# Evaluation of Intramyocellular Lipids Concentration in the Different Thigh and Calf Muscles before and after Endurance Exercise

M. Zehnder[1], P. Saillen[1], R. Kreis[1], C. Boesch[1], P. Vermathen[1]

[1]Dept. Clinical Research, University & Inselspital, Berne, Switzerland

**Introduction:** Several studies have shown that intramyocellular lipids (IMCL) are important fuels during prolonged moderate-intensity exercise in well-trained athletes [e.g. 1-3], and that the fat content of the diet influences the IMCL recovery rate [4]. However, little is known about specific IMCL usage in different muscle groups during exercise. Previously, we have shown the feasibility of $^1$H-MRSI to measure IMCL changes after exercise simultaneously in different muscles [5]. In the present study we employed and extended this technique to measure IMCL changes in thigh and calf using a highly standardized exercise protocol to allow for a detailed muscle specific analysis of metabolite changes.

## Methods

Subjects: Eight trained cyclists (6 males, 2 females, 31±9 y, 8±4 training h/week) and 8 trained male runners (29±5 y, 8±4 training h/week) exercised 3 h on a cycle ergometer (at 49±3 % of max workload = 69±4 % of max heart rate) or on a treadmill (at 52±8 % of max speed = 75±5 % of max heart rate), respectively. The subjects added supplementary 0.75 g fat/kg body weight to their diet during 2 days prior to exercise to ensure full IMCL stores. In 2 male cyclists right and left thigh muscles as well as in 4 runners right and left calf muscles were examined pre- and post-exercise. IMCL concentration was determined before and after endurance exercise in *m. rectus femoris (RF), vastus intermedius (VI), medialis (VM), lateralis (VL1, VL2)* and *adductor magnus (AM)* for the cyclists and in *m. soleus (S), gastrocnemius (G), tibialis anterior (TA)* and *extensor digitorum (ED)* for the runners. Blood triacylglycerol (TAG) was measured at rest and at regular intervals until the end of the test.

MRSI Measurements: Measurements were performed using a 2D MRSI sequence in transverse orientation with PRESS volume pre-selection (1.5T system, SIGNA, GE). Spectra were acquired with TR 1200 ms, TE 35 ms, water presaturation, Matrix: 36×36 (circular sampling) over a FOV of 20 cm. Postprocessing of the MRSI data included spatial zerofilling to 64x64, moderate spatial apodization and lipid extrapolation [6]. The spectra were fitted using an iterative non-linear least squares fitting algorithm ("TDFDFIT" [7]), using prior knowledge. IMCL of each voxel was assigned to a specific muscle (see above) using image segmentation. For each muscle an average IMCL signal area was calculated and the concentration was determined using bone marrow lipid signal as internal reference.

**Results:** In Figs. 1 and 2 are shown individual IMCL concentrations in all investigated muscles, plotted before versus after exercise. In Fig. 3 are shown IMCL concentrations averaged for all muscles before (b) and after (a) exercise. The Figures demonstrate a) a good correlation of IMCL before and after exercise in thigh and calf muscles ($R^2$=0.75, p<0.0001), b) higher resting IMCL concentration in S than in G and TA for calf and higher resting IMCL in vastus muscles than in RF for thigh muscles, c) strongly reduced IMCL after exercise (values below identity line), d) stronger absolute IMCL depletion in muscles with high IMCL resting concentration (slope of 0.52 and 0.43 in thigh and calf respectively) e) with increasing resting IMCL a trend towards approaching a lower threshold IMCL after exercise (Fig. 2, dashed line) has been observed (statistically significant for calf muscles).

Between muscles, beside significant differences in *absolute* IMCL depletion, also significant differences in *relative* IMCL reduction (relative to resting IMCL) were detected with higher reductions in "*slow*" than in "*fast*" muscles, e.g. between S (*slow*) and G (*fast*) for calf muscles, and between the vastus muscles (*slow*) and RF (*fast*) for thigh muscles. This is demonstrated for S and G in Fig. 4 showing that for all but one subject relative IMCL reduction was lower in G than in S. IMCL levels in right and left legs were highly correlated. Blood TAG increased significantly during both kinds of exercise ($p < 0.01$) but no correlation was found between this increase and IMCL utilization or between resting TAG and resting IMCL in any muscle. In addition, it was found that the TMA peak increased significantly with exercise.

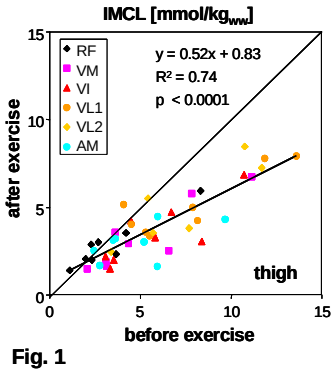


Fig. 1

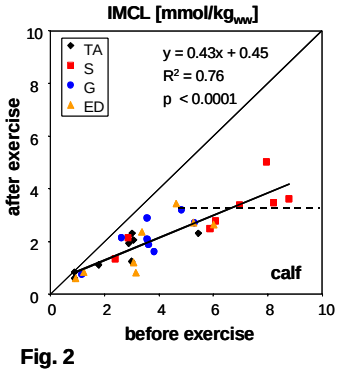


Fig. 2

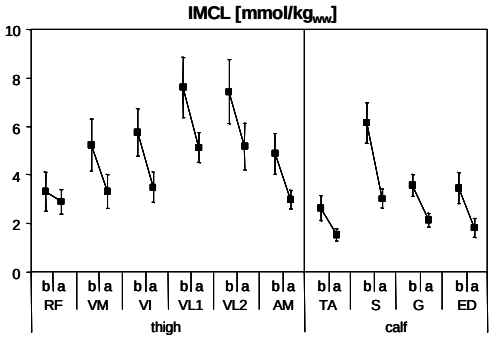


Fig. 3

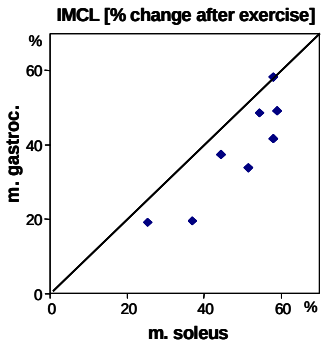


Fig. 4

**Discussion:** The good correlation of IMCL before and after exercise confirmed the feasibility of the method. The results showed significant and muscle specific reductions of IMCL after exercise in different muscles. In general, resting IMCL and absolute as well as relative IMCL reduction after exercise was higher in slow compared to fast muscles. The results demonstrate the potential of the method and may help to shed further light on the physiological role of IMCL. The increase in TMA is probably related to the appearance of acetyl-carnitine with exercise and differing MR-visibility between carnitine and acetyl-carnitine [8]. Acetyl-carnitine is thought to buffer the ratio of free to acetylated coenzyme A in heavy exercise. This is measured in a spatially resolved manner for the first time, but needs further elucidation.

**References:** **1.** van Loon et al. Am J Physiol Endocrinol Metab 285: E804, 2003; **2.** Watt et al. J Physiol 541: 969, 2002; **3.** Boesch et al. Proc Nutr Soc. 58:841, 1999; **4.** Décombaz et al. Eur J Nutr 39: 244, 2000; **5.** Vermathen et al. Proc. ISMRM 11: 786, 2003; **6.** Haupt et al, Magn. Reson. Med. 35: 678, 1996; **7.** Slotboom et al. Magn. Reson. Med. 39: 899, 1998; **8.** Kreis et al. NMR Biomed. 12:471, 1999

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