SHORT REPORT

Association of T2 relaxation time determined by magnetic resonance imaging and intramyocellular lipid content of the soleus muscle in healthy subjects

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ABSTRACT

The level of intramyocellular lipids (IMCL) is a physiological marker of skeletal muscle function. ^{1}H-magnetic resonance spectroscopy (MRS) is an established method to measure IMCL contents *in vivo*. However, all of the MR systems do not always contain measurement instruments for ^{1}H-MRS, thus in a clinical setting, alternative methods for estimation of IMCL content are needed. Here, we investigated the association between T1 and T2 relaxation times, determined by MR imaging, and IMCL measured by ^{1}H-MRS in the soleus and tibialis anterior muscles of 15 healthy male subjects. Intriguingly, in the soleus muscle, but not in the tibialis anterior muscle, T2 relaxation time correlated significantly with IMCL ($r = 0.65$, $P < 0.05$). The result suggests the possibility that T2 relaxation time of the soleus muscle can be used to estimate IMCL in a clinical setting. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00108.x, 2011)

KEY WORDS: T2 relaxation time, Intramyocellular lipid, T1 relaxation time

INTRODUCTION

The level of intramyocellular lipids (IMCL), which consists mainly of triglyceride, has been the focus of research in recent years as a physiological marker of skeletal muscle function. In sedentary individuals, high levels of IMCL are associated with insulin resistance[1–3]. In contrast, in lean healthy subjects, IMCL levels correlate with maximal oxygen uptake[2,4]. Accordingly, IMCL levels do not seem to correlate with a particular factor, but rather reflect various factors. However, at least under certain conditions, the level of IMCL can be potentially used as a physiological marker in a clinical setting and also as a research tool in the fields of diabetes, obesity and exercise physiology.

IMCL can be measured non-invasively by proton-magnetic resonance spectroscopy (^{1}H-MRS)[1,2,4,5]. However, in a clinical setting, all of the MR systems do not always contain measurement instruments for ^{1}H-MRS. For a wide clinical application of IMCL, it is important to develop alternative methods to measure these levels.

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The muscular system consists of various types of muscles that contain different proportions of muscle fiber types. Type I fibers are oxidative slow-twitch fibers containing high IMCL and many mitochondria. In contrast, type II fibers are glycolytic fast-twitch fibers containing low IMCL and have low aerobic capacity[6]. The soleus muscle (SOL) is a typical muscle containing a high proportion of type I muscle fibers, whereas the tibialis anterior muscle (TA) is a muscle with a relatively high proportion of type II fibers[7].

Magnetic resonance imaging (MRI) is widely used in clinical setting. T1 and T2 relaxation times measured by MRI provide useful information regarding tissue characterization. Recent studies showed a longer T2 relaxation time for type I fibers compared with type II fibers in animal models and humans[8,9]. These data suggest that T2 relaxation time might be useful for estimation of IMCL. Thus, in the present study, we investigated the association between IMCL and T2 relaxation time in healthy subjects.

MATERIALS AND METHODS

Subjects

The non-obese healthy male subjects were recruited by a bulletin board in Juntendo University from 2006 to 2007 and 15 subjects were included. They were in good health as determined by medical history, a physical examination and standard blood chemistry analyses. All subjects gave written informed consent

to the study, which was approved by the Ethics Committee of Juntendo University Study Protocol.

Study Design and Measurement of Various Parameters

Vigorous physical activity was prohibited from 3 days before measurement in all subjects. Each subject was instructed to eat a weight maintained normal-fat diet (25% fat, 55% carbohydrate, 20% protein) from 3 days before measurement. Then, blood samples were taken after overnight fasting. The IMCL levels and T1, T2 relaxation times were measured by ^{1}H-MRS[4,5,10,11] and MRI[12], respectively (VISART1.5T-EX V4.40; Toshiba, Tokyo, Japan). Resonance of ^{1}H-MRS was quantified by reference to the methylene signal intensity (S-fat), with peaks being observed at approximately 1.25 p.p.m. IMCL was quantified by the S-fat and using a creatine signal at 3.0 p.p.m. (Cre) as the reference (S-fat/Cre). Then, peripheral insulin sensitivity was evaluated by a euglycemic hyperinsulinemic clamp (target plasma glucose level of 95 mg/dL and insulin infusion rate of 100 mU/m^2/min)[5]. The steady-state glucose infusion rate (GIR) was used as peripheral insulin sensitivity.

Statistical Analysis

All data are expressed as mean ± SD. Differences of magnetic resonance data between the SOL and TA were compared by the Wilcoxon signed-rank test. Spearman's rank correlation coefficient was used to evaluate the correlations of T1 and T2 with IMCL of the TA and SOL. Statistical significance was set at $P < 0.05$.

RESULTS

The subjects' characteristics are shown in Table 1. All data were within the normal ranges. As reported previously[4], IMCL of the SOL was approximately threefold higher than that of the TA (Table 2). The T1 of the SOL was comparable to that of the TA. In contrast, similar to the amount of IMCL, T2 relaxation time of the SOL was longer than that of the TA (Table 2).

Next, we investigated the correlation of T1 and T2 with IMCL of the TA and SOL. T1 did not correlate with IMCL values of both TA and SOL. In contrast, T2 correlated significantly with IMCL of the SOL ($r = 0.65$, $P < 0.05$; Figure 1), but not with that of the TA. The IMCL of the SOL significantly correlated with GIR ($r = -0.58$, $P < 0.05$), whereas T2 in the SOL did not.

