subjects have been classified as overreached when the following criteria were met: 1) a reduction in performance in laboratory tests and 2) increased affirmative responses to questionnaires that assessed impaired general health status, negative mood, psychological status, and feelings of fatigue. In a series of studies over a 10-year period, Morgan et al. (30) determined that mood state disturbances increased in a dose-response manner as training stimuli increased. It was concluded that monitoring mood state was a method of quantifying distress during periods of intense training (30). One of the proposed mechanisms for the development of overreaching is a disturbance in the hypothalamic-pituitary axis. This disturbance might be responsible for a large number of the symptoms associated with overreaching, including altered mood state and decreased performance (9). It is generally believed that sustained overreaching especially in combination with other stressors can eventually result in the development of an overtraining syndrome, a complex clinical condition with a variety of symptoms and pathophysiological abnormalities.

Dietary carbohydrate (CHO) and muscle glycogen may play an important role in the development of overreaching. In a study performed in 1971 by Costill et al. (4), five moderately-trained runners ran 16.1 km at ∼80% maximal O\(_2\) uptake (V\(_{\text{O}_2}\)\(_{\text{max}}\)) on 3 consecutive days. During the study period, the subjects consumed a diet containing 40–60% CHO. Subjects were unable to completely resynthesize the glycogen that was utilized during exercise on any of the days, and preexercise muscle glycogen concentration was reduced by 50% by the third day. This suggests that consecutive days of intense running while consuming a diet containing a moderate amount of CHO can severely diminish muscle glycogen stores. In a later study, Costill et al. (5) found that, during a period of intensified training in well-trained swimmers, athletes consuming large amounts of CHO seemed to be able to perform the prescribed intense training more easily than athletes consuming only moderate amounts of CHO. In addition, changes in mood state were more frequently reported in the moderate-CHO group than in the high-CHO group (5). Thus low muscle glycogen concentrations due to consecutive days of intensive exercise training are likely to be associated with fatigue and decreased performance. It is therefore likely that muscle glycogen depletion is one of the causes or at least a contributor to overreaching (39).

Already in the 1970s, it was shown that muscle glycogen stores could be manipulated by dietary interventions. Berg-
strom and Hultman (1) showed that reduced training and increased dietary CHO could increase the amount of glycogen to very high levels. However, few studies have investigated the effects of different dietary strategies on glycogen availability during periods of daily training. Simonsen and colleagues (38) studied 22 male and female rowers who were randomly divided into a high-CHO group receiving 10 g CHO·kg⁻¹·day⁻¹ and a moderate-CHO group receiving 5 g CHO·kg⁻¹·day⁻¹. All subjects were monitored for 4 wk during which they trained twice daily 6 days per week. After 4 wk, the subjects in the high-CHO and moderate-CHO groups had 65 and 8% higher muscle glycogen levels, respectively, compared with the start of the experiment. Sherman et al. (37) compared the same diets in runners and cyclists during a week-long training period. In this study, no changes in muscle glycogen content were found in the high-CHO group, whereas significant decreases of 25–35% were found in the athletes consuming the moderate-CHO diet.

Only one study has investigated the effects of glycogen availability during periods of intensified training. Kirwan et al. (23) measured glycogen content in the gastrocnemius muscle of 10 male distance runners before and after they performed 5 days of intensified training consuming either 8 or 4 g CHO·kg⁻¹·day⁻¹. The muscle glycogen content decreased significantly on both diets (35 and 55% for the 8- and 4-g groups, respectively). Snyder and colleagues (40) investigated the effect of providing additional CHO to an athlete’s diet during a period of intensified training on symptoms of overreaching. It was found that even with additional CHO feedings all subjects became overreached, suggesting that muscle glycogen content does not play a role in the development of overreaching. Unfortunately, no control group was used in this study, and neither was the diet accurately controlled, so it is difficult to draw firm conclusions about the effect of dietary CHO on markers of overreaching. As far as we are aware, no well-controlled studies have systematically examined the effect of dietary CHO on a combination of physical performance and markers of overreaching in well-trained subjects. Furthermore, it is important that the dietary manipulations are within a range that athletes could reasonably be expected to adopt on a long-term basis.

Therefore the purpose of this study was to determine whether subjects who consume a diet containing ~8.5 g CHO·kg⁻¹·day⁻¹ during a period of intensified training are better able to maintain physical performance and mood state (i.e., show fewer symptoms of overreaching) than subjects consuming a diet containing ~5.5 g CHO·kg⁻¹·day⁻¹ [65 vs. 41% of total energy intake (E), respectively].

METHODS

Subjects

Sixteen male endurance-trained runners were recruited for this study, which was approved by the South Birmingham Local Ethics Committee. The subjects had to meet the following inclusion criteria: they had to have run >50 km/wk for the previous 2 mo, have at least 5 yr of running experience, and have a personal best for 10 km below 40 min. Seven of the 16 subjects were able to complete both 11-day trials fully. Before participation, the health of the subjects was assessed by a General Health Questionnaire. Each volunteer gave written, informed consent to participate after explanations of the experimental procedures and possible risks and benefits. The characteristics of the subjects were as follows: age 28.7 ± 2.6 yr, body mass 73.4 ± 2.4 kg, body fat percentage 13.2 ± 2.3%, and \( \text{Vo}_2\max \) 64.7 ± 2.6 ml·kg⁻¹·min⁻¹.

