Metabolic Changes Induced by Sustained Exhaustive Cycling and Diet Manipulation


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Abstract


The results showed that while consuming a normal diet, the cyclists developed a negative energy balance (–9 MJ·day⁻¹) and regulated their hormone levels in such a way that fat oxidation and protein breakdown were increased and CHO oxidation became depressed.

When supplemented with MF, the subjects showed increased blood glucose, insulin and decreased glucagon levels. Fat metabolism was significantly depressed as indicated by the levels of blood fatty acids, glycerol, and ketones. A significant glycogen sparing, as well as supercompensation within 24 h of recovery, was observed after MF supplementation. The normal CHO-rich diet, available ad libitum, was insufficient to fully restore glycogen within 24 h. The changes in substrate availability and glycogen depletion were accompanied by a significant performance improvement, 126% when cycling a final 90% Wmax bout, when supplemented with MF compared with N.

Key words

Nutrition, diet manipulation, exercise, hormones, substrate metabolism, muscle glycogen, muscle fat, performance, cycling

Introduction

The human body is able to adapt to extremely heavy work loads, and as a result of long-term adaptations, professional cyclists are able to increase their energy output to very high levels on a day-to-day basis. In one of the world’s most extreme cycling competitions, the Tour de France, lasting 23 days, it was shown that energy expenditure (EE) can exceed 32 MJ on a hot competition day in the mountains, while mean EE amounted to 24 MJ per day (109). The same study revealed that the cyclists were only capable of competing at such an extreme level by matching energy intake to energy expenditure. This was achieved mainly by intake of liquid carbohydrate- and energy-rich meals throughout the day in addition to the normal diet. Cyclists who were not able to maintain energy balance (EB) frequently experienced malperformance and sometimes were forced to quit prematurely. Such imbalance normally resulted from diarrhea or inadequate food intake caused by changes in food tolerance and appetite, due to the extremely fatiguing exercise. Furthermore, time for recovery is limited.
In contrast to this diet behavior of professional cyclists, nutritionists and physiologists generally believe that a normal well-balanced diet supplying 50–60 En% carbohydrate (CHO) and 0.8–1 g of protein kg⁻¹ day⁻¹ will be sufficient for any sportsman or woman in any situation. However, the question then arises how athletes can meet their daily energy and CHO needs, when both are increased to extreme levels.

Apart from problems due to the time available to eat and digest, the volume of the food, and the changes in appetite (14), one may question the possible interaction between changes in energy metabolism as a result of long-term training and the influence of changed eating habits or diet manipulation.

It is known that liver and muscle glycogen stores limit endurance performance capacity whenever exercise intensities increase above 60% VO₂max (8, 108, 112). As a result of long-term training, the body adapts toward enhanced fat metabolism, to minimize this limitation, leading to muscle glycogen sparing and delaying the onset of fatigue (55, 56, 92, 105, 114).

On the other hand, it has been discussed that maximal power output decreases with increasing contribution of fat in energy metabolism, such as after endogenous CHO depletion, or following a high-fat diet, both of which will enhance blood fatty acid concentration and utilization. As a result, when fat is the main substrate, power output will drop to 50% of maximal capacity (88), possibly as a result of a decreased maximal energy flow from fat compared with CHO (83). Studies from the early 1960s showed that glycogen stores were increased when CHO intake was increased drastically during the days preceding exercise and that this increase was associated with longer exercise times to exhaustion (8, 54, 108). The same holds for ingestion of substantial amounts of CHO during exercise, possibly delaying glycogen depletion and thereby improving performance (24, 35, 67).

However, it has also been shown that fat and CHO behave as competitive substrates when passing the muscle cell membrane for oxidation (89, 101, 104). Infusion of fat decreases CHO oxidation and enhances fat metabolism while CHO infusions cause the opposite effect (19, 39, 57). As a consequence, the quantity of CHO degraded may be larger than the quantity ingested when the latter is small. Intake at rest prior to exercise, enhancing insulin secretion, may also lead to enhanced rates of glycogen degradation and reduced times until exhaustion (45, 58). From this it may be postulated that the effectiveness of diet manipulation to a large extent depends on the quantity of nutrients involved and on the moment of intake.

Apart from this it has been shown that protein degradation during exercise is increased, whenever glycogen stores are depleted, to supply precursors for gluconeogenesis (1, 31, 77, 102) and that CHO intake and/or enhanced glycogen availability limits this protein degradation (77, 79, 86, 102).

The fact that protein degradation is also increased at rest whenever energy balance is negative for a prolonged period of time, such as during fasting, is also related to glycogen depletion and enhanced gluconeogenesis (44, 110). Taking into account these interrelationships, it can be hypothesized that performance capacity can be maintained at a higher level, and that protein degradation will be limited, whenever energy balance is maintained and CHO intake matches CHO degradation.

