

Original paper

## The influence of leg positioning on the appearance and quantification of <sup>1</sup>H magnetic resonance muscle spectra obtained from calf muscle

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### Abstract

**Purpose:** To study proton magnetic resonance spectra (<sup>1</sup>H-MRS) of the muscle metabolite of a leg muscle in neutral (NEU), internal rotation (INT), and external rotation (EXT) leg positioning.

**Material and methods:** The volunteers were selected for this study. The tibialis anterior (TA), soleus (SOL), and gastrocnemius (GAS) muscles of a non-dominant leg were determined by using single-voxel spectroscopy 8 × 8 × 20 mm<sup>3</sup> in size. <sup>1</sup>H-MRS measurements were performed on a 1.5-Tesla magnetic resonance imaging (MRI) scanner.

**Results:** The results showed that metabolite spectrum of muscle in each NEU, INT, and EXT of leg positioning were not similar. Additionally, the quantification of IMCL (CH<sub>2</sub>) and EMCL (CH<sub>3</sub>) is significantly different in SOL.

**Conclusions:** Our study showed that leg positioning influences the appearance and quantification of <sup>1</sup>H-MRS in the calf muscle. Hence, it is necessary to pay close attention to positioning because it interferes with spectral fitting and quantification.

**Key words:** intramyocellular lipids, extramyocellular lipids, MRS, magnetic susceptibility.

### Introduction

One of the important components of normal skeletal muscle is fat. This fat located inside muscle cells occurs as droplets composed of intramyocellular lipids (IMCL) and as adipocytes spread between muscle cells. These are called extramyocellular lipids (EMCL) [1]. Recently, evidence has suggested that IMCL are positively correlated with insulin resistance in type 2 diabetes [2-4], and they are also an important energy supply [5,6]. The IMCL has traditionally been measured by muscle biopsy. Furthermore, non-invasive proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) has been developed for measuring the IMCL. Compared with a muscle biopsy, <sup>1</sup>H-MRS presents several advantages for measuring IMCL. The advantages are that the *in vivo* measurement can be done, the variation coefficient is low, and the short-term changes can be determined [7]. Although the measure-

ment of IMCL with <sup>1</sup>H-MRS has been used in many previous studies, some influences of <sup>1</sup>H-MRS spectral still remain.

<sup>1</sup>H-MRS spectra of muscle are influenced by two effects: bulk magnetic susceptibility and residual dipolar coupling [8,9]. These effects depend on the angle between the muscle fibres with respect to the main magnetic field [10]. Such effects do not only affect <sup>1</sup>H-MRS spectra, but also the IMCL and EMCL quantification [11]. The <sup>1</sup>H-MRS has previously been measured by IMCL in calf muscles [12,13]. However, a correlation between the angle of calf muscle fibres and IMCL and EMCL quantification has yet to be determined. In this study, we aim to evaluate three leg positions (neutral [0°], internal rotation [-45° from neutral position], and external rotation [+45° from neutral position]) based on IMCL and EMCL quantification in the calf muscles using <sup>1</sup>H-MRS.

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### Authors' contribution:

A Study design · B Data collection · C Statistical analysis · D Data interpretation · E Manuscript preparation · F Literature search · G Funds collection

## Material and methods

### Subjects and equipment

The subjects included 39 healthy male volunteers (mean age, 21 years) who were studied after written informed consent was obtained. All procedures were approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.  $^1\text{H}$ -MRS measurements were performed on a 1.5-Tesla Magnetic Resonance Imaging (MRI) scanner equipped with a knee coil (Philips Achieva Release 2.5 series, Philips Healthcare, Netherlands).

### Data acquisition and analysis

$^1\text{H}$ -MRS spectra were measured in the calf muscle of a non-dominant leg in these subjects. The leg was placed in three positions: neutral [ $0^\circ$ ], internal rotation [ $-45^\circ$  from neutral position], and external rotation [ $+45^\circ$  from neutral position]. Angles were obtained by semicircle angle using the anterior crest of the tibia as a surface point of reference, with the long axis of each leg placed parallel to the main magnetic field. Gel bags and foam were used to support the leg and fix position throughout the scan as shown in Figure 1A.