General Design

Each subject undertook two 11-day trials in a randomized cross-over design separated by a 10-day washout period (Fig. 1). During each trial, subjects received a diet containing either ~65% E as CHO or ~40% E CHO. Subjects were tested in the laboratory on days 1, 5, 8, and 11 of both trial periods. Laboratory tests consisted of 1 h steady-state running at two different speeds, followed by a self-paced all-out 8-km run. On days 2–4, subjects trained for 1 h at an intensity eliciting 75% maximal heart rate (HR\(_\text{max} \)). On days 6, 7, 9, and 10, subjects were asked to run a 16-km outdoor course as fast as possible.

Pretrial Measurements

Graded exercise test. Approximately 3–4 days before the start of both experimental trial periods, subjects were asked to visit the laboratory for a graded exercise test to exhaustion. Subjects reported to the laboratory after a 10-h overnight fast and body mass and height were determined. Body fat was estimated from skinfold thickness measurements at four sites according to the methods of Durnin and Womersley (7). A Teflon catheter (Quickcath, Baxter, Norfolk, UK) was introduced into an antecubital arm vein and connected to a Lectrocathe (Vygon, Cirencester, UK) with a three-way stopcock (Sims Portex, Kent, UK). The catheter was maintained patent with isotonic saline (0.9%, Baxter). Subjects were then asked to mount the treadmill, and a resting blood sample (~1 ml) was collected. The test was started at 2.78 m/s at a gradient of 1%. Speed was increased by 0.56 m/s every 2.5 min until exhaustion. At the end of each exercise intensity, a small blood sample was collected for plasma lactate analysis. Heart rate was recorded continuously during the test by use of radio telemetry (Polar Vantage, Polar Electro Oy, Kempele, Finland). Breath-by-breath measurements were performed throughout exercise by use of an online gas analysis system (Oxycon Pro, Jaeger, Wuerzburg, Germany). The volume and gas analyzers of the system were calibrated using a 3-liter calibration pump and calibration gas (15.12% \( \text{O}_2 \); 5.10% \( \text{CO}_2 \)), respectively. Oxygen uptake (\( \text{Vo}_2 \)) was
considered to be maximal when at least two of the following three criteria were met: 1) a leveling off of \( \dot{V}O_2 \) with increasing speed (increase of no more than 2 m\( \text{kg}^{-1}\cdot\text{min}^{-1} \)), 2) a heart rate within 10 beats/min of the predicted maximum (220 beats/min minus age), and 3) a respiratory exchange ratio \( >1.05 \). \( \dot{V}O_2\text{max} \) was calculated as the average \( \dot{V}O_2 \) over the last 60 s of the test. The results of the first \( \dot{V}O_2\text{max} \) test were used to calculate the heart rate zone for the training runs on days 2, 3, and 4 and the speeds for the sub maximal treadmill run on days 5, 6, 8, and 11. The results of the second \( \dot{V}O_2\text{max} \) test were used to check whether the subject’s fitness level was equal before both trials.

Dietary analysis. After the graded exercise test to exhaustion, subjects kept a 3-day weighed-food record. They were asked to report accurately what they consumed during 2 weekdays and 1 weekend day. To increase the accuracy of the food analysis, subjects were carefully instructed and given electronic scales to weigh all food products they consumed. Diets were analyzed by use of nutrition analysis software (Compeat 5.2, Nutrition Systems, Carlson-Bengston Consultants). The habitual energy intake of the subjects was \( 37.7 \pm 3.0 \text{kcal} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) and the diet consisted of CHO \( 58.6 \pm 3.6\% \) E, fat \( 22.0 \pm 2.1\% \) E, protein \( 14.2 \pm 0.8\% \) E, and alcohol \( 4.5 \pm 2.6\% \) E.

Experimental Trial Periods

Diet. During the experimental trial periods, subjects consumed two diets with differing nutrient composition. The order of the diets was counterbalanced. All foods were provided to the subjects. For each diet period, three menus were composed, which were given in a three-day cyclic order. Food and drinks were consumed in three meals and two snacks, and all meals and snacks were of the same nutrient composition. Providing food in identical meals has been found to be crucial for obtaining accurate and reliable measures of CHO and fat composition. The order of the diets was passed through a filter that eliminates undesirable premature beats and noise. After filtering, the interval is referred to as N-N interval, in which N stands for normal. The details of the filtering technique have been described previously (43). The time-domain analyses were performed at rest from 2–14 min. The following variables were calculated: the mean of the N-N intervals, the standard deviation of the N-N intervals, the root mean square successive difference of all N-N intervals, and the percentage of intervals that differed >50 ms from the previous interval. The orthostatic test (transition from supine to upright position) was used to determine baroreceptor sensitivity.

