Effects of Dietary Fat on Muscle Substrates, Metabolism, and Performance in Athletes

MICHAEL VOGT[1], ADRIAN PUNTSCHART[1], HANS HOWALD[1], BRUNO MUELLER[3], CHRISTOPH MANNHART[2], LILIANE GFELLER-TUESCHER[1], PRIMUS MULLIS[3], and HANS HOPPELER[1]

[1]Department of Anatomy, University of Bern, Bern, SWITZERLAND; [2]Institute of Sport Sciences ISS, Federal Office of Sports, Magglingen, SWITZERLAND; and [3]Inselspital, University Hospital, Bern, SWITZERLAND

ABSTRACT

VOGT, M., A. PUNTSCHART, H. HOWALD, B. MUELLER, CH. MANNHART, L. GFELLER-TUESCHER, P. MULLIS, and H. HOPPELER. Effects of Dietary Fat on Muscle Substrates, Metabolism, and Performance in Athletes. *Med. Sci. Sports Exerc*., Vol. 35, No. 6, pp. 952–960, 2003. **Introduction:** The present investigation aimed at identifying differences in muscle structural composition, substrate selection, and performance capacity in highly trained endurance athletes as a consequence of consuming a high-fat or a low-fat diet. **Methods:** Eleven duathletes ingested high-fat (53% fat; HF) or high-carbohydrate diets (17% fat; LF) for 5 wk in a randomized crossover design. **Results:** In m. vastus lateralis, oxidative capacity estimated as volume of mitochondria per volume of muscle fiber (HF: 9.86 ± 0.36 vs LF: 9.79 ± 0.52%, mean ± SE) was not different after the two diet periods. Intramyocellular lipid (IMCL) was significantly increased after HF compared with LF (1.54 ± 0.27% vs 0.69 ± 0.09%, $P = 0.0076$). Glycogen content was lower after HF than after LF, but this difference was not statistically significant (487.8 ± 38.2 vs 534.4 ± 32.6 mmol·kg^{-1} dry weight, $P = 0.2454$). Maximal power and $\dot{V}O_{2max}$ (63.6 ± 0.9 vs 63.9 ± 1.2 mL O_2·min^{-1}·kg^{-1} on HF and LF) during an incremental exercise test to exhaustion were not different between the two diet periods. Total work output during a 20-min all-out time trial (298 ± 6 vs 297 ± 7 W) on a bicycle ergometer as well as half-marathon running time (80 min 12 s ± 86 s vs 80 min 24 s ± 82 s) were not different between HF and LF. Blood lactate concentrations and respiratory exchange ratios (RER) were significantly lower after HF than after LF at rest and during all submaximal exercise loads. **Conclusions:** Muscle glycogen stores were maintained after a 5-wk high-fat diet period whereas IMCL content was more than doubled. Endurance performance capacity was maintained at moderate to high-exercise intensities with a significantly larger contribution of lipids to total energy turnover. **Key Words:** EXERCISE, HIGH-FAT DIET, MITOCHONDRIA, INTRACELLULAR LIPID, RESPIRATORY EXCHANGE RATIO, HUMAN

The role of carbohydrates (CHO) in muscle fatigue has extensively been investigated. Classical studies have established that diets rich in CHO can increase glycogen stores in muscle and are thus implicated in increasing endurance performance (1). Likewise, the ingestion of CHO during prolonged bouts of exercise has been shown to increase time to exhaustion whereby ingested CHO may provide as much as 50% of the total CHO oxidation rate (39). By contrast, many aspects of muscle lipid metabolism, such as the mechanisms regulating lipid oxidation and the role of intra- versus extramyocellular lipid sources have remained elusive (36). Moreover, there is considerable uncertainty in judging the influence of high-fat diets on performance capacity (21,35). It is recognized that the major determinant of substrate selection is exercise intensity (4). At exercise intensities close to $\dot{V}O_{2max}$, CHO oxidation dominates. At 75% of $\dot{V}O_{2max}$, lipids contribute about 25% of the total energy. At 50% of $\dot{V}O_{2max}$, just over half of total energy is derived from fat and increasingly more below that intensity (38). It is also generally accepted that there is an increased reliance on fat as fuel with increasing exercise duration (7,33), underpinning the role of lipid substrates in athletic activities of long duration. Endurance exercise training leads to a greater reliance on lipids as substrates. The increase in total fat oxidation induced by endurance exercise training is well supported by a number of more recent studies (20,23,39). The upregulation of lipid metabolism in endurance trained subjects is observed at the same relative and at the same absolute work intensities (37). Van Loon et al. (37) also noted that at 50% of $\dot{V}O_{2max}$, the extra energy requirement of trained subjects to reach the same relative workload compared with untrained subjects was entirely covered by lipid oxidation. The increased contribution of lipids to oxidative metabolism after endurance training is seen as an important means of sparing muscle glycogen and postponing muscle fatigue (15). Taken together, the evidence attributes a key role to lipid metabolism whenever highly trained subjects engage in moderate exercise for very long periods such as in ultra-endurance events or in bicycle road races as the maximal rate at which ingested CHO can be oxidized is capped at approximately 1 g·min^{-1} (20,39).

In principle, the goal of enhancing skeletal muscle lipid metabolism can be met by ingesting a high-fat diet. Several

Address for correspondence: Prof. Dr. med. Hans Hoppeler, University of Bern, Institute of Anatomy, Buehlstrasse 26, 3000 Bern 9, Switzerland; E-mail: hoppeler@ana.unibe.ch.
Submitted for publication January 2002.
Accepted for publication January 2003.