Table 1 | Subjects' characteristics

Age (years)	23.9 ± 2.5
Body mass index (kg/m^2)	22.1 ± 1.3
Glucose (mg/dL)	90.9 ± 4.0
Insulin (μU/mL)	3.5 ± 1.1
Free fatty acid (mmol/L)	0.42 ± 0.19
HbA_{1c} (%)	5.2 ± 0.27
Total cholesterol (mg/dL)	159.6 ± 44.3
HDL cholesterol (mg/dL)	54.2 ± 10.9
Triglyceride (mg/dL)	84.4 ± 45.3
Leptin (ng/mL)	2.1 ± 1.2
Adiponectin (mg/mL)	6.4 ± 1.4
Glucose infusion rate (mg/kg/min)	11.3 ± 2.2

Data are mean ± SD. HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein.

Table 2 | Intramyocellular lipids, T1 and T2 levels in the tibialis anterior muscle and soleus muscle

	TA	SOL
IMCL (S-fat/Cr)	2.3 ± 1.3	7.7 ± 3.5*
T1 (ms)	1219.1 ± 222.9	1167.4 ± 203.4
T2 (ms)	29.8 ± 1.6	32.5 ± 1.3**

Data are mean ± SD. *$P < 0.001$, **$P < 0.005$. IMCL, intramyocellular lipids; SOL, soleus muscle; TA, tibialis anterior muscle.

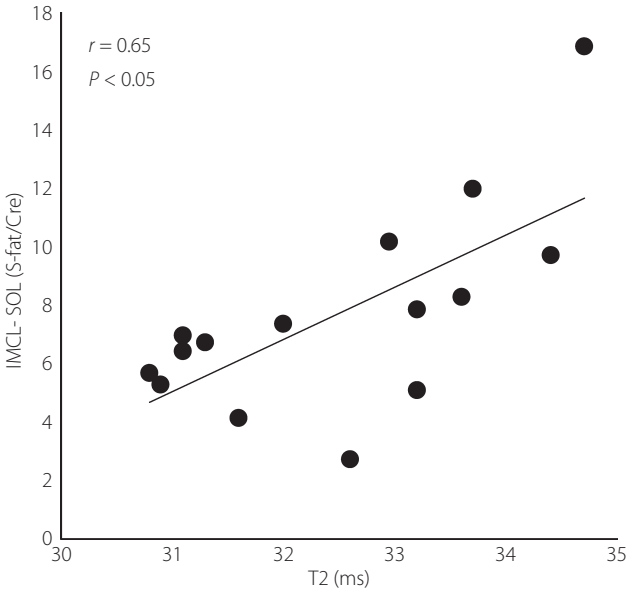

Figure 1 | The relationship between intramyocellular lipids (IMCL) level and T2 relaxation time of the soleus (SOL) muscle ($n = 15$). IMCL was quantified by the methylene signal intensity (S-fat) with creatine signal (Cre) as the reference, and was expressed as a ratio relative to Cre (S-fat/Cre). Start value of the *x*-axis was set at 30 ms.

DISCUSSION

In the present study, we investigated the association between T1 and T2 relaxation times, determined by MRI, and IMCL measured by ^{1}H-MRS in the SOL and TA muscles. We found that IMCL of the SOL was significantly higher than that of the TA. Similarly, T2 relaxation time of the SOL was longer than that of the TA. T2 relaxation time correlated significantly with IMCL in the SOL, but not in the TA. The result suggests the possibility that T2 relaxation time might be used to estimate IMCL in the SOL in a clinical setting.

We observed that T2 relaxation time of the SOL was significantly longer than that of the TA. This result is similar to a previous study showing that T2 relaxation time of the SOL is significantly higher than that of the gastrocnemius muscle[9]. It

has been shown that longer T2 relaxation time was positively associated with the proportion of type I muscle fiber[12]. In addition, other studies showed a longer T2 relaxation time for type I fibers compared with type II fibers in animal models and humans[8,9,13,14]. The SOL contains a high proportion of type I muscle fibers, whereas the TA and gastrocnemius are muscles with a relatively large fraction of type II fibers[7]. Thus, it is speculated that the differences of T2 relaxation time in each muscle might be partly explained by differences of muscle fiber composition.

We found the positive correlation between T2 relaxation time and IMCL in the SOL, but not in the TA. The reason why IMCL correlated to T2 relaxation time only in the SOL is not clear at present. However, at least in the SOL, this finding might be also related to muscle fiber composition, because type I fibers contain higher IMCL compared with type II fibers[6]. Because of the technical difficulties in assessing the fiber type of the SOL by muscle biopsy, it is difficult to clarify the relationship between IMCL, fiber type and T2 relaxation time in the present study. Nevertheless, it is reasonable that the relationship between T2 relaxation time and IMCL is observed only in a certain type of muscle.

One limitation of the present study is that we included only non-obese healthy male subjects. Thus, it is still unclear whether the findings in the present study can be applied to the general population, including female, obesity or diabetic subjects. Obviously, further study is required to generalize the result of the present study.

In summary, the results of the present study suggested the possibility that T2 relaxation time might be used to estimate IMCL in the SOL in a clinical setting.

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