The T1-weighted axial and sagittal image MRI scans had the following parameters: repetition time (TR) = 700 ms, echo time (TE) = 20 ms, slice thickness = 5 mm, and field of view (FOV) =  $20 \times 20 \text{ cm}^2$ . The single-voxel MRS had the following parameters: PRESS pulse sequence with TR = 2000 ms, TE = 30 ms, NSA = 128 with automatic shimming protocol, and voxel =  $8 \times 8 \times 20 \text{ mm}^3$ . We placed the voxel on the tibialis anterior (TA), gastrocnemius (medial head) (GAS), and soleus (SOL), as shown in Figure 1B.

Post-processing spectra were analysed by Java-based MRUI software (jMRUI version 5.2) [10,14]. The residual tail of water peak spectrum at 4.7 ppm was removed by applying the HLSVD water filtering algorithm. The spectrum was fitted by AMARES algorithm [15] for IMCL,

EMCL, and creatine [16]. The zero-order phases were estimated by AMARES, and the first-order phase was fixed at 0 ms with the first two points of data being truncated for baseline correction. The spectrum line shape was estimated by Lorentzian function. The fitted spectrum showed metabolite spectrum peaks at different chemical shifts: IMCL ( $\text{CH}_3$ ) at 0.9 ppm, IMCL ( $\text{CH}_2$ ) at 1.3 ppm, EMCL ( $\text{CH}_3$ ) at 1.1 ppm, EMCL ( $\text{CH}_2$ ) at 1.5 ppm, and creatine at around 3.02 ppm.

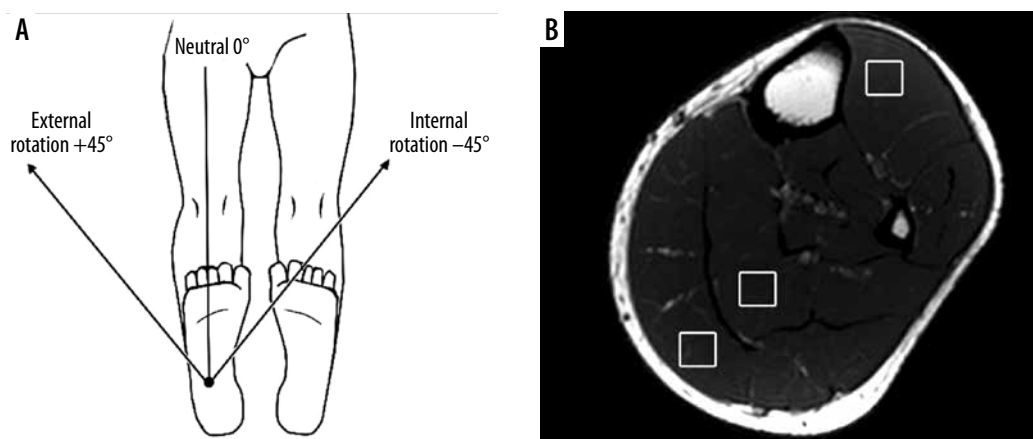
To reduce biological factors that may affect the IMCL level in each individual, such as gender, training, and diet, each IMCL ( $\text{CH}_2$ ) and EMCL ( $\text{CH}_2$ ) amplitude was calculated as the ratio to the amount of methylene IMCL and EMCL signal intensity (IMCL [ $\text{CH}_2$ ] + EMCL [ $\text{CH}_2$ ]). Each IMCL ( $\text{CH}_3$ ) and EMCL ( $\text{CH}_3$ ) was calculated as the ratio based on the amount of methyl IMCL and EMCL signal intensity (IMCL [ $\text{CH}_3$ ] + EMCL [ $\text{CH}_3$ ]). Creatine was calculated as a percentage differently than the creatine signal intensity in the neutral position.