0195-9131/03/3506-0952
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/01.MSS.0000069336.30649.BD

studies show a reduction of the RER at submaximal workloads after increasing the fat content of the diet, indicating a more pronounced oxidation of fatty acids (1,7,26,28,30). However, the role of high-fat diets on endurance performance has remained controversial. Short-term high-fat diets (up to 5 d) generally showed a negative effect on endurance performance (1,11,29). In longer-term high-fat diets (7–49 d), performance was found decreased (13,32), unchanged (5,6,7,12,14,30,31), or improved (18,26,28). It is difficult to define reasons for these equivocal results. Different diet protocols as well as different training and testing procedures could all contribute to the different outcomes reported in these studies.

The purpose of the present investigation was to evaluate the influence of a long-term high-fat and of a low-fat diet on substrate stores in skeletal muscle, substrate selection, and performance capacity in highly trained endurance athletes for whom training conditions were kept constant during the dietary interventions. The fat content of the diets was chosen within the range practiced by athletes such that subjects could prepare and control their diet at home. It was hypothesized that on a high-fat diet, performance would be improved at moderate exercise intensity (i.e., running time in a half-marathon) but remain unchanged at high exercise intensities (power delivered over a 20-min cycling time trial). Based on results of a previous study (16), muscle oxidative capacity and muscle glycogen content were expected to remain unchanged while intramyocellular fat content was expected to increase with the high-fat diet.

MATERIAL AND METHODS

Subjects. Eleven healthy male subjects participated in this study after giving written informed consent. They were selected among the first 100 finishers of a National Duathlon. Selection criteria were: a) closeness to the laboratory facilities and b) availability of an IBM-compatible home computer capable of running the nutritional software. The study design was approved by the Ethical Committee of the University of Bern. The subjects were nonsmoking, between 19 and 43 (31.6 ± 2.0; mean ± SE) yr of age, and 176.9 ± 1.8 cm in height. They were regularly participating in duathlon and triathlon competitions. The subjects were required to keep a training diary and to maintain the training load constant over the entire experimental period.

Diets. This study was designed such that each subject served as his own control in a randomized cross-over design. In the 2 wk preceding the first diet intervention, the subjects were familiarized with recording all food items immediately after each meal in a dietary log and with using the software (EBIS 2.0, Stuttgart, Germany) on their own PC for the analysis of diet composition. Based on body mass and training volume, individual daily energy requirements were calculated, and the subjects were instructed to adjust their diet to meet these requirements. Based on food preferences, the subjects were given sample menus and instructed how to vary these to reach the target compositions of macronutrients during the diet periods. In principle, the fat content of the diet was increased by selecting food items with a higher fat content and/or by supplementing olive oil to reach the target composition. Throughout the dieting periods, subjects mailed their diet records weekly and were additionally contacted by phone at least twice weekly to check for diet and training compliance. As a rule, consumed food was weighed on calibrated balances. Subjects were free to choose their preferred food items as long as they stayed within the prescribed composition of macronutrients (high-fat diet: 50–55% of total energy from fat; low-fat diet: 15–20% of total energy from fat; 15% of total energy from proteins on both diets). Subjects were paired according to individual $\dot{V}O_{2max}$ and randomly assigned to start dieting on either the low fat or the high-fat diet. Diet periods lasted 5 wk for the high-fat and low-fat period. Diets were matched for caloric content. All exercise tests were performed during the last dieting week of each period (see below). After the first diet period, subjects immediately switched to the alternate diet. Body weight and body fat were recorded at the end of each dietary period at an identical time of day. Body fat content was determined by a seven-point skinfold measurement.

Analysis of muscle structure. Muscle biopsies were performed 48 h after the last training session and immediately before the first performance test (see below). They were taken from the mid-thigh region of m. vastus lateralis according to the technique of Bergstrom et al. (1). One part of the biopsy was immediately frozen in isopentane cooled with liquid nitrogen. Fiber type composition was determined from the first biopsy taken before the dietary intervention, processing 12-μm cryostat cross-sections for the demonstration of myofibrillar ATPase according to the technique described in detail by Billeter et al. (2). All fiber type counts were obtained from sections treated with acid preincubation (pH 4.5). We analyzed three to five individual sections from each muscle biopsy depending on the size of the biopsy specimens. All fibers that appeared reasonably cross-sectioned (minor to larger fiber axis > 0.5) were counted.

The remainder of the biopsy was fixed in a 6.25% solution of glutaraldehyde in 0.1 mM sodium cacodylate buffer (adjusted to 430 mosmol·L^{-1} with NaCl; the total osmolarity of the fixative was 1150 mosmol·L^{-1}, pH 7.4) and fixed for 1 h before blocks suitable for electron microscopy (EM) processing were cut. Blocks were postfixed for 2 h in a 1% solution of osmium tetroxide and block-contrasted with 0.5% uranyl acetate. After dehydration with ethanol, they were embedded in Epon. Sections of 50- to 70-nm thickness were cut and picked up on 200-mesh copper grids covered with a carbon-coated Parlodion film (41). Sections were contrasted with lead citrate and uranyl acetate. From each biopsy, four blocks were sectioned, and 10 micrographs were taken with a Philips 300 transmission electron microscope and recorded on a 35-mm film to achieve a final magnification of about ×24,000 on the morphometric screen (Fig. 1). On each film, a carbon-grating replica was recorded for calibration. The volume densities of muscle fiber structures were estimated using well-established stereological procedures (41). Point counting was performed

