

## Intramyocellular lipid content is increased after exercise in nonexercising human skeletal muscle

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**Schrauwen-Hinderling, V. B., L. J. C. van Loon, R. Koopman, K. Nicolay, W. H. M. Saris, and M. E. Kooi.** Intramyocellular lipid content is increased after exercise in nonexercising human skeletal muscle. *J Appl Physiol* 95: 2328–2332, 2003. First published August 15, 2003; 10.1152/jappphysiol.00304.2003.—Intramyocellular lipid (IMCL) content has been reported to decrease after prolonged submaximal exercise in active muscle and, therefore, seems to form an important local substrate source. Because exercise leads to a substantial increase in plasma free fatty acid (FFA) availability with a concomitant increase in FFA uptake by muscle tissue, we aimed to investigate potential differences in the net changes in IMCL content between contracting and noncontracting skeletal muscle after prolonged endurance exercise. IMCL content was quantified by magnetic resonance spectroscopy in eight trained cyclists before and after a 3-h cycling protocol (55% maximal energy output) in the exercising vastus lateralis and the nonexercising biceps brachii muscle. Blood samples were taken before and after exercise to determine plasma FFA, glycerol, and triglyceride concentrations, and substrate oxidation was measured with indirect calorimetry. Prolonged endurance exercise resulted in a  $20.4 \pm 2.8\%$  ( $P < 0.001$ ) decrease in IMCL content in the vastus lateralis muscle. In contrast, we observed a substantial ( $37.9 \pm 9.7\%$ ;  $P < 0.01$ ) increase in IMCL content in the less active biceps brachii muscle. Plasma FFA and glycerol concentrations were substantially increased after exercise (from  $85 \pm 6$  to  $1,450 \pm 55$  and  $57 \pm 11$  to  $474 \pm 54$   $\mu\text{M}$ , respectively;  $P < 0.001$ ), whereas plasma triglyceride concentrations were decreased (from  $1,498 \pm 39$  to  $703 \pm 7$   $\mu\text{M}$ ;  $P < 0.001$ ). IMCL is an important substrate source during prolonged moderate-intensity exercise and is substantially decreased in the active vastus lateralis muscle. However, prolonged endurance exercise with its concomitant increase in plasma FFA concentration results in a net increase in IMCL content in less active muscle.

intramuscular triglycerides; inactive muscle; fat oxidation; magnetic resonance spectroscopy

DURING SUBMAXIMAL exercise, fat and carbohydrate form the main substrates for the exercising muscle. Stable isotope studies have shown that ~50% of total fat oxidation is accounted for by plasma free fatty acid

(FFA) oxidation, suggesting that other fat sources play a substantial role in energy provision (8, 14, 21). Other fat sources include intramuscular lipid stores (4, 5) and lipoprotein-derived triglycerides (10). Recent studies using <sup>1</sup>H-magnetic resonance spectroscopy (MRS) have shown that intramyocellular lipid (IMCL) content is decreased after prolonged endurance exercise in active muscle (4, 7, 13, 17). This suggests that IMCL oxidation contributes significantly to total energy expenditure. From the net depletion of IMCL content measured before and after a marathon run, Boesch et al. (2) estimated that between 11 and 26% of the energy provided by fat oxidation had originated from IMCL breakdown.

As stated above, oxidation of IMCL and lipoprotein-derived triglycerides contributes substantially to fat oxidation during exercise; however, the largest contribution is thought to come from plasma FFA. During prolonged endurance exercise, adipose tissue lipolysis is substantially increased to enable ample provision of plasma FFA to the working muscle. However, the release of fatty acid (FA) by lipolysis exceeds the oxidation capacity of the working muscle, as it has been shown that only 70–90% of the rate of disappearance of FFA is oxidized to CO<sub>2</sub> (20, 21). The remaining part of FA taken up might be reesterified in adipose tissue, liver, and exercising or nonexercising skeletal muscle. The contribution of adipose tissue is considered to be very small (19), and hepatic reesterification was estimated to account for 24% of total reesterification (19). Skeletal muscle has been shown to take up and reesterify FFA during exercise (19). Muscle contraction strongly increases the percentage of plasma FFA that is routed toward oxidation, leading to a net decrease in muscle triglyceride during exercise. In noncontracting muscle, however, the abundance of FFA during exercise could lead to an increase in muscle triglycerides.