### Statistical analysis

Statistical analysis was done using Origin version 8 with Friedman test for three pairwise analyses, with statistical significance being less than 0.05 between neutral, internal rotation, and external rotation on each metabolite (IMCL [ $\text{CH}_2$ ], EMCL [ $\text{CH}_2$ ] and IMCL [ $\text{CH}_3$ ], EMCL [ $\text{CH}_3$ ]) ratio and the percentage difference of Cr. The Wilcoxon Signed Rank Test was used when the three pairwise comparisons were shown to be significantly different, having a statistical significance of less than 0.05. Any metabolite that jMRUI software is unable to detect from fitted algorithm or that yielded unreliable results was excluded from the data analysis.

## Results

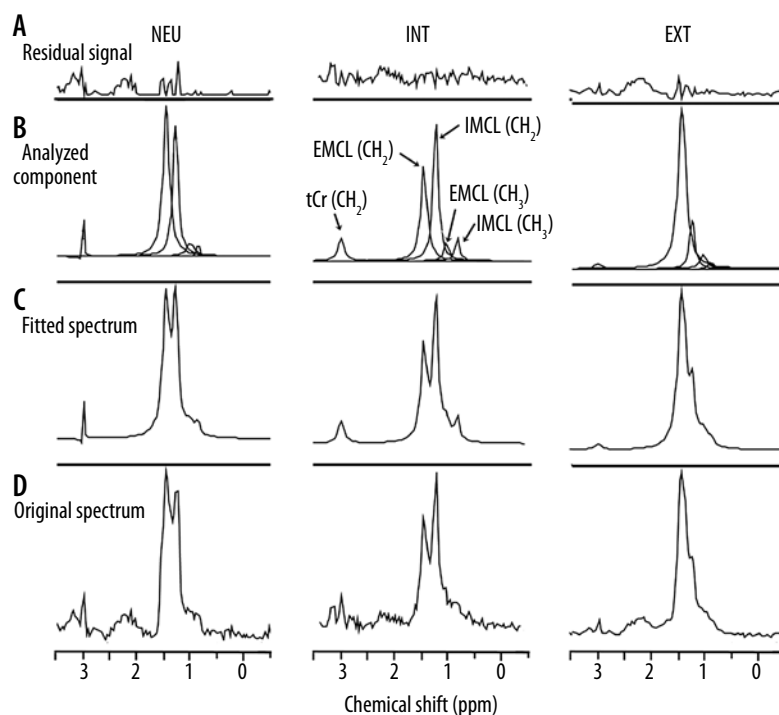
The  $^1\text{H}$ MRS measurements were obtained using spectra from 39 volunteers. All spectra were fitted to the jMRUI AMARES algorithm. The spectrum was scaled to water peak at 4.7 ppm.



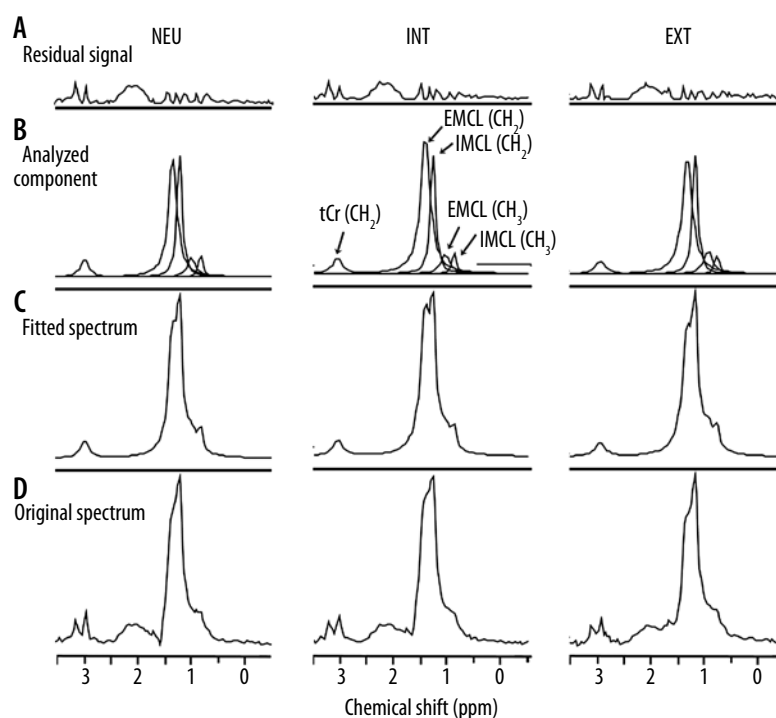
**Figure 1.** A) The angle and position of the leg in neutral ( $0^\circ$ ), internal rotation ( $-45^\circ$ ), and external rotation ( $+45^\circ$ ). B) The position of voxel in TA, SOL, and GAS