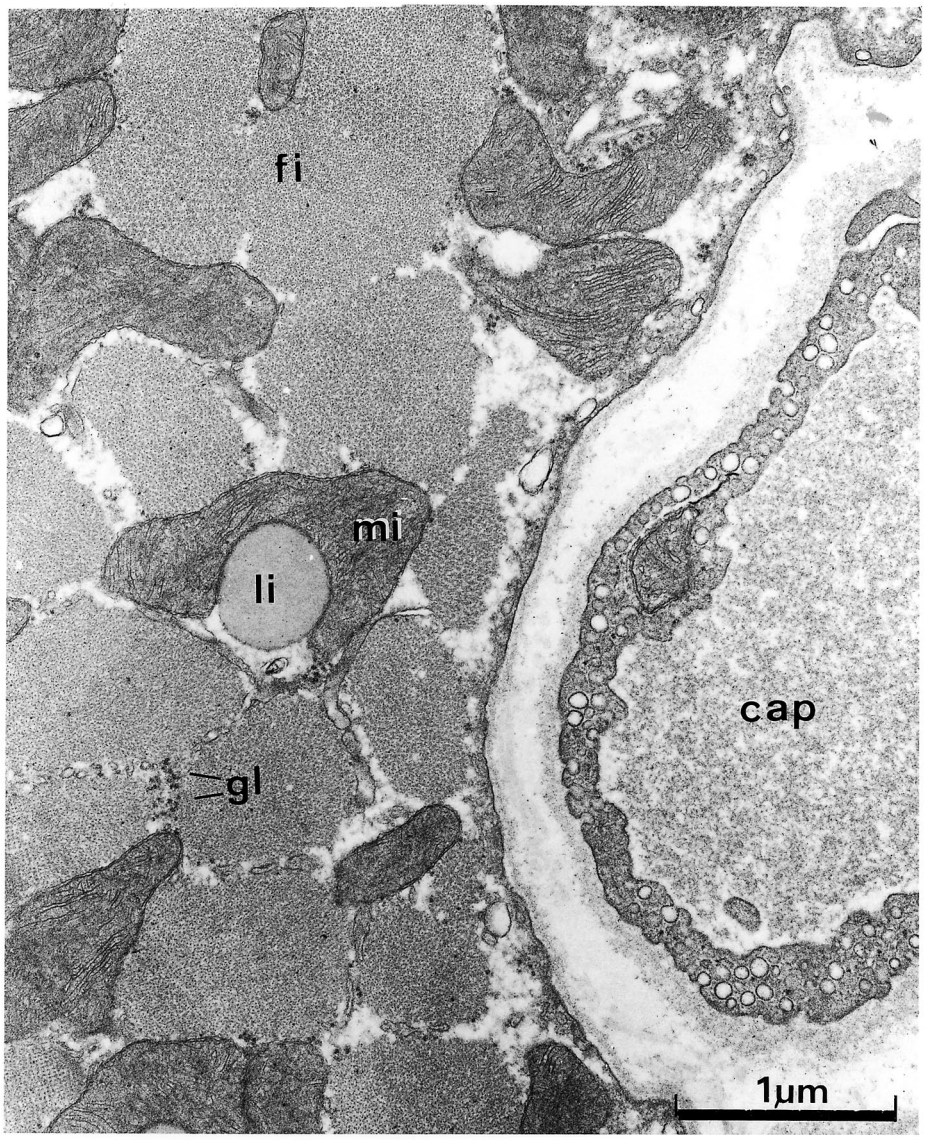

FIGURE 1—EM picture of cross-sectional muscle area. li: lipid droplet; fi: myofibrils; mi: mitochondria; gl: glycogen rosettes.; cap: capillary; magnification: ×28,500; scale bar: 1 μm.

on a grid containing 100 test points for mitochondria and 400 points for lipid droplets. Statistical analyses for all 40 micrographs per sample were performed for the volume of total mitochondria per fiber volume and for the volume of total lipid deposits per fiber volume. Applying this methodology, intramyocellular lipid (IMCL) is expressed in percentage of fiber volume, but it may be transformed to millimoles per kilogram wet weight using a multiplication factor of 10.1 (19).

Glycogen determination. To determine muscle glycogen content, 15–20 sections of frozen muscle tissue of thickness 30 μm were freeze dried and dissected from blood and connective and adipose tissue. The weighed, powdered muscle tissue was boiled in 1 M HCl for 30 min to break up the glycogen chains; 6-phospho-gluconolacton and NADPH were formed from the resulting glucose by enzymatic reaction with hexokinase and glucose-6-P dehydrogenase. The formation of NADPH, which is proportional to the initial amount of glycogen, was then determined fluorometrically (excitation: 340 nm, emission: 460 nm). Glycogen content is expressed as millimoles glycogen per kilogram muscle dry weight.