Under resting conditions, skeletal muscle is capable of clearing FFA from plasma, and elevated plasma FFA concentrations lead to a net accumulation of IMCL. Infusions of triglyceride-heparin (1), 72 h of fasting

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(18), as well as 3 days of high-fat diet all lead to elevated plasma FFA levels and an increased IMCL content. Therefore, it is generally thought that, during resting conditions, elevated plasma FFA concentrations stimulate reesterification of FA in skeletal muscle. However, during exercise, lipolysis is stimulated in adipose tissue as well as in skeletal muscle. Although prolonged moderate- to high-intensity exercise leads to a net decrease in IMCL content in contracting muscle, it could be speculated that, similar to the observations at rest, IMCL content is increased in noncontracting muscle.

In the present study, we measured IMCL content in both exercising leg muscle and nonexercising arm muscle before and after a 3-h cycling protocol. If oxidation rate outweighs the rate of reesterification in contracting muscle, we would expect IMCL content in the exercising leg muscle to decrease. Analogously, if reesterification rate exceeds FFA oxidation rate in noncontracting muscle, a net increase in IMCL content in the nonexercising arm muscle should be observed.

## MATERIALS AND METHODS

### Subjects

Eight young [age:  $24 \pm 3$  (SD) yr], highly trained [maximal energy output ( $W_{\max}$ ) per kilogram:  $5.8 \pm 0.46$ ] male cyclists participated in this study. The study was approved by the institutional Medical Ethics Committee. Subjects gave their written, informed consent after the nature of the procedure and possible risks were explained.

### Experimental Protocol

Before the test days, subjects received a standardization diet ( $13 \pm 1\%$  of energy as protein,  $56 \pm 3\%$  as carbohydrate, and  $30 \pm 1\%$  as fat) for 3 days during which they refrained from exercise. On the test day, subjects reported to the laboratory in the early afternoon. A baseline  $^1\text{H-MRS}$  scan was performed to quantify IMCL content in the vastus lateralis and the biceps brachii muscle. Subsequently, a blood sample was collected (7 ml). A 3-h cycling protocol was then performed. After 5 min of warming up at 100 W, subjects cycled for 3 h at 55% of their  $W_{\max}$ , which averaged  $231 \pm 19$  W. During the exercise trial, oxygen uptake ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) were measured every 30 min continuously over 10-min intervals (Oxycon- $\beta$ , Mijnhardt, Mannheim, Germany). From respiratory measurements, total fat and carbohydrate oxidation rates were calculated by using the nonprotein respiratory quotient as given below (12)

$$\text{Fat oxidation rate} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2 \quad (1)$$

$$\text{Carbohydrate oxidation rate} = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2 \quad (2)$$

with  $\dot{V}O_2$  and  $\dot{V}CO_2$  in liters per minute and oxidation rates in grams per minute.

Subjects were allowed to drink water ad libitum during cycling. After cessation of exercise, subjects were allowed a quick shower (maximum 5 min), after which a second blood sample was taken. Immediately after this, a second MRS measurement was performed to quantify IMCL content in the leg and subsequently in the arm muscle.

### Procedures

**MRS measurement.** Image-guided, localized, single-voxel  $^1\text{H-MRS}$  was performed in the vastus lateralis and the biceps

brachii muscle before and immediately after the 3-h cycling test. The measurements were performed on a 1.5-T whole body scanner (Intera, Philips Medical Systems, Best, the Netherlands) with a flexible surface coil wrapped around the upper leg and arm, with the extremity placed in the parallel position with the  $B_0$  magnetic field.