Since cyclists competing successfully in the Tour de France were able to maintain energy balance only by using liquid CHO-rich foods in addition to the normal diet (109) and no controlled studies in the field of ultraendurance sports supporting this practice are available, it was decided to design a cross-over study in which the nutritional and biochemical changes of cyclists ingesting a CHO-rich diet composed of conventional but CHO-rich food (treatment N) could be compared with those after diet manipulation, in which the same diet was supplemented with a substantial amount of CHO concentrated liquids (treatment MF). The study was performed in a controlled laboratory setup in which 2 heavy days with an energy expenditure comparable to that observed in the Tour de France (109) were simulated.

Materials, Procedures, and Methods

The complete experimental setup of the study has been described in a separate paper (15). Therefore, only the details of this section specific to the metabolic part of the study will be outlined here. The total group of 13 subjects receiving the normal diet (N) was divided into two subgroups for diet manipulation. In this paper only the data from N and from one subgroup, receiving an experimental high-maltodextrin, low-fructose beverage, MF (Perform, Wander Ltd) (n = 6) will be described and discussed. During the normal diet treatment the subjects were supplemented with a placebo beverage. For compositions of the drinks see Table 1.

Analyses

Muscle glycogen content in the biopsy sample (37) from the m. vastus lateralis, which was freeze-dried (Leybold Hereaus, GT2, FRG), was determined fluorimetrically after HCl hydrolysis (91) and expressed as mmol of glycogen units·kg⁻¹ dry weight.

Muscle triacylglycerol content was assessed as described elsewhere (116). In short, freeze-dried tissue specimens were extracted with chloroform-methanol (2:by vol). The various lipid classes were separated using one-dimensional thin-layer chromatography. The fatty acid moieties in the triacylglycerol spot were transmethylated with BF3 in methanol. The fatty acid methyl esters were quantified by gas-liquid chromatography.

Blood Chemistry

The following methods were applied for determinations in blood plasma:

Blood glucose = glucose oxidase method (Boehringer 124036); lactate = oxidometric analysis (Roche analyzer 640); acetoacetate and 3-hydroxybutyrate = enzymatically with 3-hydroxybutyrate dehydrogenase (7); glycerol = glycerol kinase UV test (Boehringer 297771);
Table 1: Composition of beverages

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Maltodextrin (g; l⁻¹)</th>
<th>Fructose (g; l⁻¹)</th>
<th>K⁺ (mmol)</th>
<th>Osmolality (mosm)</th>
<th>pH</th>
<th>Energy (kJ; l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0</td>
<td>0</td>
<td>8.5</td>
<td>106</td>
<td>4.38</td>
<td>-</td>
</tr>
<tr>
<td>Mf</td>
<td>150</td>
<td>33</td>
<td>8.5</td>
<td>390</td>
<td>4.45</td>
<td>3158</td>
</tr>
</tbody>
</table>

Table 2: Summary of observations with respect to nutritional indices and performance time

<table>
<thead>
<tr>
<th>Day</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Cumulative (days 4 + 5 + 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO intake (En%)</td>
<td>N</td>
<td>61.6 ± 1.7</td>
<td>62.9 ± 1.3</td>
<td>62.4 ± 1.8</td>
<td>62.7 ± 1.6</td>
</tr>
<tr>
<td>Mf</td>
<td>66.6 ± 2.7</td>
<td>80.5 ± 1.5**</td>
<td>79.7 ± 1.3**</td>
<td>67.0 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Nitrogen intake (g)</td>
<td>N</td>
<td>19.3 ± 0.9</td>
<td>16.9 ± 1.2</td>
<td>18.6 ± 1.1</td>
<td>21.5 ± 1.0</td>
</tr>
<tr>
<td>Mf</td>
<td>19.7 ± 1.0</td>
<td>15.7 ± 1.5</td>
<td>17.7 ± 1.4</td>
<td>17.9 ± 1.1***</td>
<td>51.3 ± 2.8</td>
</tr>
<tr>
<td>Energy balance (MJ)</td>
<td>N</td>
<td>1.18 ± 0.20</td>
<td>-9.78 ± 0.82</td>
<td>-8.12 ± 0.88</td>
<td>2.65 ± 0.92</td>
</tr>
<tr>
<td>Mf</td>
<td>4.90 ± 0.90</td>
<td>0.28 ± 1.23***</td>
<td>-0.54 ± 1.75***</td>
<td>2.13 ± 1.32</td>
<td>1.86 ± 2.36</td>
</tr>
<tr>
<td>Nitrogen balance (gN)</td>
<td>N</td>
<td>1.93 ± 0.46</td>
<td>-2.56 ± 1.34</td>
<td>-3.65 ± 1.08</td>
<td>-2.47 ± 1.44</td>
</tr>
<tr>
<td>Mf</td>
<td>1.93 ± 0.56</td>
<td>-2.13 ± 0.59</td>
<td>1.17 ± 2.23</td>
<td>-1.12 ± 1.01</td>
<td>-2.09 ± 2.50</td>
</tr>
<tr>
<td>Final performance at 90% Wmax, (min)</td>
<td>N</td>
<td>9.9 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mf</td>
<td>22.4 ± 7.9*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values ± 1SEM (for N, n = 13; Mf, n = 6). CHO intake was determined by weighed food intake procedure using a computerized food table for analysis. Nitrogen intake was determined by weighed food intake after previous determination of the nitrogen content of all food items by the chemiluminescence method. Energy balance was determined by indirect calorimetry and weighed food intake after previous determination of energy content of all food items by bomb calorimetry. Nitrogen balance was calculated from total nitrogen intake and nitrogen losses in urine, sweat, feces, and blood. Statistical significance with respect to the initial values on day 3 is indicated by: **P < 0.01; ***P < 0.001. Statistical significance of Mf with respect to N (each subject was his own control) is indicated by: *P < 0.05; **P < 0.01; ***P < 0.001. For complete description of the nutritional data see refs. 15, 16.