Performance tests. Subjects performed three identical sets of performance tests, the first in the week immediately preceding the first diet period (baseline), the second and third set during the last week of each diet period. For each set of tests, subjects appeared at the identical time in the laboratory to minimize the effects of diurnal fluctuations on performance variables. The subjects were required to abstain from training or vigorous exercise 48 h before the tests. They were asked to consume a set pretest meal of 400–500 kcal adjusted to the fat content of the dieting period 2 h before the start of the first test. $\dot{V}O_{2max}$ was measured with an incremental exercise test on a cycle ergometer. After 2 min at rest, the protocol started with a load of 100 W. Power was increased every second minute by 30 W until the subjects were unable to maintain a cadence of 80 RPM. Maximal power was calculated by linear interpolation of power over time when the performance test was not stopped at the time of an exercise increment. Blood lactate was

measured at the end of each incremental step and 2 min after the end of the $\dot{V}O_{2max}$ test. Heart rate (Accurex Plus, Polar Electro Finland Oy, Kempele, Finland) and respiratory gases (Oxycon alpha, Jaeger GmbH, Würzburg, Germany) were monitored continuously. Two days later, subjects performed a submaximal exercise test at four submaximal workloads on a calibrated cycle ergometer followed by an all-out 20-min time trial. After 10 min of sitting on the ergometer at rest, they exercised for 10 min each at 20%, 40%, 60%, and 75% of the individual maximal power as determined in the $\dot{V}O_{2max}$ test. The final 20 min of this test consisted of a time trial, whereby subjects could individually select the power output of the ergometer to maximize total work over this time period. Respiratory gases were measured during the last 2 min of each submaximal exercise step and during the entire 20 min of the time trial. Heart rates were recorded continuously during the whole test. Blood lactate concentration was measured at the end of each exercise step and after every 5 min of the time trial. For the submaximal exercise test respiratory parameters of one subject are missed due to technical problems. At the end of each test week, minimally 48 h after the last laboratory performance test, all subjects competed in a half-marathon race (21-km, outdoors) during which heart rates and running time were recorded.

Statistics. Data are presented as means ± SE. The statistical analysis was performed using a statistic software package (Statistica 5.1 for Windows, Statsoft Inc., Hamburg, Germany). Differences between values of the high-fat and low-fat diet periods were analyzed by Student's paired *t*-test. Differences were considered to be significant for $P < 0.05$.

RESULTS

Body composition and training. Body weight and body fat content of the subjects did not change over the experimental period (Table 1). On the average, total physical activity per week was 10% or 38 min higher while eating the low-fat diet as compared with the period with the high-fat diet. However, this difference was not statistically significant ($P = 0.28$).

Diets. Self-reported fat intake was 52.9 ± 0.7% of total energy during the high-fat diet and 16.5 ± 0.6% during the low-fat diet. The composition of the ingested diets is reported in Table 2. During the high-fat diet period, subjects had a significantly larger caloric intake (+12%) than under the low-fat diet.

TABLE 1. Anthropometric data and weekly training volume.

Mean Values ± SE	PRE	HF	LF
Body weight (kg)	69.1 ± 5.1	69.1 ± 5.5	68.9 ± 5.1
Body fat content (%)	7.7 ± 0.7	7.2 ± 1.6	7.0 ± 1.7
Fat mass (kg)	5.3 ± 0.4	5.0 ± 0.7	4.8 ± 1.0
Body mass index ($kg \cdot m^{-2}$)	22.1 ± 1.1	22.1 ± 0.9	22.0 ± 1.0
Training volume ($min \cdot wk^{-1}$)	401 ± 35	371 ± 35	409 ± 44

PRE, values during prestudy familiarization test week; HF, values after the 5-wk high-fat diet period; LF, values after the 5-wk low-fat diet period.

TABLE 2. Composition of diets.

Mean Values ± SE	PRE	HF	LF
Energy intake ($kcal \cdot d^{-1}$)	3014 ± 105	3269 ± 160	2905 ± 159*
Fat intake (%)	30.6 ± 1.2	52.9 ± 0.7	16.5 ± 0.6*
($g \cdot d^{-1}$)	103 ± 6.8	192 ± 10.5	53 ± 3.1
Carbohydrate intake (%)	53.6 ± 1.5	31.4 ± 0.7	68.2 ± 0.9*
($g \cdot d^{-1}$)	385 ± 15.0	246 ± 14.3	475 ± 298
Protein intake (%)	14.4 ± 0.5	14.4 ± 0.4	14.3 ± 0.8
($g \cdot d^{-1}$)	104 ± 4.8	112 ± 5.3	100 ± 8.3

Percentage of total energy intake given for each macronutrient. PRE, values during prestudy familiarization test week; HF, values after the 5-wk high-fat diet period; LF, values after the 5-wk low-fat diet period.
* Significantly different between high-fat and low-fat diet; $P < 0.05$.

Muscle structure and glycogen content. Fiber typing performed in the first biopsy taken before the dietary intervention disclosed 73.5 ± 3.6% of Type I (range 55–95%) and 26.5 ± 3.6% of Type II fibers. Average values for muscle structure and substrate content at the end of the three dietary periods are reported in Table 3. The volume density of mitochondria remained unchanged over all dietary periods. The average total mitochondrial density was close to 10% of the muscle fiber volume. There was a statistically significant 2.3-fold higher content of IMCL after the high-fat diet compared with the low-fat diet. Average muscle glycogen content did not significantly change during the dietary interventions.