Heart rate variability measurements. On the morning of days 1 and 11 of both trial periods, heart rate variability (HRV) was measured before the start of the laboratory test. The time between R-peaks of subsequent heart beats (R-R intervals) were recorded (R-R recorder, Polar Electro Oy) during spontaneous breathing for 15 min while subjects were lying down. Thereafter, recordings proceeded for 5 min while subjects were standing quietly. The R-R interval series was passed through a filter that eliminates undesirable premature beats and noise. After filtering, the interval is referred to as N-N interval, in which N stands for normal. The details of the filtering technique have been described previously (43). The time-domain analyses were performed at rest from 2–14 min. The following variables were calculated: the mean of the N-N intervals, the standard deviation of the N-N intervals, the root mean square successive difference of all N-N intervals, and the percentage of intervals that differed >50 ms from the previous interval. The orthostatic test (transition from supine to upright position) was used to determine baroreceptor sensitivity.

Training diary and questionnaires. All subjects were provided with a training diary. The subjects were asked to provide information on their sleeping patterns (hours and quality of sleep) and the exercise they performed, on a daily basis. Furthermore, they were asked to fill out the Daily Analysis of Life Demands of Athletes questionnaire (DALDA) (36), short Profile of Mood States questionnaire (Short POMS) (12, 28) and muscle soreness chart (42). The DALDA questionnaire is divided into parts A and B, which represent the sources of stress and the manifestation of this stress in the form of symptoms, respectively. For both parts, the number of items that were marked as “worse than normal” was reported. The questions of the POMS were divided into three categories: fatigue, vigor, and tension. Global mood score of the POMS was calculated as the scores for fatigue plus tension, minus the score for vigor, plus 100. On days 1 and 11 of each trial period, subjects were provided with the 65-item version of the POMS questionnaire (POMS-65) (28). The questions of the POMS were divided into six categories: tension, depression, anger, vigor, fatigue, and confusion. Global mood score of the POMS-65 was calculated as the sum of all negative categories minus the score for vigor, plus 100. Subjects were asked to fill out the questionnaires at the same time of day.
Analysis

All blood samples were collected into prechilled test tubes containing 20 μl of 0.2 M EDTA/ml blood. The samples were spun immediately for 10 min at 3,000 rpm at 4°C. Aliquots of the samples were frozen in liquid nitrogen and stored at −70°C until further analysis. Plasma samples collected at all time points were enzymatically analyzed for glucose (Infinity glucose, Sigma), lactate (Lactate reagent, Sigma), free fatty acids (NEFA-C kit, Wako Chemicals), and glycerol (Triglycerides kit, Sigma) concentrations by using a semiautomatic analyzer (Cobas-Bio, Roche). The volume of urine collected during the treadmill exercise was measured, and a 10-ml sample was frozen at −70°C until analysis. The urine samples were analyzed for urinary nitrogen concentration by use of the modified micro-Kjeldahl method, consisting of digestion, distillation, and titration of the samples.

For determination of plasma glucose enrichment, plasma samples were deproteinized and derivatized with butyl boronic acid (34). Briefly, 250 μl of plasma were deproteinized with 2 ml of methanol-chloroform (2:3:1), 2 ml of chloroform, and water with pH 2.0. The samples were then derivatized with 250 μl of a butyl boronic acid-pyridine mixture (1 mg/10 ml) and 250 μl of acetic anhydride. Thereafter the enrichment of the derivative was measured by gas chromatograph mass spectrometer (GC/MS) by injecting 1 μl of the derivative on an Agilent 6890N gas chromatograph equipped with a split-splitless injector and 7683 autosampler (Agilent Technologies, Stockport, UK). Mass spectrometric detection was obtained with an Agilent 5973N mass-selective detector. Data were acquired by using selected ion monitoring for masses mass-to-charge ratio 297 and 303.

Breath samples were analyzed for 13 C-to- 12 C ratio by continuous-flow elemental analyzer isotope ratio mass spectrometry (Europe Scientific, Crewe, UK). From indirect calorimetry and stable isotope measurements (13 CO2-to-12 CO2 ratio), total energy expenditure and oxidation rates of total fat, total CHO, plasma glucose, and muscle glycogen were calculated.

Calculations

From VO2, CO2 production (VCO2), and grams of excreted urinary nitrogen (n), CHO and fat oxidation rates were calculated by using the following equations (8)

\[
\text{CHO oxidation} = 4.55 \times V_{\text{CO2}} - 3.21 \times V_O2 - 2.87 \times n
\]

\[
\text{Fat oxidation} = 1.67 \times V_O2 - 1.67 \times V_{\text{CO2}} - 1.92 \times n
\]

with VO2 and VCO2 in liters per minute and oxidation rate in grams per minute.

Plasma glucose oxidation was calculated according to the formula

\[
\text{Plasma glucose oxidation} = \frac{(E_{\text{br}} - E_{\text{br}_{\text{basal}}}) \times 1}{k} \times \frac{1}{(E_{\text{pl}} - E_{\text{pl}_{\text{basal}}})}
\]

in which Ebrbas is the 13 C enrichment of expired air during exercise at rest before tracer infusion, Eplbas is the 13 C enrichment of plasma glucose during exercise at different time points, Ebr is the 13 C enrichment of plasma glucose at rest before tracer infusion, and k is the conversion factor [amount of CO2 (in liters) produced by the oxidation of 1 g of glucose; k = 0.7467 liter CO2/g glucose].