Statistics

Wilcoxon signed rank test was used to compare the data of the first standardized rest day with those of the following exercise and recovery days as well as for comparison of differences between the two treatments N (n = 13) and Mf (n = 6). For the latter each subject served as his own control. For all analyses, the 0.05 level was used as the minimum level of confidence for statistical significance.

Results

The results of the study dealing with food consumption, energy, fluid, and nitrogen balance, as well as relative contribution of CHO and fat in energy metabolism, have been described and discussed extensively in other papers (15, 16). The results most important with respect to the biochemical parameters studied are summarized in Table 2 and Fig. 1.

Plasma volume Fig. 1. On both rest days plasma volume in M and Mf showed a significant increase at 12:00 AM and 4:00 PM compared with 7:00 AM on the first day. Although on exercise days Mf subjects tended to have decreased plasma volumes below the initial level compared with N treatment, this was not statistically significant.

Blood glucose changes are represented in Fig. 2. During N treatment blood glucose remained almost the same throughout the entire test. As a result of exercise there was a small but significant elevation on days 4 and 5. During Mf treatment blood glucose values were significantly more elevated on the exercise days than during N treatment. There was no difference between the two rest days.

Blood lactate was significantly elevated after 2 h of exercise and in the blood sample taken 1 min after exhaustion. This elevation was the same for both N and Mf. Mean maximal lactate values did not exceed 4 mmol l⁻¹, despite the fact that work load was increased to 90% Wmax before reaching the moment of exhaustion (Table 3).
Plasma free fatty acids. During the first resting day fatty acid levels were significantly lower at 7:00 AM, after an overnight fast (7:00 AM of day 4 served as reference value). This was in contrast to the post-exercise rest day where morning levels were elevated. Both treatments showed similar responses during the rest days. During exercise days, however, fatty acids increased significantly more in N than in MF. This increase remained present during the post-exercise night. In both treatments fatty acids increased more on the second exercise day than on the first (Fig. 2).

Plasma ketones. Acetocacetate at rest in N was significantly elevated at 4:00 PM compared with 7:00 AM of the first rest day. As a result of exercise, the level increased significantly, remained elevated during the night, increased further during the second exercise day, and remained significantly elevated throughout the post-exercise recovery day. In MF plasma acetocacetate increased less and tended to be significantly lower throughout the experiment (Fig. 2). Beta-hydroxybutyric acid response was similar to that of acetocacetate, except that it returned somewhat faster to basal levels (Fig. 2).

Plasma urea was stable in N during day 3 and increased significantly during both exercise days, reaching the highest level at the moment of exhaustion. In MF plasma urea was significantly lower on day 4. There was no significant difference in response between N and MF on days 5 and 6 (Fig. 3). On day 6 plasma urea remained elevated and increased to a level comparable to days 4 and 5.

Plasma ammonia responses during both treatments were equal. During exercise days plasma ammonia increased significantly and reached the highest values at exhaustion. The values at the moment of exhaustion on the second exercise day tended to be lower than on the first exercise day (Fig. 3).

Plasma insulin levels in N and MF were equal at 7:00 AM of the first rest day (Fig. 4). During this day insulin increased significantly. This increase was more pronounced in MF. During the following exercise days insulin in N was significantly lowered at all measuring times. The food-induced insulin response during the day remained entirely absent during the second exercise day. The post-exercise recovery day showed a normal response again. However, the insulin level at 7:00 AM and 12:00 AM remained significantly lower compared with the pre-exercise rest day. The subjects under MF treatment responded in a similar manner. However, their insulin levels were significantly elevated compared with N during the exercise days, whereas food-induced insulin increase on the second exercise day was not observed at all.

Plasma glucagon responses in N and MF were not different. Plasma glucagon was significantly elevated at the end of both exercise days compared with the 3:30 PM sample of the pre-exercise rest day. The overnight fasting level between both exercise days was not different from that following the first rest day and from that post-exercise (Fig. 4).

