Quantification of lipids in human lower limbs using yellow bone marrow as the internal reference: gender related effects

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Introduction
Assessment of the lipids in skeletal muscle has been shown to be of importance, both in the investigation of muscle lipid metabolism in healthy volunteers and in studies of patients with metabolic disorders. In muscle tissue, lipids are stored either as extra- (EMCL) or as intramyocellular (IMCL) lipids [1]. Whereas EMCL are metabolically relatively inert, IMCL are built up, and used within several hours. A number of reports showed that IMCL levels are dependent on many factors, such as diet [2], physical activity [3], obesity [4], gender [5], age [6], muscle type [7], etc. These factors underline the importance of being aware of all potential relations if a prospective study is not to be influenced by uncontrolled, concomitant factors. The aim of this study was to investigate whether there is a gender difference in the lipid concentrations in different muscle compartments of the legs of normal volunteers.

Methods and Materials
The study groups consisted of ten females and ten males. The subjects were healthy, normal-weight, passive in free time physical activity and with sedentary occupations. Mean age and body mass index (BMI) of the females were 27.5 ± 4.7 (range, 23 - 35 years) and 21.7 ± 1.6 kg/m² (range, 19.6 - 25.1), respectively. The mean age and BMI of the males were of 28 ± 3.5 (range, 24 - 35 years) and 23.6 ± 1.4 kg/m² (range, 21.7 - 25.7), respectively.

All data were acquired on a 1.5 T Gyroscan Intera MR scanner (Philips). MRS session was performed after a ~12 h overnight fast period. The thigh muscles were measured in the axial slice approximately 20 cm above the centre of knee. The axial slice of the calf was placed in the most extended part of the calf. The measurements were performed using the high-spatial-resolution MRSI technique [4, 8]. The method uses 2D rf spoiled gradient-echo sequence with step increments of TE, while TR and bandwidth per pixel were kept constant. The slice thickness was 15 mm. Methods, such as noncontinuous and irregularly shaped volume of interest (VOI), Thigh muscles were segmented in three groups: quadriceps (qua), biceps (bic), and adductor (add) compartments (Fig. 1a, c). Calf muscles were divided in six VOIs: gastrocnemius medialis (gm), gastrocnemius lateralis (gl), soleus (so), and deep posterior (dp), anterior (ac), and lateral (lc) compartments (Fig. 1b, d). VOI slice_1 (Fig. 2a) represents the whole slice without subcutaneous fat, bones and bone marrow (white and gray pixels in Fig. 1c, d) VOI slice_2 (Fig. 2b) represents the lean muscles (gray pixels in Fig. 1c, d) and was made from slice_1 by omitting the white voxels with dominant EMCL spectral lines. The white voxels were selected by thresholding the fat image [8]. Module spectra were computed from VOIs depicted by white and/or gray pixels shown in Fig. 1c and d. Total fat was quantified using the spectra computed from the white and gray pixels in the considered VOI. IMCL concentrations were evaluated from the VOIs which contained gray voxels with well resolved IMCL spectral lines. The fat content in % was computed using the methylene EMCL intensity of the VOI with 100% fat content as the internal standard. The VOI was defined in the central part of the tibial or femur bone marrow.

Results
Figure 2 shows the examples of water suppressed magnitude spectra of the calf. The spectrum of the whole slice with the exception of bones, and bone marrow is shown in Fig. 2a (VOI, slice_1). Figure 2b shows the spectrum of lean muscles (VOI, slice_2) and spectrum processing results. Figure 3 shows the mean lipid content in the thigh and calf muscles. No significant differences in total fat and IMCL concentrations of correspondent VOIs were found between genders (P > 0.05). A common feature for both genders was a higher total fat content in the thigh compared with the calf. Total fat content was higher by factor ~2.5 (Fig. 3a, b, VOI, slice_1). The mean IMCL level was, however, more than 3 times higher in the calf muscles compared with the thigh (Fig. 3c, d, VOI, slice_2).

Discussion
This study found insignificant differences in total fat and IMCL levels between males and females in both thigh and calf muscles. The lack of significance in these results is probably due to the large between-subject variability in both genders. To our knowledge, there are no previous MRS estimates of the total fat and IMCL content of the thigh muscles of normal and untrained volunteers that can be compared with our results. The positive feature of our MRSI method is high spatial resolution. It provides advantages over the conventional spectroscopic methods, such as noncontinuous and irregularly shaped VOIs (Fig. 1, 2), post acquisition VOIs selection and avoiding the influence of relaxation effects because the fat spectral line is used as the internal concentration reference. It was possible to improve the IMCL estimations by excluding the voxels with a large EMCL content (Fig. 2b).

Conclusion
This work, investigating normal-weight volunteers with the sedentary life style, revealed insignificant differences in total fat (EMCL + IMCL) and IMCL concentrations of correspondent VOIs of the legs between genders. The high spatial resolution MRSI technique enables a more detailed study of muscle lipid distribution and can therefore improve our understanding of muscle lipid metabolism in healthy volunteers and in studies of patients with metabolic disorders.

References

Fig. 1: VOIs at thigh (a, c) and calf (b, d) muscles.

Fig. 2: (a) Spectrum of the whole slice without subcutaneous fat, bones, and bone marrows (VOI slice_1), fits, and residue. (b) Spectrum of lean muscles (VOI slice_2), fitting results, and residue.

Fig. 3: Total fat content in thigh (a) and calf (b) muscles. IMCL content in thigh (c) and calf (d) muscles.