The rate of appearance (Ra) and rate of disappearance (Rd) of glucose were calculated by using single-pool non-steady-state Steele equations (41), as described elsewhere (47)

\[
R_a = \frac{F - V[(G_{pl} + G_{pl_{basal}})/2][(E_{pl} - E_{pl_{basal}})/(t_2 - t_1)]}{(E_{pl} + E_{pl_{basal}})/2}
\]

\[
R_d = \frac{(G_{pl} - G_{pl_{basal}})}{(t_2 - t_1)}
\]

in which F is the infusion rate (in μmol·kg⁻¹·min⁻¹), V is the plasma volume of distribution = 160 ml/kg, Gpl and Gplbasal are the glucose concentrations at times 2 and 1 (i.e., t2 and t1), respectively, and Epl and Eplbasal are the plasma glucose enrichments at times 2 and 1, respectively.

Muscle glycogen oxidation (in g/min) was calculated as

\[
\text{Muscle glycogen oxidation} = \text{total CHO oxidation} - \text{plasma glucose oxidation}
\]

Statistical Analyses

Experimental data were presented as means ± SE. Two-way analysis of variance for repeated measures was used to identify differences between the HCHO and Con trials in variables measured over time (i.e., time to complete 8- and 16-km runs, heart rate during steady-state runs, etc.). In case of significant interactions, analysis of variance for repeated measures was repeated for the HCHO and Con trial separately, followed by Student’s t-tests with a Bonferroni correction between individual days to detect differences within each trial over time. Nutrient components of the two diets, global mood scores of the POMS, and substrates used on the last day of the two trials were compared by using a Student’s t-test. For all statistical analyses, significance was accepted at P < 0.05.

RESULTS

Exercise and Diet Regime

No differences were observed in energy intake between the two diet periods (HCHO: 219 ± 4 kJ/kg vs. Con: 225 ± 4 kJ/kg). As intended, CHO intake was significantly different between the trials (HCHO: 8.45 ± 0.17 g·kg⁻¹·day⁻¹; Con: 5.41 ± 0.09 g·kg⁻¹·day⁻¹). CHO, fat, and protein contributed 65.0 ± 1.0, 21.9 ± 0.4, and 13.1 ± 0.4%, respectively, to energy intake during the HCHO trial. During the Con trial, the different nutrients provided 40.6 ± 0.1, 43.6 ± 0.4, and 15.6 ± 0.4%, respectively. Subjects remained weight stable during both diet periods. Subjects ran for a total of 830.1 ± 12.6 min with an average heart rate of 150.6 ± 3.5 beats/min during the 11 days of the HCHO trial. Both exercise time and average heart rate were of the same magnitude during the Con trial (830.1 ± 12.5 min, 150.4 ± 3.7 beats/min).

Subjects in both trials decreased their performance significantly and showed significant alterations in mood state (see below); they were therefore classified as overreached at the end of both the HCHO and the Con trial.

Performance

Performance during the 8-km time trial significantly decreased over the course of both the HCHO and Con trials. From H1 (day 1 of the HCHO trial) to H11 (day 11 of the HCHO trial), average time to complete the 8-km self-paced run increased by 61 ± 23 s (P < 0.05). In contrast, the increase from day 1 to day 11 of the Con trial (C1 to C11) was 155 ± 38 s (P < 0.05). In Fig. 2, running speeds on days 1, 5, 8, and 11 are displayed. Running speed decreased significantly from day 1 to day 11 during the HCHO trial (3.2 ± 1.2% difference; P < 0.05). During the Con trial, speed decreased from 4.15 ± 0.11 to 3.84 ± 0.10 m/s (a 8.2 ± 2.1% difference; P < 0.05). Running speed on day 11 was significantly lower during the Con trial compared with the HCHO trial (P < 0.05). Average %HRmax during the 8-km runs decreased significantly from 92 ± 4% on H1 to 84.9 ± 1.4% HRmax on H11 (P < 0.05). A
similar decrease was seen on the first and last day of the Con trial (92.1 ± 0.6% to 84.1 ± 0.7% $\text{HR}_{\text{max}}$; $P < 0.05$). Time to complete the 16-km runs on the road was increased only in the Con trial ($67.53 \pm 2.40 \text{ min}$ on day 6 to $71.04 \pm 2.85 \text{ min}$ on day 10; $P < 0.05$). In Fig. 3, running speeds on days 6, 7, 9, and 10 are presented. When subjects consumed the HCHO diet, no statistically significant changes were seen in average running speed. During the Con trial, on the other hand, running speed was significantly lower on day 10 ($P < 0.05$) compared with day 6. When running speed was compared between diets, significant differences were found on days 9 (HCHO: $67.98 \pm 3.18$ vs. Con: $71.04 \pm 2.85$ min; $P < 0.05$) and 10 (HCHO: $67.21 \pm 2.75$ vs. Con: $70.64 \pm 2.77$ min; $P < 0.05$).

**Muscle Soreness, Global Mood State, Resting Heart Rate, and HRV**

General muscle soreness scores were $1.8 \pm 0.5$ at the start of the HCHO trial and did not increase significantly over the 11 days of the trial. During the Con trial, subjects started with a muscle soreness rating of 0.7 ± 0.4 that increased to 4.2 ± 1.2 on day 10 ($P < 0.05$), and 4.9 ± 1.2 by day 11 ($P < 0.05$). Muscle soreness scores were not different between trials at any time point during the two trials.