In every subject, voxels were carefully placed at the same position for repeated measurements. Care was taken to avoid vascular structures and adipose tissue deposits within the voxel. To reproduce the same voxel position, the longitudinal distance of the voxel from the intercondylar eminence of the knee joint or from the elbow was determined in a coronal image. T2-weighted turbo spin-echo magnetic resonance images, consisting of five transversal slices, were acquired at this position (slice thickness: 5 mm, repetition time: 2,000 ms, echo time: 85 ms, echo train length: 12, field of view: 210 mm, and matrix size:  $256 \times 256$ ). The patterns of the fat distribution were used to verify the longitudinal position and as landmarks to reproduce the voxel position in the transversal plane.

$^1\text{H-MRS}$  spectra from the regions of interest were acquired by using a point-resolved spectroscopy sequence with the following acquisition parameters: repetition time: 3,000 ms, echo time: 25 ms; 16-phase cycles, 128 averages; 1,024 data points over 1,000-Hz spectral width. The voxel volume was  $(12 \times 11 \times 18) \text{ mm}^3$ . The water signal was suppressed by using chemically selective saturation. The unsuppressed water signal was subsequently measured in the same voxel under the same shimming conditions and was used as a reference signal.

**Postprocessing.** The spectra were fitted in the time domain by using a nonlinear least squares algorithm [AMARES (22)] in the jMRUI software package (9) (<http://www.mrui.uab.es/mrui/>). Three peaks were fitted for IMCL (I1, I2, and I3 in Fig. 1) and three peaks for extramyocellular lipids (EMCL) (E1, E2, and E3 in Fig. 1). Prior knowledge of the relative peak positions (3) and area ratios (C. Boesch, private communication) as determined by Boesch and colleagues was used as a constraint. To increase the accuracy and reliability of the fitting procedure in the vastus lateralis muscle, the line width of the  $\text{CH}_2$  peak of IMCL (I2 in Fig. 1A) was fixed relative to the line width of the unsuppressed water peak (measured in the same voxel), and soft constraints were used on the line width and the position of the EMCL peak. In the biceps brachii muscle, these additional constraints were unnecessary. IMCL and EMCL peaks were fitted with a Gaussian curve, whereas water peaks were fitted with a Lorentzian curve. The signals were corrected for T1 and T2 relaxation by using the T1 and T2 relaxation times as determined by Schick et al. (16). The corrected area of the  $\text{CH}_2$  peak of IMCL was expressed relative to the area of the water peak.

### Analysis

**Blood analyses.** Samples of venous blood (7 ml) were obtained in EDTA-coated tubes and immediately centrifuged at high speed (1,000 g,  $4^\circ\text{C}$ ) for 10 min. Plasma was immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analyses for FFA (Wako NEFA test kit, Wako chemicals, Neuss, Germany), free glycerol (148270, Roche Diagnostics, Indianapolis, IN), and triacylglycerol (GPO-trinder 337B, Sigma Diagnostics, St. Louis, MO) on a COBAS FARA semi-automatic analyzer (Roche, Basel, Switzerland).

**Statistics.** Characteristics of subjects and diet composition are reported as means  $\pm$  SD, whereas results are reported as means  $\pm$  SE. Statistical analyses were performed with SPSS for Windows 10.0.0 software (SPSS, Chicago, IL). Differences