For TA muscle, the change in spectral appearance of TA is observed by metabolite intensity. Despite the change, the spectrum has still shown good separation of metabolite peaks in both the methyl group and the methylene group of IMCL and EMCL, as indicated in Figure 2.

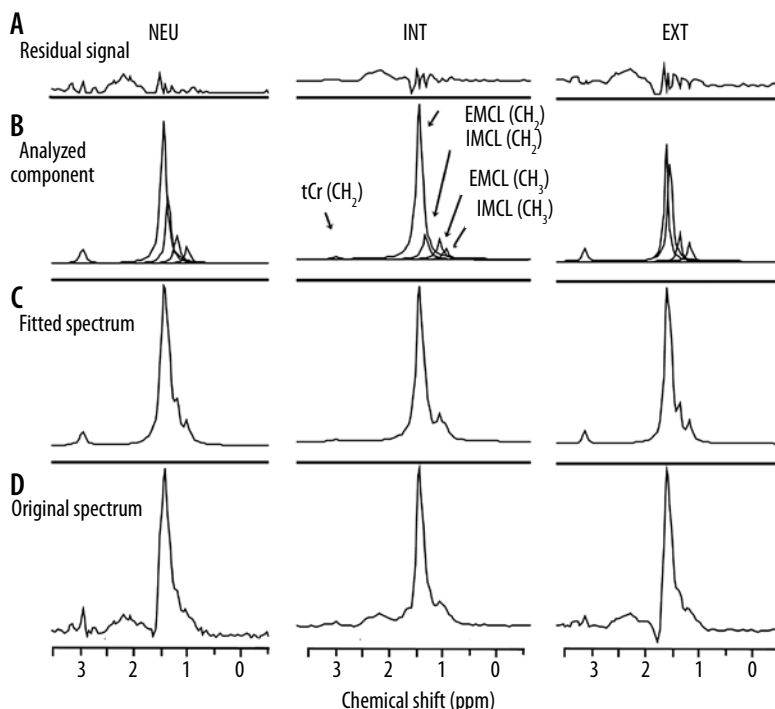
The mean line width of EMCL  $\text{CH}_2$  changed from  $7.51 \pm 2.8$  (NEU) to  $13.7 \pm 7.1$  (INT) and  $17.4 \pm 2.8$  Hz (EXT), and the mean line width of EMCL  $\text{CH}_3$  changed from  $9.54 \pm 6.4$  (NEU) to  $10.8 \pm 5.4$  (INT) and  $15.2 \pm 4.1$  Hz (EXT).



**Figure 2.**  $^1\text{H}$ MRS spectrum obtained from 1.5 T MRI scanner from tibialis anterior (TA), (A) residual signal, (B) analysed individual component, (C) fitted spectrum from jMRUI AMARES algorithm, (D) original spectrum. The spectrum shows IMCL ( $\text{CH}_2$ ) at 1.3 ppm, EMCL ( $\text{CH}_2$ ) at 1.5 ppm, IMCL ( $\text{CH}_3$ ) at 0.9 ppm, EMCL ( $\text{CH}_3$ ) at 1.1 ppm, and creatine at 3.02 ppm



