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ORIGINAL CONTRIBUTION

J. Décombaz
M. Fleith
H. Hoppeler
R. Kreis
C. Boesch

Effect of diet on the replenishment of intramyocellular lipids after exercise

Summary *Background* Muscle triglycerides are important as a source of energy and in relation to metabolic sensitivity. However, the classic biopsy method does not distinguish intra- from extracellular fat, and their regulation by exercise and diet is largely unknown. Magnetic resonance spectroscopy (MRS) is available to assess the intramyocellular lipid (IMCL) pool non-invasively in humans. *Aim of the study* The aim of this work was to use sequential MRS measurements of IMCL and glycogen to explore the role of three levels of dietary fat on the replenishment of these energy stores after exercise. *Methods* Following 2 h of exercise, two subjects (S1, S2) were fed one of three diets (15 %, 40 % or 70 % fat energy), each on a separate occasion. IMCL and glycogen were measured by MRS in the *tibialis anterior* muscle before, after exercise, and at 10 and at 32 h of recovery. *Results* Initial IMCL concentration (mmol · kg^{-1} : 3.0 in S1 and 1.8 in S2) was reduced to 70 % after exercise. The rate of replenishment was minimal with the low-fat (mmol · kg^{-1} · 24h^{-1} : 0.7 and 0.0) and much higher with both higher fat diets (mmol · kg^{-1} · 24h^{-1} : 3.1 and 3.2 in S1, 0.7 and 0.9 in S2). Glycogen and IMCL replenishments were inversely correlated. *Conclusions* IMCL and glycogen can vary acutely in response to diet after exercise. Studies are needed to determine if such variations occur within the range of ordinary diets and to clarify the functional significance of IMCL in differently active individuals.

Key words Muscle triglycerides – dietary fat – glycogen – exercise – magnetic resonance spectroscopy

C. Boesch · R. Kreis
Department of Clinical Research
MR-Spectroscopy and Methodology
MR-Center 1
University and Inselspital
3010 Bern, Switzerland

H. Hoppeler
Department of Anatomy, University
Bühlstrasse 26
3010 Bern, Switzerland

J. Décombaz (✉) · M. Fleith
Nestlé Research Center
Nestec Ltd
PO Box 44
1000 Lausanne 26, Switzerland
e-mail: jacques.decombaz@rdls.nestle.com

Introduction

Fat is a major fuel for mammalian daily physical activities. It is available for oxidation in muscle cells both from extramuscular (circulating lipids) and intramuscular (triglyceride stores) sources. Research in man has shown that a significant proportion of energy expenditure during endurance exercise may be derived from muscle triglycerides [4, 6, 9], suggesting that the provision of these stores may need to be secured for optimal performance. One recent study looking at the effect of dietary composition found an increase in total muscle triglyceride concentration 24 h after exercise [13]. However, the biopsy technique as used in that study fails to distinguish adipocytes interspersed between muscle fibers and the active intracellular compartment identified by ultrastructural morphometry and composed of triglyceride vesicles (IMCL, intramyocellular lipids).

^{1}H-magnetic resonance spectroscopy (MRS), by taking advantage of the difference in magnetic susceptibility between spherical IMCL droplets and cylindrical lipid layers, has demonstrated the ability to distinguish and quantify IMCL specifically and non-invasively [3, 12]. Using this method, we recently observed large differences in IMCL repletion after a marathon run with two post-exercise diets [2]. The aim of the present work was to combine ^{1}H-MRS and ^{13}C-MRS to observe IMCL and glycogen [8], respectively, and to explore the effect of three different levels of fat intake on the post-exercise replenishment of both muscular energy stores.