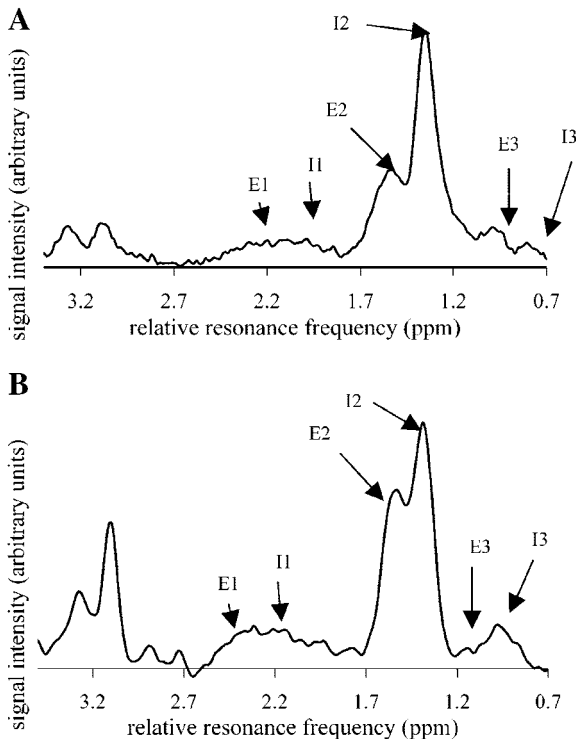


Fig. 1. Typical spectra of the vastus lateralis muscle (A) and biceps brachii muscle (B). Extramyocellular lipid peaks are depicted as E1–E3; intramyocellular lipid (IMCL) peaks are depicted as I1–I3. ppm, Parts/million.

in values before and after exercise were detected with a paired *t*-test. Results were considered significant if  $P < 0.05$ .

## RESULTS

### Substrate Oxidation

Whole body fat oxidation increased continuously during the 3-h cycling protocol, whereas carbohydrate oxidation decreased (see Fig. 2). Subjects oxidized on average  $91 \pm 4$  g of fat and  $494 \pm 17$  g of carbohydrate during the exercise protocol. The total energy expenditure amounted to  $11.7 \pm 0.2$  MJ. The relative contribution of fat oxidation to the total energy expenditure

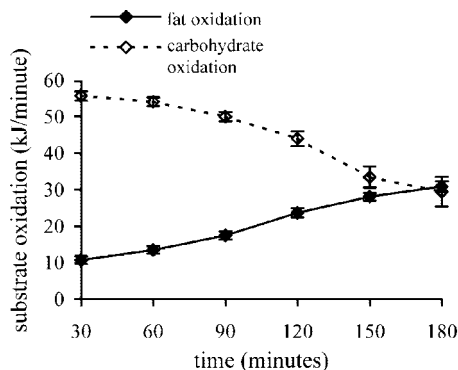


Fig. 2. Average fat and carbohydrate oxidation during the 3-h cycling protocol. Values are means  $\pm$  SE. Solid line and  $\blacklozenge$ , fat oxidation; dashed line and  $\diamond$ , carbohydrate oxidation.

was  $32 \pm 1\%$ , whereas the relative contribution of carbohydrates was  $68 \pm 1\%$ .

### Blood Parameters

Plasma FFA concentrations increased during exercise ( $85 \pm 6$  and  $1,450 \pm 55$   $\mu$ M before vs. after exercise, respectively,  $P < 0.001$ ). Glycerol concentrations also increased ( $57 \pm 11$  and  $474 \pm 54$   $\mu$ M before and after exercise, respectively,  $P < 0.001$ ), whereas triacylglycerol concentrations decreased during exercise ( $1,498 \pm 139$  and  $703 \pm 57$   $\mu$ M before and after exercise, respectively,  $P < 0.001$ ).

### IMCL and EMCL Content

Baseline IMCL content was four times higher in the vastus lateralis muscle compared with the biceps brachii muscle ( $P < 0.001$ ). IMCL content at baseline was  $0.61 \pm 0.05\%$  of the water resonance in the vastus lateralis muscle and decreased by  $20.4 \pm 2.8\%$  ( $P < 0.001$ , see Fig. 3A). The EMCL content did not change significantly ( $0.39 \pm 0.06\%$  before vs.  $0.36 \pm 0.06\%$  after exercise,  $P = 0.3$ ). In the nonexercising biceps brachii muscle, IMCL content at baseline was  $0.14 \pm 0.02\%$  of the water resonance and increased after exercise by  $37.9 \pm 9.7\%$  ( $P < 0.01$ , see Fig. 3B). The EMCL content did not change significantly ( $0.15 \pm 0.03\%$  before vs.  $0.15 \pm 0.01\%$  after exercise,  $P = 1.0$ ).