**Figure 3.**  $^1\text{H}$ MRS spectrum obtained with 1.5 T MRI scanner from soleus (SOL), (A) residual signal, (B) analysed individual component, (C) fitted spectrum from jMRUI AMARES algorithm, (D) original spectrum. The spectrum shows IMCL ( $\text{CH}_2$ ) at 1.3 ppm, EMCL ( $\text{CH}_2$ ) at 1.5 ppm, IMCL ( $\text{CH}_3$ ) at 0.9 ppm, EMCL ( $\text{CH}_3$ ) at 1.1 ppm, and creatine at 3.02 ppm



**Figure 4.** <sup>1</sup>H MRS spectrum obtained with 1.5 T MRI scanner from gastrocnemius (GAS), (A) residual signal, (B) analysed individual component, (C) fitted spectrum from jMRUI AMARES algorithm, (D) original spectrum. The spectrum shows IMCL (CH<sub>2</sub>) at 1.3 ppm, EMCL (CH<sub>2</sub>) at 1.5 ppm, IMCL (CH<sub>3</sub>) at 0.9 ppm, EMCL (CH<sub>3</sub>) at 1.1 ppm, and creatine at 3.02 ppm

For SOL muscle, the spectra appearance did not change for difference leg positions. Additionally, there was well-defined splitting of the spectrum peak occurring between both the methyl and methylene group of IMCL and EMCL in all positions, as indicated in Figure 3. The mean line width of EMCL CH<sub>2</sub> changed from 12.8 ± 4.4 (NEU) to 16.0 ± 4.9 (INT) and 10.5 ± 4.8 Hz (EXT), and the mean line width of EMCL CH<sub>3</sub> changed from 1.17 ± 3.8 (NEU) to 12.1 ± 3.5 (INT) and 8.72 ± 3.9 Hz (EXT).

For GAS muscle, the fitted spectrum obtained also showed no difference between the various leg positions. However, it was shown that leg position affects IMCL

(CH<sub>2</sub>) and EMCL (CH<sub>2</sub>) splitting because the spectrum peaks tend to overlap, making it difficult to distinguish between each one, as indicated in Figure 4. The mean line width of EMCL CH<sub>2</sub> changed from 9.46 ± 6.7 (NEU) to 9.51 ± 3.1 (INT) and 7.92 ± 4.0 Hz (EXT), and the mean line width of EMCL CH<sub>3</sub> changed from 7.24 ± 2.6 (NEU) to 9.05 ± 2.3 (INT) and 8.24 ± 2.9 Hz (EXT).

The spectra have shown that variation of leg position affects spectral intensity, spitting, and line width. Some spectral changes in appearance are barely visible; the AMARES quantification algorithm showed that the change in EMCL CH<sub>2</sub>, EMCL CH<sub>3</sub> line width varies

**Table 1.** Median of IMCL (CH<sub>3</sub>) and EMCL (CH<sub>3</sub>) ratio to the sum of methyl IMCL and EMCL concentration (IMCL [CH<sub>3</sub>] + EMCL [CH<sub>3</sub>]), IMCL (CH<sub>2</sub>) and EMCL (CH<sub>2</sub>) ratio to the sum of methylene IMCL and EMCL concentration (IMCL [CH<sub>2</sub>] + EMCL [CH<sub>2</sub>]). Statistical analysis with Friedman test at *p* < 0.05

| Muscle | Metabolite              | NEU                 | INT                 | EXT                 | <i>p</i> -value |
|--------|-------------------------|---------------------|---------------------|---------------------|-----------------|
|        |                         | Concentration ratio | Concentration ratio | Concentration ratio |                 |
| TA     | IMCL (CH <sub>3</sub> ) | 0.466               | 0.410               | 0.373               | 0.687           |
|        | EMCL (CH <sub>3</sub> ) | 0.534               | 0.590               | 0.627               | 0.607           |
|        | IMCL (CH <sub>2</sub> ) | 0.443               | 0.257               | 0.337               | 0.417           |
|        | EMCL (CH <sub>2</sub> ) | 0.557               | 0.743               | 0.696               | 0.717           |
| SOL    | IMCL (CH <sub>3</sub> ) | 0.388               | 0.279               | 0.083               | 0.004*          |
|        | EMCL (CH <sub>3</sub> ) | 0.688               | 0.721               | 0.917               | 0.004*          |
|        | IMCL (CH <sub>2</sub> ) | 0.374               | 0.309               | 0.270               | 0.121           |
|        | EMCL (CH <sub>2</sub> ) | 0.626               | 0.691               | 0.730               | 0.121           |
| GAS    | IMCL (CH <sub>3</sub> ) | 0.440               | 0.260               | 0.283               | 0.513           |
|        | EMCL (CH <sub>3</sub> ) | 0.594               | 0.740               | 0.717               | 0.607           |
|        | IMCL (CH <sub>2</sub> ) | 0.294               | 0.158               | 0.274               | 0.246           |
|        | EMCL (CH <sub>2</sub> ) | 0.706               | 0.842               | 0.744               | 0.417           |