Plasma epinephrine and norepinephrine responded to exercise in a similar way. It should be mentioned, however, that the catecholamines were only determined at the moment of exhaustion and at the same measurement time during days 2 and 6. Interestingly norepinephrine tended to increase more in MF than in N, whereas the opposite was the case for epinephrine (Fig. 4).
Fig. 2 Changes in CHO and fat metabolism as measured by substrate levels in blood. Blood glucose and fatty acids are presented as absolute values. Ketones and glycerol are corrected for changes in plasma volume. Statistical significance with respect to the initial value on day 3 is indicated by: $- - = P < 0.05; - - = P < 0.01$; $--- = P < 0.001$. For fatty acids the initial value is day 4, 7:00 AM. Statistical significance of MF with respect to N (each subject was his own control) is indicated by: $* = P < 0.05$. $- - - - = N$, $--- = MF$ exercise.

Plasma cortisol decreased during the day on both rest days. This decrease was absent on both exercise days resulting in a significant elevation halfway through exercise and at the end of exercise. The response in N and MF was the same (Fig. 4).

Muscle glycogen on rest day 2 was not different among treatments. As a result of exercise, glycogen decreased significantly in both N and MF; however, MF post-exercise levels were significantly higher than the values of the same subjects under N treatment. After 24 h of recovery N subjects did not restore their muscle glycogen to initial levels, whereas in MF subjects glycogen was restored, showing supercompensation (Fig. 5).

Muscle triacylglycerol. The muscle content of triacylglycerol (TG) was comparable in N and MF subjects prior to exercise (Fig. 6). In both groups the content of TG significantly decreased by about 40%–50% as a result of the heavy exercise bout. In the N group extremely high TG values

Fig. 5 Mean muscle glycogen content at rest as determined from a biopsy sample on day 2, 45 min after exhaustion on day 5, and after 24 h recovery on day 6. Statistical significance with respect to the initial value on day 3 is indicated by \( \ldots = P < 0.01; \ldots = P < 0.001 \). Statistical significance of Mf with respect to N (each subject was his own control) is indicated by \( * = P < 0.05 \). Vertical bars indicate 1 SEM (for N, \( n = 13; Mf, n = 6 \). □ = N, □ = Mf.

Fig. 6 Muscle triacylglycerol content (expressed as fatty acid mole-

ules per gram dry weight of tissue) as determined in biopsy samples, before exercise on day 2, 45 min after exhaustion on day 5, and after 24 h recovery on day 6. Statistical significance with respect to the initial value on day 3 is indicated by \( \ldots = P < 0.05; \ldots = P < 0.01 \). Bars refer to mean ± 1 SEM (for N, \( n = 13; Mf, n = 6 \). □ = N, □ = Mf.

were measured in the post-exercise tissue specimen obtained in 2 of the 13 subjects. Since these two deviating values were most likely caused by subcutaneous fat associated with the muscle tissue in the biopsy material, these data were not taken into account for statistical analysis. The changes observed in tissue TG during exercise were not significantly different between the two groups. The muscle TG content did not restore to initial levels in both N and Mf during 24 h after the exercise bout (Fig. 6).
Cycling performance quantified by the time that the subjects were able to perform at 80% and finally 90% \( W_{\text{max}} \), was substantially effected by the two treatments. The subjects increased their mean final 90% \( W_{\text{max}} \) performance over 2 days from 9.9 \( \pm \) 2.6 to 22.4 \( \pm \) 7.9 min when being under \( M_F \) treatment as compared with \( N \) treatment (Fig. 7).

Discussion

The present study was designed to analyze the effect of repeated long-lasting exercise on nutritional indices and metabolic changes and to compare these effects with those present after supplementing the same diet with a high-maltodextrin, low-fructose beverage while performing the same exercise program. A survey among professional cyclists competing in the Tour de France showed that liquid CHO supplements provided the major part of total CHO intake. Moreover, the study indicated that the athletes were only able to maintain \( E_B \) by using these supplements in addition to the normal conventional meals (109). During a part of this competition the cyclists were competing in the Alps. At this stage finishing on sequential days takes place on the top of a mountain and daily energy expenditure exceeded 35 MJ. It is especially during this extremely intensive part of the competition that the athletes have to cope with exhaustion.

Higher exercise intensities will deplete CHO stores of the body and this may be related to a decrease in power output and physical exhaustion (8, 9, 88, 108, 112). Cyclists in practice indicate that the only way to prevent this is to ingest CHO. Meanwhile a number of studies showed that time to exhaustion is increased when substantial amounts of CHO are ingested (24, 35, 67) and that training may enhance the capacity to take up blood-borne glucose during exercise (74). In contrast to this it was shown that ingestion of small amounts of CHO may have no effect on performance (40), which suggests that the amount of nutrients ingested may be critical with respect to possible benefits. None of the available studies however have dealt with exhausting exercise over a prolonged period of time during sequential days in highly trained subjects.

The amateur cyclists in the present study were of international level. The athletes cycled 4.5 h per day while simulating two intensive competition days. During this exercise approximately 1 h 20 min were performed at an intensity of 80% \( W_{\text{max}} \) and final exercise to exhaustion was performed at 90% \( W_{\text{max}} \).