If values are converted to millimoles per kilogram muscle wet weight, as described by Boesch et al. (2), IMCL content is  $7.8 \pm 0.4$  and  $6.2 \pm 0.5$  mmol/kg in the vastus lateralis muscle and  $1.8 \pm 0.2$  and  $2.5 \pm 0.3$  mmol/kg in the biceps brachii muscle before and after exercise, respectively.

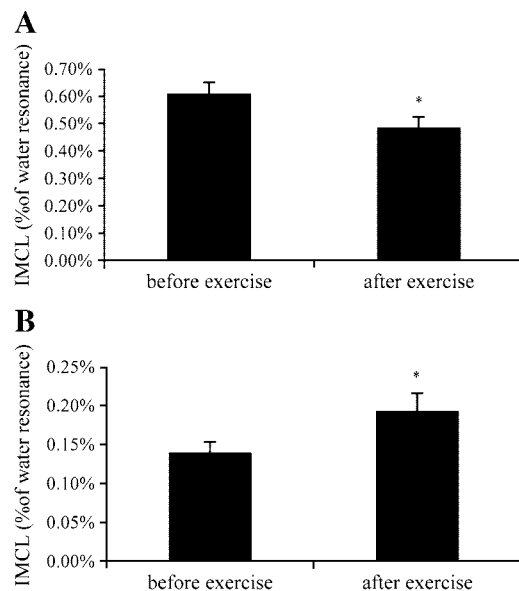


Fig. 3. IMCL content in the vastus lateralis muscle (A) and biceps brachii muscle (B) before and after the 3-h cycling protocol ( $n = 8$ ). Content is expressed as percentage of the  $\text{CH}_2$  peak of IMCL relative to the water signal. Values are means  $\pm$  SE. \*Significant differences between values before and after exercise ( $P < 0.05$ ).



## DISCUSSION

The most important finding of the present study is that IMCL content in the less active muscle of the arm increased during prolonged submaximal cycling exercise, whereas we confirm earlier reports of decreasing IMCL content in the working leg muscle (4, 7, 13, 17). We suggest that, from the increased plasma FFA levels and subsequent increase in FFA uptake during exercise, a large portion of FA is routed toward oxidation in contracting muscle, whereas, in noncontracting muscle, plasma FFA uptake leads to a net accumulation of IMCL. This is in line with results from stable isotope studies from Sacchetti et al. (15), who describe a net uptake and partial resynthesis of FFA by nonexercising leg muscle during one-legged exercise.

Others have reported that elevated FFA plasma concentrations increase uptake under resting conditions. Boden et al. (1) reported that an increase in FFA (due to lipid-heparin infusions) during 3 h resulted in an increased IMCL content in the soleus muscle, with the increase in IMCL content correlating with the FFA plasma concentrations. In addition, Stannard et al. (18) observed that the increase in FFA concentration during 72 h of fasting was accompanied by elevated IMCL content. Similarly, in the present study, FFA levels increased during exercise, and the IMCL content in the biceps brachii muscle was elevated after exercise. These results suggest that, also in a physiological situation such as during exercise, relatively short-term elevated FFA concentrations lead to accumulation of IMCL in nonexercising muscle, thereby contributing to the clearance of FFA from plasma. As FFA are generally thought to be toxic at higher concentrations, it is important that plasma FFA concentrations be controlled. Inactive skeletal muscle seems to contribute to this control by acting as a buffer for FFA.

