INTRAMUSCULAR LIPID CONTENT IN FEMALE ENDURANCE-TRAINED ELDERLY PERSONS BY IN VIVO $^1$H-MR SPECTROSCOPY

YOSHIHAO NAKAGAWA$^1$, MASAAKI HATTORI$^2$, KUNIAKI HARADA$^3$, RYUJI SHIRASE$^3$, MICHIRO BANDO$^3$ and GOROHI OKANO$^4$)

Abstract

The aim of this study is the evaluation of intramyocellular (IMCL) and extramyocellular lipids (EMCL) in skeletal muscle in elderly female endurance-trained individuals. The subjects comprised endurance-trained elderly persons (END: n=7, age=66.1±2.0) and healthy elderly control subjects (CON: n=7, age=70.1±3.0). All subjects were female and matched by age and lower BMI. We quantified differences in IMCL and EMCL concentrations in the tibialis anterior (TA), soleus (SOL), and medial gastrocnemius (MG) muscles using $^1$H-MR spectroscopy. The IMCL and EMCL contents in SOL and MG in END were significantly lower than those in CON (p<0.01). Total lipid content in SOL and MG was lower in END. The IMCL and EMCL contents in TA in END were slightly lower than those in CON. Water contents of all types of muscle in END were higher than those in CON. These results suggest that stored IMCL and EMCL in END are less than in CON.


key word: intramuscular lipid, exercise, elder, muscle, water

Introduction

Lipid contained within skeletal muscle plays an important role in its function, as demonstrated in recent studies linking muscle triglyceride (TG) content to a reduced insulin-stimulated glucose uptake and to reductions in glycogen synthase activity within muscle$^1)$. An increased muscle lipid content has also been noted in older persons$^2)$ and in association with muscle-wasting diseases$^3)$. However, muscle TGs are capable of providing a substantial proportion of energy to exercising muscles. Endurance-trained elderly persons are known to have higher VO$_2$max, which can compensate somewhat for reduced physical functional capacity that occurs with aging.

More recently, a quantification method based on magnetic resonance spectroscopy has greatly facilitated the study of intramuscular lipids. Moreover, this noninvasive technique has facilitated the distinction between lipids within muscle cells (intramyocellular lipids, IMCL) from lipids interlaced between muscle fibers (extramyocellular lipids, EMCL). It has also been demonstrated to be as accurate as biochemical or histological methods$^4)$. However, it remains unknown how much is intramuscular lipid content in endurance-trained elderly persons compared to older adults. This study is intended to estimate IMCL and EMCL levels in muscle in endurance-trained elderly persons. Additionally, water concentrations in muscle were assessed using $^1$H-MR spectroscopy.

Methods

Subjects. Fourteen healthy subjects (64−74 yr) participated in this study. Subjects consisted of endurance-trained older women (END: n=7, age=66.1±2.0, BMI=19.6±1.6, %FAT=20.5±4.9) and healthy elderly controls (CON: n=7, age=70.1±3.0, BMI=20.4±1.6, %FAT=25.8±5.3) END

$^1)$Health and Sport Sciences, Otaru University
$^2)$Department of Community Development, Hokkaido Tokai University
$^3)$Division of Radiology, Sapporo Medical University Hospital
$^4)$Department of Exercise Science, Sapporo Medical University
Correspondence to: Yoshinao Nakagawa, e-mail: nak@res.otaru-uc.ac.jp
who had consistently trained for 10 or more consecutive years were recruited to participate in this study. All subjects were female and matched by age and lower body mass index (BMI).