The results of the study show that the subjects ingesting the conventional CHO-rich diet at rest and during exercise were not able to maintain \( E_B \) during days of exhaustive physical work, in contrast to when the diet was supplemented with \( M_F \) (Table 2).

As a result of the supplementation total CHO intake increased significantly as did the relative contribution of CHO to total daily energy intake. From these observations it becomes apparent that the two main factors influencing energy exchange and performance capacity – \( E_B \) and CHO availability – were significantly different between the two treatments. Although the subjects receiving the conventional
CHO-rich diet were in negative EB (for a detailed description see refs. (15, 16), they did not develop hypoglycemia (Fig. 2). A possible explanation may be that the ad libitum food intake during exercise conserved sufficient liver glycogen and/or supplied adequate amounts of CHO to the blood to avoid a fall in blood glucose. A second explanation may be that highly trained individuals have developed an enhanced capacity to synthesize glucose from lactate, glyceral, and alanine during exercise.

A contribution of gluconeogenesis to the maintenance of the blood glucose level is supported by the observed increase of hormones that favor gluconeogenesis in the liver and by the observation that blood lactate was significantly increased half way during exercise and at exhaustion. The fact that mean maximal blood lactate did not increase above 3.8 mmol l⁻¹, despite the final exercise intensity of 90% Vmax, indicates that lactate clearance in these subjects must have been substantial. Recently, it has been shown that lactate turnover during continuous exercise is quantitatively larger than glucose turnover (13), whereas trained subjects have an enhanced lactate clearance capacity rather than a change in production (33).

Although it may be assumed that lactate production in the exercising muscle is related to the availability of CHO, in such a way that in a state of CHO depletion less lactate will be produced, no difference could be detected in blood lactate between the two treatments. This occurred despite the large difference in CHO availability (Table 2). Blood glucose was significantly increased in MF. This is most probably due to the ad libitum intake of the CHO beverage, which must have led to a continuous supply of CHO from the gastrointestinal tract to the blood. The intake of 300 g CHO from the beverage supplemented during the rest days hardly influenced the blood glucose level. Oxidation and/or storage in endogenous energy depots at the same rate as entry into the blood may have been the reason for this. It has been shown that an increase of the CHO content in the diet enhances CHO metabolism at rest and during exercise (54, 122) and that blood glucose uptake at the site of the muscle membrane increases with both increasing exercise intensity and blood glucose levels (6, 42, 106, 118, 119).

Insulin is not required for glucose uptake in the muscle cell during exercise, because of an insulin-like factor having the same effect on glucose transport through the muscle cell membrane (63). However, it is known that insulin in the presence of muscle contractions has an additive effect on glucose uptake (25, 68, 96, 97). Therefore, it may be assumed that during the present study where both blood glucose and insulin were increased in MF (compared with N), substantial amounts of CHO will have been available for oxidation in the muscle cell and consequently must have induced glycogen sparing. In studies in which labeled glucose was infused or orally ingested, the observation was made that exogenous CHO is highly oxidized during exercise leading to a sparing of endogenous CHO. It appeared that the amount of CHO sparing seems to be related to the dose of the load infused or ingested, i.e., greater loads induce more elevated blood glucose levels and subsequently an enhanced oxidation, thereby reducing the degradation of local glycogen pools (28, 85, 90, 94, 95).

Sparing of glycogen may take place in the liver and the muscle. Although liver glycogen was not measured, it may be assumed that there was no reason to increase liver CHO output from liver stores because of the high amount of CHO ingested. Glucose infusion studies have shown that liver glucose output drops to a low level or is almost entirely blocked when 2 mg·kg⁻¹·min⁻¹ is infused. Although exercise seems to reverse this inhibition, it was observed that during exercise splanchnic glucose output of glucose and saline infused subjects was identical. This indicates that infusion limits the magnitude of rise in glucose output to an amount equal to infusion rate (41). In the present study CHO supply to the vena porta must have been substantially greater in the MF treatment than the above-mentioned infusion rate, both at rest and during exercise.

A statistically significant difference in post-exercise muscle glycogen level was observed after exercise on day 5 between N and MF. Subjects supplemented with MF showed a significant glycogen sparing, although cycling time to exhaustion was significantly longer (Table 2). Since the muscle biopsy was taken at approximately 45 min after exercise, it can be hypothesized that glycogen resynthesis will have taken place. It has been observed that post-exercise glycogen resynthesis can occur from the conversion of lactate (61, 65).

However, blood lactate levels at exhaustion were equal in both N and MF so that this cannot account for the difference observed. Although a substantial amount of CHO may still have been present in the gastrointestinal tract, this can only account for a small part of the difference observed. From a recent study (75), in which the same beverage was supplied, it was calculated that the average maximal rate of post-exercise glycogen synthesis in the trained cyclist amounted to 37 μmol·g⁻¹·dry weight·h⁻¹ (range 27–52), this rate was comparable to maximal synthesis rates after exercise as calculated from data of other investigators (73, 82, 95). Assuming an average maximal post-exercise synthesis rate of 37 μmol·g⁻¹·dry weight, only 20% of the difference observed (141 μmol·g⁻¹·dry weight) can be explained by de novo synthesis.