In Fig. 4, the total number of “worse-than-normal” scores of part B of the DALDA questionnaire are presented for both trials. In both trials, the scores increased significantly over the course of the 11 days. No significant differences in the scores were detected between the trials. The results of the short POMS were used to calculate a global mood state during each day of the trials. Whereas the global mood state score was significantly lower during the Con trial compared with the HCHO trial on some days, the average global mood state was not significantly different between the two diets. The global mood state determined by the POMS-65 is displayed in Fig. 5, A and B, and demonstrated a significant increase from 107 ± 7 on C1 to 126 ± 7 on C11 ($P < 0.05$). No change in the global mood score was observed during the HCHO trial. When we look at the subscales of the POMS-65 in Fig. 5, vigor is significantly decreased after 11 days in both trials ($P < 0.05$). Scores for fatigue are markedly increased after 11 days of Con ($P < 0.05$), whereas no changes were seen after 11 days of HCHO ($P < 0.05$ from Con). On days 1 and 11 of both trials, resting heart rate was measured after the subject had rested for 10 min in a quiet room. Resting heart rate was 50 ± 4 beats/min at the start of both trials, and it changed to 45 ± 4 and 47 ± 4 beats/min in the HCHO trial ($P < 0.05$) and Con trial ($P < 0.05$), respectively. There was no significant time × diet interaction, but there was a significant time effect on resting heart rate ($P < 0.05$). Standard deviation of NN intervals was 117 ± 22 and 119 ± 21 ms at the start of the HCHO and Con trial, and remained the same during both trials (127 ± 10 and 110 ± 25 ms for HCHO and Con, respectively). Also, no changes were found in root mean square successive difference or percentage of intervals that differed >50 ms from the previous interval in either group. Subjects did not experience any sleeping problems because the number of hours slept and the quality of sleep did not change over the training period.

**Submaximal Exercise**

Heart rate, plasma lactate concentration, plasma catecholamine concentrations, and RPE. Average heart rate during low-intensity exercise did not change over the 11 days of intensive training. A significant time × diet interaction was seen for heart rate during moderate-intensity exercise. Heart rate decreased significantly from H1 to H11 (from 160 ± 5 to 152 ± 3 beats/min; $P < 0.05$), but no significant reduction in heart rate was seen from C1 to C11 (158 ± 5 and 154 ± 4 beats/min). RPEs during the first part of the submaximal test
were similar from the start to end of both trials. During the second part, RPE was significantly higher on day 11 ($P < 0.05$) compared with day 1 of the Con trial. Furthermore, RPE on C11 was significantly higher than on H11 ($P < 0.05$). The plasma lactate concentration at rest did not change markedly over the 11 days of both trials. There was no time × diet effect for lactate concentration at either intensity. Lactate did decrease significantly from C1 (1.01 ± 0.12 mmol/l) to C8 (0.69 ± 0.03 mmol/l; $P < 0.05$) and C11 (0.73 ± 0.03 mmol/l; $P < 0.05$) during 30 min of running at 58% $V_{\text{O}_2\text{max}}$

Plasma norepinephrine and epinephrine concentrations at rest, after 30 and 60 min of steady-state exercise, and on completion of the self-paced time trial are displayed in Fig. 6, A and B, respectively. No differences in norepinephrine and epinephrine concentrations were detected between the trials. Both increased significantly over time.

Substrate utilization. During 30 min of exercise at 58% $V_{\text{O}_2\text{max}}$, no changes occurred in the plasma glucose concentration. During the following 30 min at 77% $V_{\text{O}_2\text{max}}$, a small but nonsignificant increase was observed. On day 1 of the HCHO trial and Con trial, the plasma glucose concentration increased significantly above the concentration measured after 60 min of steady-state exercise (HCHO: 5.24 ± 0.20 mmol/l; Con: 5.04 ± 0.20 mmol/l) at the end of the 8-km time trial (HCHO: 6.78 ± 0.36 mmol/l; Con: 6.38 ± 0.40 mmol/l). On day 11 of both trials, no increase in plasma glucose was observed during the 8-km time trial.

In Table 1 the average protein, fat, and CHO oxidation over the low-intensity and moderate-intensity steady-state runs are presented. Protein oxidation rates did not differ between the

Fig. 6. Plasma norepinephrine concentration (A) and plasma epinephrine concentration (B) during laboratory test on day 1 and day 11 of HCHO and Con trials. Values are means ± SE. 0–30 min, running at 58% $V_{\text{O}_2\text{max}}$; 30–60 min, running at 77% $V_{\text{O}_2\text{max}}$; 60 min–end, 8-km self-paced run.
trials. On C11, CHO oxidation was significantly lower compared with C1 and H11 during the exercise bout at 58% \( V_{\text{O}2\text{ max}} \). Concomitantly, fat oxidation rates on C11 were significantly higher than on C1 and H11. No changes in substrate utilization were found over the course of the HCHO trial.