**Determination of IMCL, EMCL and muscle water with $^1$H–MR spectroscopy.** Magnetic resonance images (MRI) for localization and $^1$H–MRS were acquired using a clinical 1.5 T whole body scanner system (Signa Horizon LX; GE Medical Systems). A standard head coil (28 cm diameter) was used for detection. In each examination, the subjects lay supine with the right calf placed along the coil axis. Transverse T1-weighted MR images (TR, 300 ms; TE, 8.5 ms) were acquired to determine the placement of the $^1$H–MRS voxels, at a slice thickness of 5 mm, 28-cm field, and 512×512 data matrix. Voxel positions were placed to avoid vascular structures and gross adipose tissue deposits and ensure consistent orientation of muscle fibers along the magnetic field. The voxel was 1 cm$^3$. As a consequence of this selection process and high spatial resolution, the lipid values determined in these studies represent the lower boundary, particularly for EMCL. Localized proton spectra were obtained using a PRESS sequence with TE/TR = 30/2000 ms and 128 averages with water suppression. Spectra were processed using the Nuts software package (Acorn NMR Inc., USA). They were line-broadened, phase-corrected, and baseline corrected. The resonances of interest were line-fitted at a mixed Lorentzian/Gaussian function. After correction for T1 and T2 relaxations, quantification of IMCL and EMCL contents was carried out to compare the intensity of (CH2)n (methylene) at 1.3 ppm and 1.5 ppm resonance to the water resonance intensity at 4.7 ppm. This experiment quantified differences in IMCL, EMCL and water concentrations in the tibialis anterior (TA), soleus (SOL), and medial gastrocnemius (MG) muscles using $^1$H–MR spectroscopy.

**Blood Analysis.** Blood samples were drawn between 17:30 and 18:00 p.m. at least 5 h after subjects had consumed identical meals. These blood samples were analyzed for plasma lipids, lipoprotein cholesterol and HbA1c (glycosylated hemoglobin).

**Statistical analysis.** Student’s t-test with an assumption of equal variance was used to compare physical characteristics and blood components between END and CON. Comparisons in intramuscular lipid concentrations (IMCL and EMCL) between END and CON were made using an analysis of variance (ANOVA) for multiple comparisons.

**Results**

Figure 1 shows IMCL (A) and EMCL (B) concentrations: IMCL and EMCL contents in SOL and MG in END were significantly lower than those in CON ($p < 0.01$). Consequently, the total lipid contents in SOL and MG were markedly lower in END. The IMCL and EMCL contents in TA in END were

![Graphs showing IMCL and EMCL concentrations in different conditions.](image-url)

**Fig 1.** IMCL (A) EMCL (B) and water (C) contents of tibialis anterior (TA), medial gastrocnemius (MG) and soleus (SOL) among control (CON) and endurance-trained elderly persons (END). Results are means±SE. *p<0.01.
Table 1. Comparisons of blood lipid, lipoprotein profiles and HbAlc in elder runners and controls.

<table>
<thead>
<tr>
<th></th>
<th>Runner (n=7)</th>
<th>Control (n=7)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>range</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.1 ± 0.3</td>
<td>4.8 - 5.5</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>242.3 ± 48.9*</td>
<td>171 - 321</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>88.0 ± 50.8</td>
<td>31 - 179</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>137.8 ± 47.3</td>
<td>56.4 - 208.7</td>
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<tr>
<td>VLDL-C (mg/dl)</td>
<td>18.4 ± 9.4</td>
<td>8.6 - 33.2</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>86.1 ± 15.2*</td>
<td>61.6 - 106</td>
</tr>
</tbody>
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Values are mean ± SD. *p<0.01: Significant difference between runner and controls. Total cholesterol; TC, triglyceride; TG, HDL-cholesterol; LDL-C, LDL-cholesterol; LDL-C and VLDL-cholesterol; VLDL-C

- Slightly lower than those in CON (NS). The IMCL contents were different in the three types of muscles that we studied, descending in the order of SOL > MG > TA for both END and CON. Water contents in all types of muscle in END were higher than that in CON (Fig. 1C)). They differed in the descending order of TA > SOL > MG in both END and CON. Total body water (TBW) was assessed using bioelectrical impedance analysis. The percent TBW was higher in END (54%) than in CON (51%). Table 1 shows the blood samples (TG, TC, LDL-C, HDL-C and HbAlc). The HDL-cholesterol and total cholesterol values in END were significantly higher than those in CON (p<0.01).