Therefore, it can be concluded that the majority of the glycogen sparing observed must have been due to glycogen sparing during exercise. In this respect it is an interesting observation that exhaustion under MF treatment was not caused by a low level of muscle glycogen. Studies in which glycogen depletion in time was related to the state of fatigue indicate that exhaustion occurs when muscle glycogen drops to a low level (8, 107, 112). In the present study mean muscle glycogen at exhaustion in the MF group was greater than 250 μmol·g⁻¹·dry weight. Thus, other factors than glycogen must have played a role in inducing the state of exhaustion.

As discussed earlier, the availability of fatty acids is of great importance for energy metabolism in the endurance athlete because of its sparing effect on endogenous CHO stores. A high CHO intake makes a high lipolytic activity superfluous while a low CHO intake or glycogen depletion requires a high lipolytic rate (89, 104). Therefore, the difference in CHO availability between N and MF must have had its impact on overall fat metabolism.
Glucagon, catecholamines, growth hormone, and sympathetic activity all raise lipolysis whereas insulin has an inhibiting effect (5, 12, 69).

The hormonal changes in the present study (Fig. 4) were all in favor of an enhanced lipolytic activity especially during N treatment where EB was negative and plasma levels of glycerol, fatty acids, and ketones increased significantly.

The presented values of all hormones and of blood glucose and fatty acids are absolute values (not corrected for changes in plasma volume) in contrast to the values of metabolic intermediates and end products such as lactate, glycerol, ketones, urea, and ammonia which are given as corrected values. The reason is that it is the absolute level of the first mentioned parameters that determines the metabolic effects. With respect to the last mentioned parameters it is important to consider the rate of mobilization or production. Therefore, a change in those parameters should be corrected for changes in plasma volume.

Since the uptake of fatty acids is directly related to the plasma fatty acid concentration (34, 46) and observed R values declined as a result of exercise, it can be concluded that fatty acids have contributed substantially to energy exchange in N. The significant increase in plasma ketones may be related directly to the enhanced fat metabolism since a high plasma fatty acid and a low insulin level enhance the rate of ketogenesis (89). In MF fat metabolism was suppressed through the high CHO intake and the related metabolic changes. Although glycerol and fatty acids also increased significantly in this group, the magnitude of this increase was significantly smaller than that observed in N. An exception to this was the plasma glycerol level at the end of the second exercise day. The fact that a significant increase in lipolysis occurred in MF, despite the high blood glucose and insulin level, can only be explained by the mutual action of factors that exert an effect on lipolytic activity. The inhibiting effects of increased blood glucose and insulin levels may at the very moment have been overruled by the effect of an increase in catecholamines, glucagon, and cortisol.

It may be assumed that sympathetic nerve activity, while performing the same exercise protocol, will have been in the same in both treatments as indicated by the norepinephrine levels. Interesting is the observation that the plasma glycerol level at exhaustion on day 5 is not significantly different between N and MF. This coincides with disappearance of the significant differences between N and MF in blood glucose and insulin despite the large CHO intake from the supplement in the MF group. Plasma fatty acids on the other hand remained significantly lower under MF treatment, but it has to be kept in mind that plasma fatty acids are a reflection of both fatty acid release and uptake so that it is difficult to draw conclusions about lipolytic activity and/or fatty acid oxidation using this parameter. Despite a small plasma pool, fatty acids may have a rapid turnover rate supplying substantial portions of substrate (32). Glycerol is a better indicator because its conversion to glucose by gluconeogenesis is believed to be slow so that its accumulation in blood will better reflect overall lipolytic activity (88). So far it can be concluded that blood-borne fatty acids have contributed significantly to energy exchange despite the high level of CHO intake during exercise.

Muscular triacylglycerol (TG) content, as determined from the biopsy samples, decreased due to exercise but was not influenced by the diet treatment. Enhanced uptake of blood-borne fatty acids and CHO may lead to a sparing of local glycogen (70, 69) but such a sparing effect was not observed with respect to muscle TG in the present study. In the trained muscle it may be that the release of fatty acids from endogenous TG during exercise depends on a local regulatory factor and that while exercising the amount released for oxidation does not exceed the oxidative capacity. Fatty acids from the blood entering the muscle cell during exercise may then additionally be oxidized. This additional fat metabolism may therefore lead to (additional) glycogen sparing by decreasing the need for glycolysis but not to a decrease of muscular lipolytic rate and sparing of TG. The observation that circulating fatty acids have no sparing effect on muscular TG (114, the present study) may be explained by the hypothesized possibility that the intracellular fatty acid content is not elevated during exercise or in other words all fatty acids taken up and/or released from endogenous stores are immediately oxidized for energy production.