Experimental

Replenishment of the energy stores in muscle after exercise was assessed in two trained men with comparable aerobic capacity (54–56 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$), S1 (runner, age 53, 60 kg) and S2 (cyclist, age 27, 74 kg). The exercise consisted of moderate intensity (mean heart rate 133 beats · min^{-1}) running for two hours on rough ground. It was repeated weekly on three occasions, each time preceded by two days of individually replicated food and activity conditions. Exercise was followed on the three occasions by different, fully controlled recovery diets with equal energy (230 kJ · kg^{-1} · day^{-1}) and protein (12 % energy) content, but either low-fat (15 % energy), normal (40 %) or high-fat (70 %) content. A high proportion (90 %) of the lipids was from one oil source, with 18:1 n–9 (59 %), 18:2 n–6 (26 %) and 18:0 (3 %) as the main fatty acids. During recovery, physical activity involving the legs was kept to a minimum and as similar as possible on all three occasions. IMCL and glycogen were measured in the *tibialis anterior* muscle pre-exercise, post-exercise, and at 10 h (after two meals) and 32 h of recovery (after three more meals) from ^{1}H [3] and ^{13}C-MRS [1] peak areas, respectively. This muscle is recruited during running [11], and it was chosen because the orientation of the fibers nearly parallel to the magnetic field gives an optimal separation of the IMCL resonance [2, 3]. Absolute quantitation of IMCL levels in mmol/kg ww has been done according to an earlier report [2]; glycogen was quantified in arbitrary units (au) relative to the creatine signal. The typical measurement error for a single IMCL determination (CV 6.1 %) has been published in an earlier report [3]. The error for a single glycogen measurement has been determined from replicate scans (SD 0.15 au). Based on standard error propagation formulas, these errors for single measurements have been used to estimate the errors for IMCL and glycogen replenishment, respectively (in legend Fig. 1). Linear rates of replenishment were calculated using all three post-exercise values and expressed per 24 h.

Results

Subject S1 had higher concentrations than subject S2 of both IMCL and glycogen in the *tibialis anterior* muscle (Table 1). In both subjects, exercise led to a reduction of each substrate to about 70 % of its initial concentration. Post-exercise replenishment of IMCL was minimal with the low-fat diet and much higher with the normal-fat diet. Feeding the fat-rich diet did not increase it further (Fig. 1). Values for IMCL accumulation with increasing levels of dietary fat were 0.7, 3.1 and 3.2 (in S1) and 0.0, 0.7, 0.9 (in S2) mmol · kg^{-1} · $24h^{-1}$. Subject S1 reached IMCL concentrations above 5 mmol · kg^{-1} · $24h^{-1}$, far above resting values, with both higher fat diets. Rates of IMCL and glycogen replenishment were inversely correlated (within-subject correlation, –1.00 and –0.88, S1 and S2), in accordance with the respective fat and carbohydrate composition of the diets.