In contrast to the biochemical quantification of lipids in biopsy studies, the MRS technique enables us to differentiate lipid signals from two compartments, namely IMCL droplets and adipose tissue between muscle cells (EMCL) (4, 16). However, possible limitations of the technique should be considered. If the peaks are not clearly separated, the IMCL signal could be contaminated by the neighboring EMCL signal. In the present study, the EMCL and IMCL peaks are separated by 0.17 parts/million on average, which is typical for the vastus lateralis muscle (6) and which is sufficient for the quantification of the two peaks. Furthermore, the EMCL-to-IMCL ratio in the population of the highly trained athletes of the present study is relatively low, and, therefore, contamination of the IMCL by the EMCL signal is expected to be relatively low as well.

Lactate, which accumulates in muscle at high-intensity exercise, resonates in the same frequency range as the lipid signals and could, in principle, contaminate the lipid signals. Therefore, we estimated the possible contribution of residual lactate to the lipid signals. The estimation is based on a half-time of lactate disappearance of 10.6 min (11), the delay of 30 min between the

end of the cycling exercise and the actual spectroscopic measurement, and a lactate concentration of 11.6–15.6 mmol/kg dry wt immediately after exercise (21). From these calculations, we conclude that the contribution of lactate to the lipid signals is <0.5% in the present study.

Care should be taken when using the water signal as concentration reference, because water and electrolyte shifts during exercise are possible. An alternative would be to use total creatine as a reference. When using total creatine instead of water as internal standard in the present study, similar results were obtained. We chose to use the water peak as internal reference, because the use of the small peak of total creatine introduced some additional variability so that the average coefficient of variation of repeated measurements increased from an average of 6% with water to 11.5% with total creatine as a reference (17). Because no trends in water signal were found before vs. after exercise, we concluded that it is justified to use the water signal as internal standard in the present study.

We report considerably lower values of IMCL content in the biceps brachii muscle, compared with the IMCL content in the vastus lateralis muscle. This agrees with the finding by Boesch et al. (2) that IMCL content is highly variable between muscle groups and is in line with findings by Ward et al. (23), who also report lower IMCL content in forearm muscle compared with leg muscle.

The EMCL content did not change significantly with exercise, either in the arm or in the leg muscle. However, EMCL data should be interpreted with care, as EMCL content generally shows a high spatial variation (4). Small changes in voxel position can have a high impact on the EMCL signal intensity. Furthermore, the ratio of EMCL-to-IMCL content in the leg of highly trained athletes of the present study is relatively low. Therefore, contamination of the EMCL signal by the IMCL signal is possible.

From the finding that IMCL content is increased during exercise in nonexercising muscle, the question arises as to whether FFA could also be reesterified in exercising muscle. This might not result in a net increase in IMCL, because of the continuous oxidation of IMCL during contraction. However, the simultaneous reesterification and even stronger hydrolysis of triglycerides could result in a turnover of IMCL with a net decrease in IMCL content in exercising muscle. The turnover is not quantifiable by <sup>1</sup>H-MRS, because only IMCL content, but not flux, is measured with this method. Using stable isotopes, Sacchetti et al. (15) reported that FFA are reesterified in both contracting and noncontracting muscle during one-legged exercise. However, the rate of esterification was about four times higher in the noncontracting leg compared with the contracting leg. Although significant esterification took place also in the active leg, intramuscular lipid content was decreased by 30% after exercise. Because reesterification has been shown to take place also in contracting muscle, the depletion data of the leg in the

present study represent net changes and cannot be directly interpreted as fat oxidation. However, the data can be interpreted as a minimal estimate of IMCL oxidation. In the study of Sacchetti et al. (15), no net increase in muscle triglyceride was found in the non-contracting leg muscle. This discrepancy with the present study could be due to a different exercise protocol used and different muscles investigated.

In conclusion, we report the capacity of noncontracting muscle to take up and reesterify FFA during physiologically elevated plasma FFA. During exercise, when peripheral lipolysis is stimulated to increase plasma FFA availability and substrate oxidation, a net accumulation of IMCL occurs in nonexercising muscle.

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#### DISCLOSURES

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