Several studies have indicated that muscular fat contributes to a significant extent to overall fat metabolism in the working muscles, particularly in the first stage of endurance exercise. Havell (60) and Paul (92) calculated in their exercise experiments that approximately 50% of the fat oxidized was derived from local stores. The observation that trained individuals have a greater capacity to rely on muscular fat (62, 105) and have an increased fat storage in the slow-twitch muscle fibers in dropouts located near the mitochondria (64) while the fractional extraction of fatty acids does not increase during exercise-induced increased blood flow, suggests that maximal fatty acid transport across the muscle cell membrane in some way exposes a limiting factor for fat metabolism during endurance exercise. There may thus be a physiologic need to store more TG in the muscle cells involved in endurance exercise. From the data obtained it may be hypothesized that degradation of locally stored TG during exercise is under direct control of sympathetic nerve activity and that circulating hormones, blood-borne substrates, and local glycogen levels may only have a limited influence. Some indirect evidence may support this hypothesis.

- Insulin, a strong lipogenic hormone was significantly elevated in MF compared with N.
- In MF muscle glycogen was higher than in N.
- In MF plasma fatty acids were lower than in N.

Nevertheless, muscle TG depletion was similar.

Moreover, elevated plasma fatty acids were shown to have no sparing effect on muscular TG (114), the major part of fat oxidized in the first stage of prolonged exercise is derived from muscular stores (21, 92), and finally, if moderate continuous exercise or intensive interval exercise with the same mean power output per hour is performed, muscle TG degradation is found to be the same (36).
Protein metabolism is influenced by the concerted action of anabolic and catabolic regulators which are activated or inhibited by physical activity, energy balance, CHO availability and the level of daily protein intake.

It is known that protein turnover is quite sensitive to immediate energy supply (120) and that the most stable turnover rates appear to be present during a state of stable hormone levels and constant supply of nutrients in a fixed ratio (53). A low or high energy intake leads to a negative or positive nitrogen balance (48, 51, 66, 103). An almost linear relationship between the level of protein intake and protein synthesis rate has been observed at low levels of protein intake (84). Das (27) observed in rats that change from a high to a low protein diet or vice versa caused an immediate change in nitrogen output which then reached a new constant level after approximately 30 h. This change occurred simultaneously with an adaptation in the activity of urea cycle enzymes. Because exercise may also affect nitrogen balance by suppressing muscle protein synthesis and increasing protein degradation (most probably in the liver and muscle (30, 31, 71, 102)), which may further be influenced by the amount of endogenous CHO stored (77, 79), it was assumed that an accurate estimation of the changes in protein metabolism due to exercise and diet manipulation could only be made if the pre-exercise nutritional and metabolic status was controlled as much as possible.

Based on this discussion it was decided to standardize a minimum level of protein intake of 1.2 g·kg⁻¹·day⁻¹ during day 2 and to maintain at least this minimum intake during the entire experimental period. According to Waterloo (120), in blood the only end products of protein indicating actual changes in protein degradation are urea and ammonia. But for an overall indication of net protein breakdown in the body, resulting in a net nitrogen loss, an accurate analysis of nitrogen excretion in urine and sweat should be performed. During exercise urinary nitrogen excretion drops and plasma urea concentration rises (102) most probably because of both a reduced urine production and decreased renal excretion. This leads to an alternative route for elimination of the protein waste products via enhanced secretion in the sweat (77). A measurement of both routes is thus essential. The results of the present study show that plasma urea increased significantly with N treatment, as a result of exercise and negative energy balance. In contrast plasma urea remained on a more or less stable level in Mf and tended only to rise at the point of exhaustion on the second exercise day. This difference in urea response is comparable to that described by Lemon and Mul- lin (78) who observed that endogenous CHO depletion increases urea production during exercise.

Nitrogen losses were affected by exercise. In N the mean cumulative nitrogen balance over 2 exercise days and the following recovery day was −8.69 ± 2.50 g. In the Mf group there was a tendency to nitrogen sparing despite the longer performance time (−2.09 ± 2.50 g). However, this effect did not reach significance, most probably due to the large variations. This difference in nitrogen balance may be explained entirely by the effect of a negative EB and CHO balance.

A complete picture of all variables regarding protein metabolism studied in the present experiment will be presented in separate papers (17, 18).

Plasma ammonia was not affected by the treatments. Ammonia may be derived from the deamination of amino acids or from the intracellular adenylate pool. The latter takes place during exercise of high intensity when immediate energy requirements exceed the amount of energy released from substrate degradation. Rises in plasma ammonia during exercise may be influenced by the availability of local gly- coen stores as is observed in McArdles disease (4, 23, 121). In the present experiment the subjects cycled until exhaustion. It may thus be that adenylate pool depletion and AMP deamination has played a role (apart from amino acid degradation for gluconeogenesis) in ammonia production. However, taking into account the substantial glycogen availability in the supplemented subjects when reaching exhaustion the existence of a real energy deficit may be questioned, unless single muscle fibers have been depleted.

From the data presented it may be concluded that protein degradation and nitrogen loss from the body is increased in athletes during intense sustained exercise and that EB and CHO intake are of direct influence.