For both IMCL and glycogen, there is a large interindividual variability with respect to the response to the dietary interventions (Fig. 2), but the two substrates react in a different way. Whereas the high-fat diet leads to variable increases of IMCL in all 11 subjects, the situation is less clear for the changes in muscle glycogen content. As a matter of fact, decreases in glycogen were observed under the high fat as well as under the low-fat diet, and in 4 of 11 subjects, muscle glycogen content even increased while they were consuming the high-fat diet (data for individual time courses not shown).

$\dot{V}O_{2max}$ tests. There was no change in maximal power, maximal oxygen consumption, maximal carbon dioxide production, maximal blood lactate concentration, or maximal heart rate due to high-fat or low-fat diet (Table 4). Whereas maximal blood lactate concentrations were similar between diets, lower submaximal lactate concentrations were measured after the high-fat diet compared with the low-fat diet (Fig. 3).

Submaximal exercise testing. As indicated in Figure 4 and Table 5, respiratory exchange ratio was significantly lower at rest and at all exercise intensities after the high-fat

TABLE 3. Muscle structure and substrate content.

Mean Values ± SE	PRE	HF	LF
Volume density of total mitochondria (% of total fiber area)	9.38 ± 0.38	9.86 ± 0.36	9.79 ± 0.52
Intramyocellular lipid content (% of total fiber area)	0.79 ± 0.08	1.54 ± 0.27*	0.69 ± 0.09
($mmol \cdot kg^{-1}$ wet weight)	8.0 ± 0.9	15.6 ± 2.8*	6.9 ± 0.9
Muscle glycogen content ($mmol \cdot kg^{-1}$ dry weight)	545.8 ± 36.3	487.8 ± 38.2	534.4 ± 32.6

PRE, values during prestudy familiarization test week; HF, values after the 5-wk high-fat diet period; LF, values after the 5-wk low-fat diet period.
* Significantly different between high-fat and low-fat diet; $P < 0.01$.

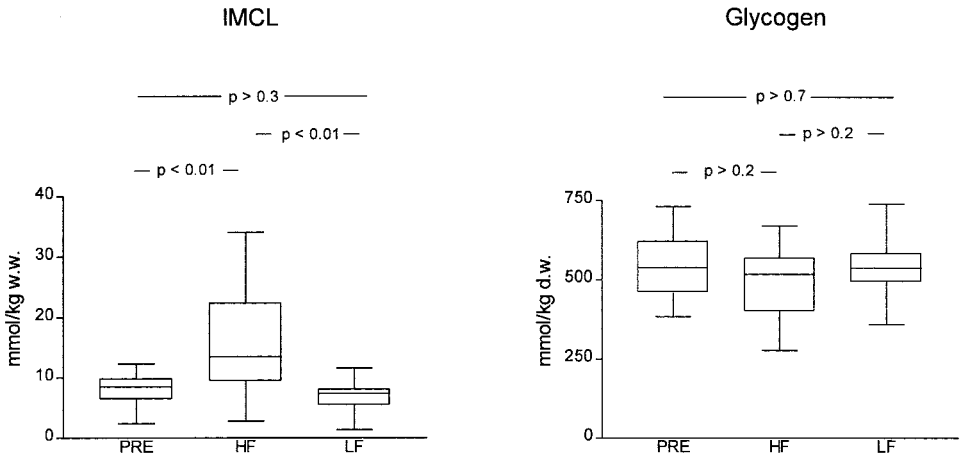

FIGURE 2—Box and whiskers plots of intramyocellular lipids (IMCL) and glycogen after the three different dietary interventions (PRE: values during prestudy familiarization test week. HF: values after the 5-wk high-fat diet period; LF: values after the 5-wk low-fat diet period). *Boxes* extend from the 25th to the 75th percentile with a *horizontal line* at the median, and *whiskers* show the range of the individual data.

diet compared with the low-fat diet. Exercise at 20%, 40%, 60%, and 75% of W_{max} intensities corresponded to 36%, 54%, 72%, and 86% of $\dot{V}O_{2max}$. Compared with the low-fat diet, oxygen consumption at rest was higher after the high-fat diet but was not different between diets during submaximal exercise steps. Carbon dioxide production was similar at rest but significantly higher during all exercise steps after the low-fat diet. Ventilation and rating of perceived exertion according to Borg remained unchanged between diets at rest and at each exercise intensity (data not shown).

Time-trial test. During the 20-min time trial test in which power output was self-adjusted by the subjects, no differences in endurance performance (work capacity) was found between diets (Table 5). Work capacity, measured as average power output that the subjects could perform over the 20-min period, corresponded to 79% of maximal power output and was close to 89% of $\dot{V}O_{2max}$. Average lactate concentration, ventilation, and rating of perceived exertion (data not shown) over the 20-min period were not different between diets.

Half-marathon run. The mean running time for the half-marathon was not different between the high fat (80 min 12 s ± 86 s) and the low-fat diet (80 min 24 s ± 82 s).

DISCUSSION

Subjects. Our subjects had a relatively high $\dot{V}O_{2max}$ of 64 mL·min^{-1}·kg^{-1} (range 59–70) and a predominance of Type I fibers in m. vastus lateralis as expected from an average competition history of over 5 yr, their ranking in competition and their weekly training volume (>6 h·wk^{-1}).

TABLE 4. Maximal values for variables obtained during incremental $\dot{V}O_{2max}$ tests.