In Table 2, glucose kinetics during the steady-state runs on day 11 of both trials are presented. This table contains data from the five subjects who received the labeled glucose infusion. There was a trend for total CHO oxidation to be higher in the HCHO compared with the Con trial (Table 1), but this did not reach statistical significance. Plasma glucose oxidation represented 25 \( \pm \) 5% (at 58% \( V_{\text{O}2\text{ max}} \)) and 23 \( \pm \) 4% (at 77% \( V_{\text{O}2\text{ max}} \)) of total CHO oxidation during the HCHO trial, compared with 29 \( \pm \) 3 and 32 \( \pm \) 7% during the Con trial, respectively. Plasma glucose oxidation was not significantly different between any of the trials. Muscle glycogen oxidation (calculated as total CHO oxidation – plasma glucose oxidation), on the other hand, showed a clear trend to be higher in the HCHO trial compared with the Con trial at both intensities (58% \( V_{\text{O}2\text{ max}} \): 96 \( \pm \) 27 vs. 64 \( \pm \) 10 glycosyl units\( \cdot \)kg\(^{-1}\)\(\cdot\)min\(^{-1}\)), 77% \( V_{\text{O}2\text{ max}} \): 137 \( \pm \) 33 vs. 92 \( \pm \) 22 glycosyl units\( \cdot \)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) for the HCHO and Con trials, respectively). The \( R_g \) glucose was 29.1 \( \pm \) 2.0 \( \mu \)mol\( \cdot \)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) and 29.9 \( \pm \) 2.7 \( \mu \)mol\( \cdot \)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) during the first 30 min of the submaximal run on H11 and C11, respectively. This rate was 33.7 \( \pm \) 2.4 and 36.0 \( \pm \) 2.8 \( \mu \)mol\( \cdot \)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) when the intensity increased to 77% \( V_{\text{O}2\text{ max}} \). No significant differences in \( R_g \) glucose were detected between the Con and HCHO trials. \( R_d \) glucose was similar to \( R_g \) glucose during both intensities in the Con and HCHO trials. During the first 30 min of exercise, \( \sim 85\% - 90\% \) of the glucose disappearing from the circulating was oxidized. During the second half hour, 95–98% glucose taken up by the muscle was oxidized.

**Table 1. CHO, fat, and protein oxidation rates during 30 min running at 58% and 77% \( V_{\text{O}2\text{ max}} \) on days 1, 5, 8, and 11 of HCHO and CON trial**

<table>
<thead>
<tr>
<th></th>
<th>( 58% ) ( V_{\text{O}2\text{ max}} )</th>
<th>( 77% ) ( V_{\text{O}2\text{ max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHO oxidation, g/min</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>1.63 ( \pm ) 0.24</td>
<td>1.61 ( \pm ) 0.22</td>
</tr>
<tr>
<td>CON</td>
<td>1.69 ( \pm ) 0.20</td>
<td>1.50 ( \pm ) 0.21</td>
</tr>
<tr>
<td><strong>Fat oxidation, g/min</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>2.21 ( \pm ) 0.21</td>
<td>2.11 ( \pm ) 0.25</td>
</tr>
<tr>
<td>CON</td>
<td>2.14 ( \pm ) 0.24</td>
<td>1.95 ( \pm ) 0.23</td>
</tr>
<tr>
<td><strong>Protein oxidation, g/min</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.06 ( \pm ) 0.01</td>
<td>0.05 ( \pm ) 0.02</td>
</tr>
<tr>
<td>CON</td>
<td>0.08 ( \pm ) 0.04</td>
<td>0.12 ( \pm ) 0.04</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 7 \). CON, control; \( V_{\text{O}2\text{ max}} \), maximal \( \text{O}_2 \) uptake. *Significantly different from day 1; †Significantly different from high-carbohydrate diet (HCHO).

**Table 2. Glucose kinetics during 30 min running at 58% \( V_{\text{O}2\text{ max}} \) and 30 min at 77% \( V_{\text{O}2\text{ max}} \) during day 11 of HCHO and CON trial**

<table>
<thead>
<tr>
<th></th>
<th>( 58% ) ( V_{\text{O}2\text{ max}} )</th>
<th>( 77% ) ( V_{\text{O}2\text{ max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>( R_g ) glucose, ( \mu )mol( \cdot )kg(^{-1})(\cdot)min(^{-1})</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>29 ( \pm ) 2</td>
<td>30 ( \pm ) 3</td>
</tr>
<tr>
<td>CON</td>
<td>29 ( \pm ) 2</td>
<td>30 ( \pm ) 3</td>
</tr>
<tr>
<td><strong>( R_d ) glucose, ( \mu )mol( \cdot )kg(^{-1})(\cdot)min(^{-1})</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>26 ( \pm ) 4</td>
<td>25 ( \pm ) 2</td>
</tr>
<tr>
<td>CON</td>
<td>26 ( \pm ) 4</td>
<td>25 ( \pm ) 2</td>
</tr>
<tr>
<td><strong>Plasma glucose oxidation, ( \mu )mol( \cdot )kg(^{-1})(\cdot)min(^{-1})</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>96 ( \pm ) 27</td>
<td>64 ( \pm ) 10</td>
</tr>
<tr>
<td>CON</td>
<td>96 ( \pm ) 27</td>
<td>64 ( \pm ) 10</td>
</tr>
<tr>
<td><strong>Muscle glycogen oxidation, glycosyl units( \cdot )kg(^{-1})(\cdot)min(^{-1})</strong></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HCHO</td>
<td>122 ( \pm ) 28</td>
<td>89 ( \pm ) 10</td>
</tr>
<tr>
<td>CON</td>
<td>122 ( \pm ) 28</td>
<td>89 ( \pm ) 10</td>
</tr>
<tr>
<td>%( R_g ) oxidized</td>
<td>90 ( \pm ) 10</td>
<td>85 ( \pm ) 3</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 5 \). \( R_g \) and \( R_d \) rates of appearance and disappearance.