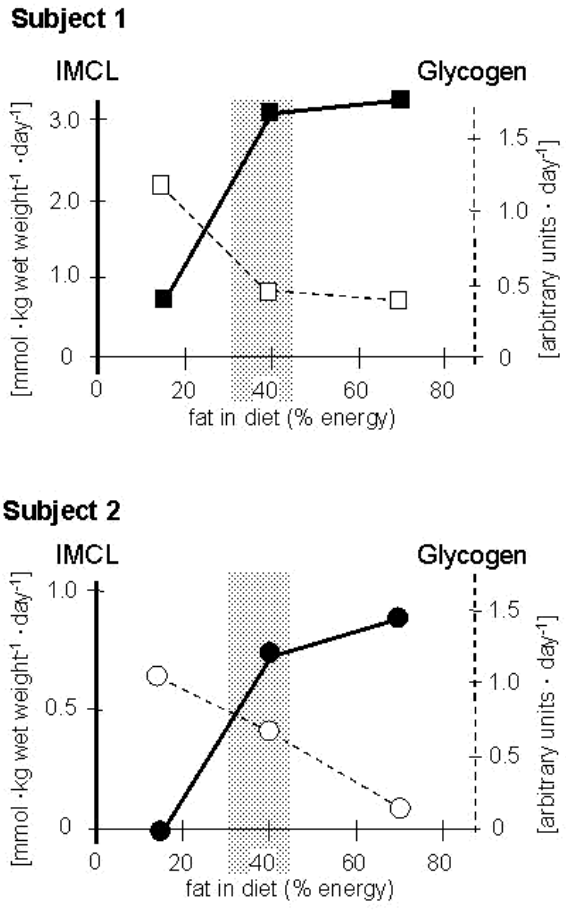

Fig. 1 Replenishment of intramyocellular lipids (filled symbols) and glycogen (empty symbols) in *tibialis anterior* muscle following a 2 h run, expressed per 24 h, on three diets with different fat content. Runner S1 (squares) and cyclist S2 (circles). *Dotted area*: normal range for dietary fat intake. The estimated errors for the replenishment rate were 0.15 mmol · kg^{-1} · $24h^{-1}$ for IMCL and 0.17 arbitrary units · $24h^{-1}$ for glycogen, respectively.

Table 1 Effect of running exercise on the concentrations of triglycerides and glycogen in *tibialis anterior* muscle

	Parameter	Pre-exercise[a]	Post-exercise[a]
IMCL	Subject 1	3.00 (0.62)	1.89 (0.30)
	Subject 2	1.79 (0.38)	1.42 (0.53)
Glycogen	Subject 1	2.59 (0.28)	1.88 (0.05)
	Subject 2	1.95 (0.06)	1.31 (0.30)

IMCL intramyocellular lipids (mmol/kg ww). Glycogen (arbitrary units). [a] Means (SD) of three trials.

Discussion

These results in two subjects bring support to the studies [e. g. 4, 6] indicating that total muscle triglycerides decrease during exercise and to the ultrastructural observations [9] localizing this change in the IMCL pool. Although classical studies have looked at the *vastus lateralis* muscle, the available evidence [2, 3] suggests that the *tibialis anterior* muscle responds to exercise qualitatively in a way comparable with thigh muscles.

The interesting finding of this study is the confirmation [2] that the IMCL content of human muscle cells can vary within a broad range and acutely in response to changes in diet. While IMCL storage was prevented when fat intake was small, similarly high rates of storage were reached with both the normal diet and the fat-rich diet, supporting the idea that fatty acid uptake by muscle is a saturable process [14]. Despite the salient similarity of their response, there was a large quantitative difference between both subjects. Differences in initial fuel stores as well as in rates of replenishment may have been caused by the training (running or cycling) influencing muscle characteristics differently, notwithstanding equal whole body aerobic capacity. Subject S1 accumulated as much IMCL in one day on the higher fat diets as his average initial muscle concentration. Supercompensation of IMCL following a high-fat diet seems indeed to have occurred, as it does in muscle glycogen following a high-carbohydrate diet.

These observations have a bearing in sport nutrition. Endurance training increases muscle triglyceride stores [5] as well as fatty acid uptake and oxidation [15]. This shift toward lipid oxidation during the submaximal periods of an exercise may spare glycogen (the obligatory fuel at high work intensity) and contribute to increase physical performance. Very high carbohydrate (hence low fat) diets that are optimal for glycogen loading before competition might simultaneously result in low muscle fat stores. Since prolonged, sustained exercise can nearly deplete IMCL [9], exercise conditions may exist where a conventional nutritional management will limit exercise capacity due to reduced availability of locally stored triglycerides. Conversely, IMCL supercompensation in trained muscles could perhaps confer a benefit in special categories of endurance performance.

The implications of these data extend to the metabolic fitness of sedentary people, for which there is growing evidence of a negative association between insulin sensitivity and total muscle triglyceride [10] or IMCL [7]. Since the largest change in IMCL replenishment occurred between 15 % and 40 % dietary fat, it would be of interest to know if a realistic dietary intervention such as shifting food habits from, say, 40 % to 30 % dietary fat would be enough to reduce IMCL stores. Why trained individuals tend to be more insulin sensitive than sedentary ones regardless of larger IMCL stores seems paradoxical and needs to be explained. It is also necessary to clarify whether modulations in IMCL concentrations by diet can be achieved in the absence of exercise prescription and with prevalent life style activities. Our early IMCL data [3] suggest that ordinary walking is capable of modulating IMCL to a significant extent.

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