Mean Values ± SE	PRE	HF	LF
Power (W)	377 ± 8.3	374 ± 8.9	375 ± 7.2
$\dot{V}O_2$ (mL·min^{-1})	4538 ± 72	4425 ± 90	4425 ± 65
$\dot{V}O_2$ relative to body weight (mL·min^{-1}·kg^{-1})	65.5 ± 1.1	63.6 ± 0.9	63.9 ± 1.2
$\dot{V}CO_2$ (mL·min^{-1})	5355 ± 107	5262 ± 109	5411 ± 144
Lactate (mmol·L^{-1})	8.4 ± 2.0	7.6 ± 1.5	8.1 ± 1.5
Heart rate (beats·min^{-1})	186 ± 2	183 ± 3	182 ± 3

$\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production; Lactate, capillary blood-lactate concentration; PRE, values during prestudy familiarization test week; HF, values after the 5-wk high-fat diet period; LF, values after the 5-wk low-fat diet period.

The high average volume density of mitochondria also points to outstanding aerobic work capacities of these subjects who were highly motivated to participate in this study. Their adherence to testing, training, and dieting procedures was excellent.

Diet. The diet intervention was designed such that the subjects themselves could monitor and adjust their diet composition on a daily basis. The protein content of the diets was kept constant at 14% over both periods and was in fact identical to the protein content of the habitual diet of the subjects. The fat content of 17% of energy during the low-fat period is typical for low-fat or high-CHO diets in many studies (14,18,26,31). During the high-fat period, the subjects consumed 53% fat energy similar to the study reported by Pogliaghi and Veicsteinas (31). This is substantially more than in the studies of Horvath et al. (18) and Hoppeler et al. (16) but considerably less than in many other studies on long-term diets (7,12–14,26,28,30,32). Some subjects had difficulties adjusting to the high-fat diet partly because of the reduced food volume, with the consequence that they inadvertently increased their caloric intake by 12%

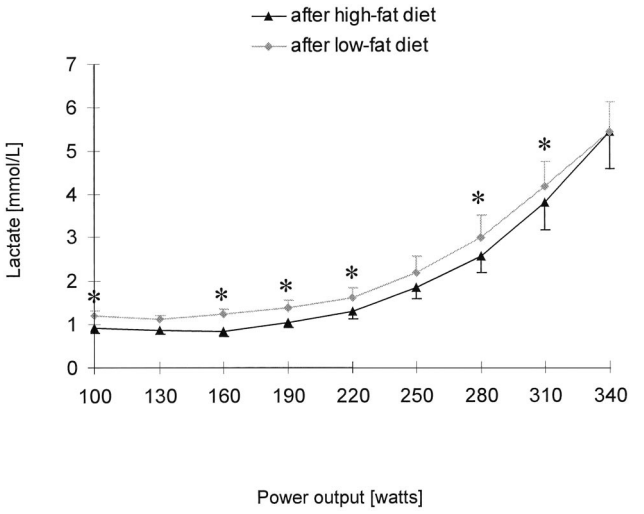

FIGURE 3—Blood lactate curves during incremental $\dot{V}O_{2max}$ test (values are means ± SE; * significantly different between high-fat and low-fat diet; $P < 0.05$).

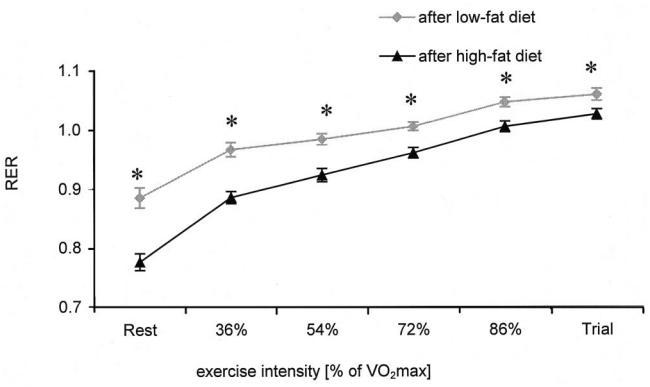

FIGURE 4—RER during 10 min at rest as well as at 20%, 40%, 60%, and 75% W_{max} and in the 20-min time-trial test (values are means ± SE; * significantly different between high-fat and low-fat diet; $P < 0.05$).

compared with the low-fat dietary period. A higher caloric intake on a high-fat diet with no changes in body mass had been noted previously (16). Despite the considerable increase in caloric intake in the present study, there was only a 0.2 kg increase for both body mass and body fat content that was not statistically significant (Table 1). Based on the observed difference in caloric intake, it can be calculated that a weight gain of approximately 1.4 kg should have resulted over the high-fat period, taking into account the reduction in energy expenditure due to the slightly lower training volume in that dietary period. If the statistically significant 9.2% increase in resting oxygen uptake after the high-fat compared to the low-fat period (Table 5) is considered to be representative for an increase in basal metabolism, the 12% increase in energy intake (Table 2) would nearly be compensated for, thus explaining the absence of a gain in body mass.