**DISCUSSION**

The main aim of the present study was to determine whether consumption of a diet containing \( \sim 8.5 \) g \( \text{CHO} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) (\( \sim 65\% \) E) compared with \( \sim 5.5 \) g \( \text{CHO} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) (\( \sim 40\% \) E) would result in better maintenance of physical performance and mood state over the course of a period of intensified training, thereby reducing the symptoms of overreaching. It was found that, although overreaching was not prevented, the higher CHO intake, reduced the symptoms of overreaching.

Subjects in the present study had significantly increased times to complete a preloaded self-paced 8-km run in the laboratory after 11 days of intensified training. However, even though average speed over the course of the HCHO trial decreased significantly, the difference between the first and last day was small compared with changes seen in the Con trial. During the 16-km runs outdoors, the differences between the diets were even more profound. In addition to the decreased running performance, a change in mood state was seen in the...
subjects during the 11-day trials, with most changes more marked in the Con trial. The worse-than-normal scores on part B of the DALDA questionnaire increased significantly in both trials. Global mood state decreased significantly in the Con trial whereas no change was seen in the HCHO trial. Furthermore, it was found that the Con trial resulted in significantly increased scores for fatigue. Differences in fatigue were observed between diets not only at rest, but also during exercise. Scores for vigor decreased significantly in both trials. RPEs during the moderate-intensity run were significantly higher during the Con compared with the HCHO trial.

Similar observations have been reported by Costill et al. (5), who studied 12 collegiate male swimmers during a 10-day period of intensified training. The subjects were allowed to consume food ad libitum during the intensified period. Subjects who consumed fewest calories and grams of CHO had highest ratings of perceived training effort. These subjects also showed changes in mood state over the training period (31). The results of this study suggest a role for CHO in the prevention of overreaching.

In the present study, it was shown that, during the Con trial, CHO oxidation decreased and fat oxidation increased. During the HCHO trial, CHO and fat oxidation rates remained stable, suggesting that muscle glycogen was completely restored between exercise bouts. Total CHO oxidation was lower on C11 compared with H11. In a subgroup of five subjects, plasma glucose oxidation was similar during H11 and C11, indicating that the observed differences in CHO oxidation are most likely completely accounted for by differences in muscle glycogenolysis between H11 and C11. A ~33% difference in muscle glycogen oxidation between the HCHO and Con trials was observed during exercise; this difference, however, just failed to reach significance ($P = 0.093$ and $P = 0.053$ for the 58% $\dot{V}O_2_{\text{max}}$ and 77% $\dot{V}O_2_{\text{max}}$, respectively). The progressive decrease in plasma lactate concentration during the Con trial also indicates that glycogenolysis decreased.

It is possible that the trend toward lower muscle glycogen oxidation in the Con trial compared with the HCHO trial might be due to differences in preexercise muscle glycogen concentration. It has been suggested that the rate of glycogenolysis is related to the amount of glycogen stored in the muscle. The majority of studies in rats and humans have reported an almost linear relationship between muscle glycogen content and the rate of glycogenolysis (14, 35). Few studies have investigated muscle glycogen concentration in subjects undergoing an intensified running training and dietary intervention comparable to the one used in the present study (23, 37). Sherman et al. (37) showed that, after 7 days of intensive running training, muscle glycogen concentration decreased by 25% when subjects were consuming a diet containing 5 g CHO·kg$^{-1}$·day$^{-1}$. The results of these studies suggest that the diet and exercise regime used in the present study substantially decreased the preexercise muscle glycogen concentration, leading to the observed trend toward reduction in glycogenolysis.

A reduced sensitivity of the muscle to catecholamines as a result of overreaching has been suggested as another possible cause for the decreased muscle glycogenolysis (27). It has been speculated that chronically elevated levels of catecholamines, which are expected during periods of very intensive training, will induce a loss of sensitivity to catecholamines (27). Jost et al. (22) found a reduced $\beta$-adrenoreceptor density in lymphocytes in distance runners and swimmers during prolonged high-volume training compared with tapering. As far as we are aware, no studies have directly measured tissue sensitivity to catecholamines after a period of intensified training resulting in overreaching, but there are, however, some indirect indications. Under normal conditions, catecholamine concentration and heart rate are closely related, with increases in heart rate seen as a response to increased catecholamine concentration. In the overreached state, this relationship is often disturbed; heart rate at submaximal- and maximal-intensity exercise is decreased, whereas catecholamine concentrations are normal (16, 44) or even raised (26). Over the course of the Con trial, no changes were seen in submaximal heart rates or catecholamine
concentrations. This finding is not entirely surprising, because it has been speculated that changes in tissue sensitivity to catecholamines are only to be expected after very prolonged intensified training programs (25). The data of the present study therefore do not support the hypothesis of reduced glycogenolysis as a result of reduced tissue sensitivity to catecholamines.