Hormone levels were greatly influenced by the exercise. Insulin increased as a result of food intake on day 3 but was significantly lowered by exercise on days 4 and 5. Interestingly is the observation that this effect was more pronounced on the second exercise day than on the first. This was not only the case in N treatment but also in Mf. However, in N the food-induced increase disappeared completely on day 5 indicating a strong inhibition of insulin secretion whereas in Mf food stimulation still remained present. This further decrease on day 5 cannot be explained by the inhibiting effect of catecholamines on insulin secretion (18a, 98, 99, 100), since the level of epinephrine and norepinephrine was not different between the two exercise days. In this respect it is tempting to speculate that local factors such as relative glycogen availability indirectly influence insulin secretion. It may be that low glycogen levels in liver and/or muscle initiate an inhibition of insulin release so that the insulin/glucagon ratio decreases to a low level, to enhance lipolytic activity as much as possible to spare endogenous CHO. Plasma glucagon as well as cortisol did not differ between the two exercise days or between the treatments so that the insulin/glucagon ratio decreased as a result of exercise, to the largest extent on day 5. These findings are surprising in the light of the large difference in CHO availability between the two treatments.

Glucagon, which in contrast to insulin has a strong lipolytic effect (52), increases hepatic glucose output (1, 43) and stimulates hepatic gluconeogenesis (22, 38, 50). It is well documented that glucagon levels will rise whenever blood glucose levels fall or whenever local CHO stores become depleted. Several authors have described that an increase in blood glucose due to CHO ingestion during or prior to exercise inhibits glucagon secretion (2, 3, 41, 81). However, in the present study such an effect was not observed.

Cortisol also stimulates hepatic gluconeogenesis (38, 111) and appears to become increased indirectly by
adrenergic hypothalamic mechanisms via ACTH secretion (115). Although it has been described that submaximal exercise may not be associated with increments in plasma cortisol (38, 111) we did observe a significant increase. In general it is known that cortisol release during exercise depends largely on exercise intensity, duration, and training status (11, 72). The increase observed may thus be explained by the submaximal exercise over a prolonged period of time and the related adrenergic activity.

Also catecholamines stimulate liver glycogenolysis, gluconeogenesis, and overall lipolytic activity (76). Catecholamine levels in plasma increase exponentially with work intensity and are correlated most to the relative exercise intensity. However, physiologic deterioration by means of hypoglycemia, hyperthermia, or hypoxia may further enhance catecholamine release, especially of norepinephrine (47, 49). As a consequence catecholamine increase is observed to be depressed as exercise trails in which glucose was ingested compared with control (water) trials (10, 40, 47). The subjects under N treatment, although being in negative energy balance, did not develop hypoglycemia.

MF subjects had significantly increased blood glucose levels but nevertheless showed the same response as N subjects. From these data it may be concluded that hormones which enhance gluconeogenesis, liver glucose output, and lipolytic activity, are released during highly intensive and exhausting endurance exercise irrespective of CHO availability. These findings contradict other studies in which during less intensive exercise of shorter duration effects due to substrate availability were observed (47, 117) suggesting that a high level of sympathetic activity over a prolonged period of time in man may be dominant with regard to metabolic regulation of substrate mobilization and availability.

Cycling Performance

The intramuscular glycogen content, a performance-limiting factor as discussed before, may have been responsible for the differences in cycling performance. The subjects in N were not able to restore their muscle glycogen levels within 24 h of recovery (Fig. 5). Assuming a similar recovery pattern, glycogen resynthesis during the 18 h of rest between days 4 and 5 will have amounted to approximately 75% of the measured value on day 6. This incomplete glycogen recovery may be related to the decreased performance of subjects in N on the second exercise day. In contrast, in MF performance on day 5 was improved in five of the six subjects. One subject could not be motivated to sustain the high work loads at 80% Wmax already and quit prematurely (Fig. 7). Of particular interest is the observation that subjects in MF were able to perform significantly longer but became exhausted without any relation to metabolic changes which normally are related to fatigue such as hypoglycemia, high blood lactate, severe dehydration associated with hyperthermia, and glycogen depletion. It might be that after the diet manipulation described a shift occurs from glycogen, as limiting factor (subjects in N), to another unknown factor (subjects in MF). It has been suggested that ammonia plays a role in processes of central fatigue (87) and it is known that ammonia influences a number of steps in the metabolic pathways of energy release (20, 80, 87). However, the plasma ammonia levels observed were similar in the two treatments after reaching the point of exhaustion.

In summary, the results described lead to the following conclusion:

- The supplementation of a conventional CHO-rich diet with a 20% maltodextrin-fructose beverage leads to a marked increase in CHO availability which induces sparing of intramuscular glycogen stores and increases exercise time until exhaustion.
- Glycogen supercompensation can be achieved within 24 h post-exercise.
- A high CHO intake during sustained exercise reduces protein degradation.
- Local glycogen stores may influence food-induced insulin secretion during exercise.
- Muscular TG degradation during exercise may be regulated by a local factor and does not seem to be influenced by substrate availability from the blood, nor by muscular glycogen content.

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References