\*Significantly difference

with leg position. In addition, the mean line width of EMCL CH<sub>2</sub> and CH<sub>3</sub> in SOL muscle is more than TA and GAS muscle in the same position.

The quantification of each metabolite in TA, SOL, and GAS muscles are expressed in the concentration ratio, as indicated in Table 1. It was found that the concentration ratio of IMCL and EMCL changed significantly in SOL muscle.

Table 1 shows the quantification results of IMCL and EMCL, which was calculated using ratios of methylene (CH<sub>2</sub>) and methyl (CH<sub>3</sub>) lipid to water in TA, SOL, and GAS. There was a significant change in the ratios of the methyl group of IMCL and EMCL in SOL. The Wilcoxon Signed Rank test was used to determine significant differences among the groups. The significantly different ratios of IMCL (CH<sub>3</sub>) and EMCL (CH<sub>3</sub>) were found when compared with NEU-EXT and INT-EXT positions.

When comparing Cr percentage differences between NEU position with INT and EXT positions (Table 2), it was seen that the EXT position of the leg effects the quantification of Cr more than INT in terms of percentage change. However, statistical analysis determined that no significant changes can be seen.

## Discussion

<sup>1</sup>H MRS spectroscopy is a non-destructive and non-invasive method used to separate and measure IMCL from EMCL. It has been used in various clinical applications and in research such as lipid metabolism studies and sport medicine. Recent studies have shown that the various foot orientations could affect the separation of IMCL and EMCL peak from MRS leg muscle spectra [12,17,18].

This study demonstrates how various legs positions affect the appearance of spectra and quantification of 1.5 T <sup>1</sup>H MRS measured in the leg muscles of 39 healthy subjects. The obtained spectra showed clear separation between the methylene group of IMCL and EMCL in TA, with less clear separation taking place in SOL and GAS. The different leg positioning was shown to affect the appearance of the metabolite profile obtained from TA, SOL, and GAS with the variation of EMCL CH<sub>2</sub> and CH<sub>3</sub> resonance line width occurring at different positions in each muscle.

Because the IMCL is considered to be composed of spherical lipid droplets distributed homogeneously in muscle cells, magnetic susceptibility of IMCL is independent of the orientation. While the EMCL occurs as fasciae between the muscle bundles, which is orientation dependent with the different types of muscle fibre arrangement or the orientation of the limbs relative to the main magnetic field, there is bulk magnetic susceptibility and induced EMCL resonance to allow extra resonance frequency shifts, resulting in the discrimination between EMCL and IMCL being maximised at around 0.2 ppm, when the muscle is relatively parallel to the main magnetic field [9].