It has been suggested that a state of overtraining and possibly overreaching is accompanied by changes in the autonomic nervous system (19). Because HRV can be used as an indicator of the autonomic nervous system, it has been brought forward as a possible marker for overreaching. In the present study, time-domain variables of HRV were determined before and after the 11-day trial periods. No changes were detected in any of the variables during both dietary periods. Very few studies have studied HRV after a period of intensified training, and only one study (46) reported time-domain variables. Uusitalo et al. (46) measured HRV in 15 female endurance-trained athletes before and after they performed either normal or intensified training. Similar to the results in the present study, no changes were found in any of the time-domain variables in either group. Further research is warranted to determine whether HRV can be used as an early marker of overreaching.

The present study is the first study to systematically determine the fate of plasma glucose after it disappears from the circulation using a single glucose tracer. In a recent study by Jeukendrup et al. (21), two glucose tracers were used to determine the fate of plasma glucose. After an overnight fast, six trained athletes cycled for 2 h at 50% \( \dot{V}_{\text{O}_2}\text{max} \) while receiving a simultaneous continuous [6,6-\( ^2 \)H\(_2\)]glucose and [U-\( ^{13} \)C]glucose infusion. The [6,6-\( ^2 \)H\(_2\)]glucose tracer was used to determine \( R_d \) glucose, whereas the [U-\( ^{13} \)C]glucose tracer was used to calculate plasma glucose oxidation. It was found that during the second hour of exercise 99 ± 3% of the glucose taken up by the muscle was oxidized. In the present study, a [U-\( ^{13} \)C]glucose tracer was infused at relatively high rates, allowing for simultaneous determination of glucose \( R_b \) by GC/MS and plasma glucose oxidation by GC/MS and elemental analyzer-isotope ratio mass spectrometer. During the last 10 min of exercise at 58% \( \dot{V}_{\text{O}_2}\text{max} \), 90 ± 10 and 85 ± 3% of the \( R_d \) glucose were oxidized in the HCHO and Con trial, respectively. These percentages are slightly lower than those found by Jeukendrup et al. Various reasons can be given to explain the slightly different findings between Jeukendrup et al. and the present study, including the use of two tracers compared with one and the longer exercise duration. Furthermore, in the present study, glucose kinetics were measured during running exercise compared with cycling in the study by Jeukendrup et al. When the intensity in the present study was increased to 77% \( \dot{V}_{\text{O}_2}\text{max} \), the percentage of \( R_d \) oxidized increased to 98 ± 4 and 95 ± 3%, indicating that nearly all glucose taken up by the muscle was oxidized. In a pilot study using only one subject, Coggan and colleagues (3) were the first to use a U-\( ^{13} \)C-labeled glucose tracer to directly compare \( R_b \) and oxidation rates of glucose during exercise. When cycling at 70% \( \dot{V}_{\text{O}_2}\text{max} \), 93% of the \( R_b \) glucose was oxidized, which is similar to the values found in the present study. It has been suggested that at low intensities part of glucose might be used for muscle glycogen synthesis (24).

High-CHO, low-fat diets will in many cases induce a rise in plasma triglyceride concentration (for review, see Ref. 32). One of the possible causes of the rise in plasma triglycerides is an increase in lipogenesis. When the fat content of the diet is too low, fatty acid synthesis will be stimulated with a characteristic rise in triglycerides (17, 18). It has also been suggested that the increased concentration of triglycerides after consumption of a HCHO, low-fat diet is the result of decreased very low-density lipoprotein-triglyceride clearance (33). On the other hand, exercise training has been shown to decrease the concentration of plasma lipids (15). In accordance with these findings, plasma triglyceride concentration in the present study decreased significantly as a result of the intensified training period. However, as expected, the diet containing the highest amount of CHO (HCHO) was associated with the smallest decrease in plasma triglyceride concentration.

In summary, the present study indicates that consuming a diet containing \( \sim 8.5 \) g CHO\( \cdot \)kg\(^{-1}\)\( \cdot \)day\(^{-1} \) (\( \sim 65\% \) E) compared with \( \sim 5.5 \) g CHO\( \cdot \)kg\(^{-1}\)\( \cdot \)day\(^{-1} \) (\( \sim 40\% \) E) results in better maintenance of physical performance and mood state over the course of a period of intensified training, thereby reducing the symptoms of overreaching. During the Con trial subjects showed a larger decrement in running performance and their mood was altered to a greater extent compared with the HCHO trial. Muscle glycogenolysis showed a trend to be lower on the last day of the Con trial compared with the last day of the HCHO trial. This decrease in muscle glycogenolysis is most likely the result of decreased preexercise glycogen concentration. It can be concluded that dietary CHO and possibly muscle glycogen content play a role in the development of overreaching.

ACKNOWLEDGMENTS

We thank Chris Mann for medical supervision during the infusion trials. We also thank Jos Stegen (Maastricht University, The Netherlands) and Dr. Peter Emery (Kings College, London, UK) for analysis of plasma catecholamines and urinary nitrogen, respectively. Finally we thank Kelloggs UK for supplying us with Nutri-grain bars to supplement the subject’s diets.

GRANTS

This work was funded by the Centre for Human Sciences of the UK Ministry of Defence Scientific Research Programme.

REFERENCES