Intracellular substrate stores. In the present experiment, the ingestion of a high-fat diet effectively doubled IMCL content. A significant 1.9-fold increase in IMCL on a high-fat diet had previously been demonstrated by Helge et al. (14), using the biochemical determination method. In a previous study on trained runners on a high-fat diet (16), a 60% increase in IMCL, estimated by morphometry, failed to reach statistical significance due to large interindividual variation. Taking all these observations together, it appears that IMCL content depends on dietary fat supply and can be increased with high-fat diets. With regard to the carbohydrate stores, it was found that the subjects on the high-fat diet were not developing chronic depletion of their muscle glycogen. This might appear surprising at first sight. Quantitative aspects of glycogen depletion and repletion have been investigated by Costill et al. (9) more than 20 yr ago. These authors have shown that nearly three times more carbohydrate must be consumed than is utilized to restore depleted muscle glycogen concentration after exhaustive exercise. The subjects in the present study consumed at least 1000 kcal from CHO (250 g) on the high-fat diet. According to the training logs, they spent 1 h of training at 60–65% of $\dot{V}O_{2max}$, expending a total of some 900 kcal of which approximately 40% (360 kcal or 90 g of CHO) must have come from muscle glycogen (38). Therefore, they needed no more than one third to one half of the CHO ingested to refill the glycogen stores after the daily exercise bouts. The subjects were not suffering from muscle fatigue, and objectively there was only a minimal reduction in training volume by an average of 5 min·d^{-1} (Table 1, not statistically significant). In contrast to the observations in the present study, maintained muscle glycogen stores were not seen in studies in which diets contained 60% or more of the energy as fat (14,26), suggesting that muscle glycogen content would have been compromised on those diets. This aspect of reduced muscle glycogen in subjects on a high-fat diet is an important consideration in judging performance capacity. It is not surprising that in both studies reporting significantly reduced muscle carbohydrate stores, high- and moderate-intensity exercise capacities were found to be affected (14,26).

Muscle oxidative capacity. The volume density of mitochondria was not affected by either the high-fat or low-fat diet (9.86 vs 9.79% of mitochondria per muscle fiber

TABLE 5. Submaximal exercise test.

% of W_{max}	Diet	Power (W)	Lactate (mM)	$\dot{V}O_2$ (mL·min^{-1})	$\dot{V}CO_2$ (mL·min^{-1})	HR (min)	RQ (–)
Rest	HF	0	0.88 ± 0.06*	535 ± 22*	407 ± 17	66 ± 2	0.78 ± 0.01*
	LF	0	1.22 ± 0.14	490 ± 23	435 ± 25	64 ± 3	0.89 ± 0.02
20%	HF	76 ± 2	0.75 ± 0.1*	1622 ± 45	1418 ± 35*	100 ± 2	0.89 ± 0.01*
	LF	76 ± 2	1.01 ± 0.18	1586 ± 49	1534 ± 55	98 ± 2	0.97 ± 0.01
40%	HF	151 ± 3	0.72 ± 0.09*	2412 ± 57	2201 ± 44*	125 ± 3*	0.92 ± 0.01*
	LF	151 ± 3	1.07 ± 0.17	2319 ± 60	2281 ± 74	120 ± 3	0.98 ± 0.01
60%	HF	226 ± 5	1.45 ± 0.19*	3190 ± 54	3030 ± 54*	151 ± 2*	0.96 ± 0.01*
	LF	226 ± 5	1.82 ± 0.27	3131 ± 67	3151 ± 79	147 ± 3	1.01 ± 0.01
75%	HF	283 ± 6	3.38 ± 0.46*	3834 ± 66	3796 ± 84*	169 ± 2	1.00 ± 0.01*
	LF	283 ± 6	3.91 ± 0.39	3822 ± 76	4002 ± 95	167 ± 3	1.05 ± 0.01
Trial	HF	297 ± 7	5.22 ± 0.61	3939 ± 70	3985 ± 71*	178 ± 2*	1.03 ± 0.01*
	LF	298 ± 6	5.68 ± 0.49	3938 ± 74	4173 ± 101	174 ± 2	1.06 ± 0.01

Values are means ± SE, measured during the last 2 min at each exercise step. For the time trial test, values are measured during the entire 20-min period.
HF, values after the 5-wk high-fat diet period; LF, values after the 5-wk low-fat diet period.
* Significantly different between high-fat and low-fat diet; $P < 0.05$.

volume), indicating that the subjects adhered to an unchanged training schedule. Furthermore, the observation of an unchanged mitochondrial volume density after a high-fat diet is in good agreement with an unchanged citrate synthase activity in subjects on a high-fat diet for 15 d as demonstrated by Goedecke et al. (12).

Metabolism. Lower RER and lower blood lactate concentrations while exercising at submaximal intensities point to a more pronounced utilization of fat and to a glycogen sparing effect after the high-fat dietary period. To simulate the practice of endurance athletes, a preexercise meal containing either about 40 g or about 90 g of carbohydrates on high-fat or on low-fat diet, respectively, was consumed 2 h before each performance test. It is well known that the composition of the preexercise meal can influence subsequent substrate oxidation at rest and during exercise (17). From the present measurements, it cannot be distinguished between the effects of the chronic diet and of the preexercise meal on the observed metabolic shifts. Whitley et al. (42) have shown that under resting conditions RER is altered by a high-carbohydrate but not by a high-fat meal. In contrast to resting conditions, the pattern of substrate oxidation during exercise was found to be very resistant to preexercise dietary alterations in this particular study. Moreover, it was recently demonstrated that a shift toward a preference of fat oxidation persisted despite high carbohydrate availability induced by specific supply before and during exercise (6,7). These data as well as the present findings of an increased oxygen consumption at rest (Table 5) and doubled IMCL stores after the high-fat period allow us to assume that at least part of the metabolic shift was caused by the chronic dietary intervention. Other authors have demonstrated that the shift in substrate preference coincides with an increase of carnitine acyltransferase (CAT) activity of 20% after 10 d, and of 37% after 15 d on a high fat-diet without changes in citrate synthase (CS) and 3-hydroxyacyl-coenzyme A dehydrogenase (3-HAD) activities (12), indicating a qualitative shift in mitochondrial composition. The same authors further showed that during the high-fat period oxidation of muscle glycogen at a work intensity of 70% of $\dot{V}O_{2max}$ was reduced from 1.5 to 1 $g \cdot min^{-1}$. This finding is compatible with similar data reported by Lambert et al. (26). Unlike in animal experiments that show increases in citrate synthase and 3-hydroxyacyl-coenzyme A activities with high-fat diets (8), these mitochondrial enzymes remain unchanged in humans (12). This finding is supported by the unchanged mitochondrial volume density in the present study and further points to the fact that results obtained in diet studies on experimental animals may vary widely from studies carried out on humans (see also 27).