The muscle pennation or the angle of muscle fibre relative to the main axis of the muscle is related to the type of

**Table 2.** Median of percentage change when compared to signal intensity in neutral position; INT-NEU = leg in internal rotation position compared with neutral position; EXT-NEU = leg in external rotation position compared with neutral position. The creatine signal intensity of each pair of INT-NEU and EXT-NEU was analysed by Wilcoxon signed rank with statistical significant less than 0.05

| Muscle | Percentage different |         | p-value |
|--------|----------------------|---------|---------|
|        | INT-NEU              | EXT-NEU |         |
| TA     | INT-NEU              | 30.64   | 0.812   |
|        | EXT-NEU              | 65.03   | 0.477   |
| SOL    | INT-NEU              | 18.79   | 1.000   |
|        | EXT-NEU              | 21.13   | 0.944   |
| GAS    | INT-NEU              | 17.72   | 0.107   |
|        | EXT-NEU              | 46.19   | 0.932   |

muscle and its function. There can be a difference in pennation angles among subjects [18] due to various factors such as sex [19], prior muscle training [20], and muscle atrophy [21]. The muscle fibre orientation is determined by mapping <sup>1</sup>H MRS if it is assumed that the long axis of the leg is the same axis as the main magnetic field, and TA muscle fibre is mostly parallel to the main magnetic field; the larger angles being with GAS and SOL [18].

Position alters the muscle angle relative to the main magnetic field, EMCL chemical shift and peak changes due to bulk magnetic susceptibility, while the IMCL remains at the same resonance and causes the variation in spectrum appearance. In this study, spectra from GAS muscle was found to have less fitting quality, because the AMARES algorithm is unable to detect and quantify some individual metabolites. When the same muscles are compared in the same subjects, EXT was found to be the position with the lowest fitting quality, despite the good signal-to-noise ratio (SNR) of the obtained signal.

This study found that lipid quantification of EMCL (CH<sub>3</sub>) and IMCL (CH<sub>3</sub>) in SOL is statistically significant between the positions at NEU-EXT and INT-EXT, in agreement with the change of spectrum appearance showing that EXT is the position that yields the lowest spectrum fitting quality.

The significant changes in EMCL and IMCL methyl resonance occur despite the lack of SOL spectral appearance, and this is thought to be caused by the overlapping of IMCL and EMCL spectra, as well as bleeding signals from the EMCL CH<sub>3</sub> concentration. This causes estimation errors of the spectrum fitting algorithm because methyl EMCL and IMCL resonance has low signal intensity when compared to the intensity of the other metabolites in the same voxel. Therefore, it can be overestimated or underestimated quite easily. Whenever the largest angle of SOL is compared to GAS and TA, it is also suggested that the orientation of the leg is the effect that most alters the soleus and the angle between the muscle and the main magnetic field. Other studies have revealed that the geometry of the leg muscle affects the spectrum appearance [10] and quantification of metabolite [22]. However, the

exact angle of the muscle relative to the main magnetic field is beyond the scope of this work.

This study showed the limitations of quantifying metabolite when Cr is used as the reference metabolite or when used as the reference concentration. Whenever the leg is not positioned parallel to the main magnetic field the spectrum fitting quality is affected, and this may result in difficulty in Cr resonance quantification.

Lower spectrum fitting quality occurs in GAS and SOL when compared to TA. This tendency suggests that there are limitations of <sup>1</sup>H MRS metabolite quantification from the non-parallel position to main magnetic field in SOL and GAS when using the same algorithm. The fitting algorithm has been used mostly to study TA muscle, which is orientated almost parallel to main magnetic field, but it may be not suitable for other muscles that have different muscle orientations relative to the main magnetic field.

Most published studies have reported IMCL and EMCL concentrations from the methylene (CH<sub>2</sub>) resonance because this is the most dominant signal and has clear separation of peaks between IMCL and EMCL. However, to study the relationship between various factors that can alter the methylene signal per mole of triglycerides in human fat [23], the methylene resonance of IMCL and EMCL concentration should not be overlooked.

## Conclusions

In this study, leg orientation affects the appearance of <sup>1</sup>H MRS spectra obtained from TA muscle and quantification in SOL muscle. The change of spectral appearance also impedes the quantification of various metabolites, and this may result in misinterpreting spectrum results used to predict or study diseases. It is also suggested that the magnetic resonance spectroscopy measured from calf muscle can minimise the effect of leg positioning. In order to reduce the possible errors in quantification, close attention to leg positioning is required when measuring <sup>1</sup>H MRS in leg muscle.

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## Conflict of interest

The authors report no conflict of interest.

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