Discussion

The measurement of lipids in muscle by in vivo 1H-MRS is complex because of the presence of two pools of lipids in skeletal muscle, IMCL and EMCL 4,5. These two pools differ kinetically: the latter is thought to turn over very slowly and serves as a long-term fat depot, whereas the former is thought to be in dynamic and rapid equilibrium with substrate utilization and supply. This experiment quantified changes in IMCL, EMCL and water concentrations in skeletal muscles using 1H-MR spectroscopy. The IMCL consists predominantly of stored fatty acids, are stored as fat droplets in the cytoplasm near the mitochondria 6,7, and provide energy for skeletal muscle during prolonged exercise 8. Further understanding of IMCL use during exercise in men and women might clarify elevation of free fatty acids, which increases IMCL 7,8 whereas prolonged endurance exercise decreases IMCL (A). Strikingly, endurance-trained athletes also show increased IMCL levels 9.

We have demonstrated that the IMCL and EMCL contents increase with aging, and that IMCL is related to body composition changes (unpublished data). Previous reports showed that increases in IMCL and intrahepatic TG were correlated with insulin sensitivity, glycosylated hemoglobin, plasma lipids, and body fat in elderly persons 10. With aging, the IMCL content in the soleus muscle was increased by ~45% in elderly people in comparison to young controls. An age-associated decline in mitochondrial function contributes to fat accumulation in muscle 10. Loss of skeletal muscle mass is also frequently associated with other body composition changes (increased fatness). Loss of skeletal muscle with aging has emerged as a major public health issue. Recent studies have indicated that the relationship between IMCL and insulin resistance is more complex and influenced by the oxidative capaci-
ity of skeletal muscle\textsuperscript{11).} The oxidative capacity of skeletal muscle, which increases with training, is in part assessed by measurement of VO\textsubscript{2max}. In this study, resting IMCL and EMCL values in END were significantly lower than those in lean control subjects. The total lipid content in SOL and MG was markedly lower in END. Soleus muscle is rich in oxidative, slow-twitch type I fibers, whereas tibialis anterior muscle is not rich in fast-twitch type II fibers\textsuperscript{12).} Therefore, the lack of an association with the predominantly oxidative muscle might reflect the higher and possibly compensating oxidative capacity of SOL and MG in response to endurance training that could prevent lipid accumulation. Mitochondrial oxidative and phosphorylative activity would be facilitated by habitual exercise. Therefore, resting IMCL and EMCL would be low in endurance-trained elderly persons.

Water in the body makes up 40\textendash80% of an individual’s body mass with aging. Differences in body water among individuals largely result from variations in body composition. Approximately 60% of the body mass is water in striated muscle(80% water), skeleton(32% water), and adipose tissue(50% water)\textsuperscript{13).} Total body water(TBW) volume is reported to decrease with age\textsuperscript{14\textsuperscript{1).}} The water concentrations in muscle and percent TBW in END are higher than that in CON. Higher water contents in muscle might require dehydration because of habitual endurance training.

An age-associated increase in all and visceral adiposity might contribute to the increase in TG, TC, and LDL-C with age\textsuperscript{15).} In blood components in BMI-matched END and elderly persons, HbAlc, TG, and LDL-C in END revealed no significant differences compared to those in CON. Higher HDL-C and TC concentrations in END were demonstrated in this study: endurance training in elderly persons might induce them.

These results show that the IMCL and EMCL would be consumed as a source of energy in endurance-trained elderly persons. Increased muscle water is associated with decreased intramuscular lipids in endurance-trained elderly persons. This difference is likely to result from increased physical activity and is independent of age-associated changes in body composition.

References


14) Chumlea, WC, Guo, SS, Zeller, CM, Reo, NV, & Siever, RM. Total body water data for white adults 18 to 64 years of age: the Fels Longitudinal Study. Kidney Int, (1999), 56, 244-52.