The shift toward a larger fat oxidation after the high-fat dietary period may simply result from a mass action effect by the increased IMCL content of the muscle. More than 20 yr ago, Standl et al. (34) showed that higher muscle triglyceride concentrations at rest were related to greater muscle triglyceride utilization during exercise. The question whether IMCL is actually being used during moderate intensity exercise is still controversially debated (for reference see 40). Some of the studies using biochemical techniques to determine muscle triglycerides did not show depletion of muscle lipid stores with prolonged bicycle exercise (23), but those stores decreased in the postexercise period (24). The authors of these studies speculated that their findings could indicate a metabolic priority of glycogen resynthesis in the postexercise period. The drawback of both studies lies in the methodological limitation to exclude extracellular triglyceride contamination when IMCL is estimated with a biochemical technique (19). A positive identification of the intracellular localization of triglycerides is possible using EM morphometric techniques (Fig. 1). Using this approach, it has been demonstrated that exercising for over 8 h during a 100-km run leads to an almost complete depletion of both IMCL and CHO stores in skeletal muscle (22). Likewise, studies using stable isotope techniques also indicate that at low to moderate exercise intensities oxidation of IMCL can account for 20–50% of total lipid oxidation (33,38). The assumption of IMCL representing a readily available substrate store for mitochondrial oxidation has received further support from noninvasive studies using proton MR-spectroscopy to identify IMCL (3,19). Making use of this technique, Kreis (25) demonstrated that moderate exercise of 2- to 3-h duration decreased IMCL by 35% in human soleus muscle. This finding is confirmed by results from our laboratory which indicate that IMCL in m. tibialis anterior are lowered by 24% after 2 h of running at 50% of $\dot{V}O_{2max}$ independent of the training status of subjects (10). Taken together, these data definitely indicate that IMCL can be utilized during low- to moderate- intensity exercise.

Performance. An important finding of the present study is that $\dot{V}O_{2max}$ and performance remained virtually identical under both diet regimes and at all exercise intensities tested. Maximal power during the $\dot{V}O_{2max}$ test, average power delivered during the time trial, and the time achieved during the half-marathon were not different. The observation of an unchanged performance capacity at moderate to high work intensities (>65% of $\dot{V}O_{2max}$) is compatible with the results published by other authors (7,12,14,30,31). In contrast, two studies have demonstrated a reduced endurance capacity on a high-fat diet (13,32). However, one of these studies combined training of previously untrained subjects with different diet protocols (13), making it difficult to assess the combined effects of diet and training. Moreover, the results of this study were not replicated in a later study of similar design (14). In the study of Pruett (32), only four subjects completed performance tests at 70% of $\dot{V}O_{2max}$ under both high-fat and low-fat conditions. Time to exhaustion in these tests was by some 30% shorter on the high-fat diet for three subjects but by 24% longer for the remaining subject. Therefore, the difference between high-fat and low-fat diets was not found to be statistically significant. Recent studies investigating short term (5-d) high-fat diets with pretest glycogen restoration found high-intensity work capacity to be essentially unaffected by the diet regime (5,6). In all of these experiments, and similar to our own results, respiratory exchange ratios were found to be lower on the high-fat diet. The data

currently available strongly suggest that short-term and long-term diets with fat contents >55% of energy reduce respiratory exchange ratios considerably but leave the capacity for moderate- to high-intensity work unaffected, provided muscle glycogen content is not compromised by the dietary intervention.

CONCLUSIONS

The main finding of this study is that in a population of highly trained subjects the ingestion of a high-fat diet for 5 wk leads to a more than twofold increase of the IMCL content without compromising muscle glycogen stores. The respiratory exchange ratio and blood lactate concentrations are decreased at rest and at all submaximal exercise intensities, indicating a metabolic shift toward a larger utilization of fat for energy supply, whereby it cannot be determined how much of the observed differences are due to the chronic diet or to the preexercise meal. However, the observed adaptations had no effect on moderate- to high-intensity exercise performance for time periods lasting 20–90 min. Similar to other reports (7,26,32), it was noted that the individual response to a diet intervention seems to depend on some undetermined characteristics of the subjects. Therefore, it may be important to keep the variation in individual response to dietary intervention in mind for the design of future studies and for counseling individual athletes.

The authors thank F. Graber and E. Wagner for excellent technical assistance.

This study was supported by Swiss National Science Foundation (Grant 3100-42449.94) to H. Hoppeler and funds of the Institute of Sports Sciences, Magglingen, Switzerland.

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