

Effect of High-Intensity Exhaustive Exercise on Intramyocellular and Extramyocellular Lipid Levels in Crural Muscles of Young Adults

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Abstract

This study aimed to investigate the effect of high-intensity exhaustive exercise on the intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL) levels in soleus muscles (SOL) and tibialis anterior muscles (TA) of young adults. Eight healthy males performed a single bout of treadmill exercise at 80% $\dot{V}O_{2max}$ until exhaustion. Localized *in vivo* proton magnetic resonance spectroscopy was used to evaluate the lipid levels in two crural muscle groups at four time points: pre-exercise, immediately after exercise (0 hr), 1 hr, and 2 hr post-exercise. IMCL and EMCL lipid levels in SOL and TA muscles were measured in response to exercise challenge. We found that IMCL concentrations in SOL were significantly lower after exercise than before exercise; however, EMCL showed no difference in response to exercise. Both IMCL and EMCL levels in TA muscles were not significantly altered after high-intensity exhaustive exercise. We conclude that more energy relied on SOL muscle fiber, independent of TA fiber, under high-intensity exhaustive treadmill exercise. The type of muscle fiber recruited during high-intensity exhaustive exercise may explain the discrepancies in the lipid levels observed in SOL and TA muscle fibers.

Keywords: soleus muscle, tibialis anterior muscle, magnetic resonance spectroscopy

Introduction

During a long period of low-intensity exercise, fatty acids and intramyocellular lipids (IMCL) are the important sources of energy for muscle contraction (Gorski, 1992). Meanwhile, extramyocellular lipids (EMCL) are also a major source of energy during low-intensity exercise. In high-intensity exercise, the rate of metabolism of IMCL as an energy source increases (Romijn et al., 1993). Carbohydrates are the main energy source of muscle. Most previous studies focused on the effect of exercise on muscle glycogen, however IMCL also play an important role in high-intensity exercise (Johnson, Stannard, & Thompson, 2004; Krssak et al., 2000; Sjöros et al., 2019).

The fat of human muscle can be roughly divided into 2 categories: IMCL and EMCL. IMCL are also called intramyocellular triglycerides (IMTG), which adhere to mitochondria in the form of triglyceride droplets. Mitochondria are the sites where fatty acids conduct aerobic metabolism; triglycerides are decomposed into 3 molecules of free fatty acids (FFA) and one molecule of glycerol by lipase when muscle contracts. FFA can only produce energy when they re-enter into mitochondria and perform β -oxidation. Therefore, the existence of fat droplets in muscle cells provides an immediate source of energy, when muscles contract (Guo & Jensen, 1998). Extramyocellular triglycerides (EMTG) present in all adipose tissues. When tissue requires energy, EMTG can be hydrolyzed by lipolytic enzymes and release FFA into the blood, which then bind to albumin and transported to the tissues that require energy, then being metabolized to produce energy (Boesch, Slotboom, Hoppeler, & Kreis, 1997; Vermathen, Saillen, Boss, Zehnder, & Boesch, 2012).

Muscles are composed of 2 different types of muscle fibers, namely slow twitch, and fast twitch. Slow twitch fibers (type I) have high mitochondrial content, high myoglobin level, and high mitochondrial enzyme activity, mainly using an aerobic energy system. Fast twitch fibers (type II) are rich in glycolytic enzymes, and provide muscles with a large amount of anaerobic capacity, mostly utilizing the anaerobic energy system (Essén, Jansson, Henriksson, Taylor, & Saltin, 1975; Pette & Spamer, 1986). The tibialis anterior muscle (TA) in crural muscle contains abundance of type II muscle fibers, while the soleus

muscle (SOL) comprised with more type I muscle fibers (~ 70%) and more mitochondria. Mitochondria are the main sites of aerobic metabolism in the human body, and mitochondrial content can affect the energy utilization and fat metabolism during exercise (Boesch et al., 1997; Saltin, Henriksson, Nygaard, Andersen, & Jansson, 1977). This study monitored the changes of IMCL and EMCL levels in different muscle fiber types (TA and SOL) of young adults after high-intensity exercise.

Some previous studies have shown that the changes of various biochemical values in muscles, most of which involved biochemical detection analysis or biopsy. The former method could not directly reflect the changes of biochemical values because it involves extraction with chemical solvents. Moreover, the latter methods are invasive, often causing subjects discomfort and pain, and the extracted muscle fibers cannot be reused for data collection. In contrast, *in vivo* ^1H magnetic resonance spectroscopy (MRS) can be used continuously to collect the biochemical data on the same tissue or organ due to ^1H -MRS's advantages of non-invasiveness and not requiring irradiation. MRS also can be used to measure the same muscle at different time points, before, and after exercise (Cole et al., 2002; Möller et al., 2005; Schick et al., 1993). Therefore, research on muscle and biochemical values has rapidly progressed due to the application of ^1H -MRS (Boesch, Décombaz, Slotboom, & Kreis, 1999; Misra, Sinha, Kumar, Jagannathan, & Pandey, 2003).

Through the traditional techniques, mostly using biopsy, or muscle fiber extraction for measuring the lipids in muscle cells, in coordination with electron microscopy and morphometric measurements, only the number and size of lipid droplets can be observed in different muscle fiber types. However, contamination by extracellular lipids can readily occur during the analysis and treatment, which causing great variability in the obtained results if the selected muscle tissue contains a large amount of adipose tissue (Kayar, Hoppeler, Howald, Claassen, & Oberholzer, 1986; Wendling, Peters, Heigenhauser, & Spriet, 1996). At present, ^1H -MRS is only the technology that can effectively distinguish between EMCL and IMCL; facilitate to determine the IMCL and EMCL content non-invasively, and allows repeated measurements in the same muscle volume. This was an important factor

behind the decision to select MRS to conduct the examinations presented here (Schrauwen-Hinderling, Hesselink, Schrauwen, & Kooi, 2006).

Method

Study Protocol

Eight healthy male college students from National Taiwan Sport University were recruited for this study. None of the subjects was taking any medicine, and all had normal liver and kidney functions, no cardiovascular disease, non-smokers/drinkers, and not consuming any nutritional supplements. Each subject was informed about the experiment and possible risks involved in the study. The experiment was initiated only after each subject had provided a written informed consent form. All subjects were required to undergo maximum oxygen uptake measurement and followed by 8 h fasting night before the experiment. Before the test days, subjects received a standardized diet ($13 \pm 1\%$ protein, $56 \pm 3\%$ carbohydrate, and $30 \pm 1\%$ fat) for 3 days during which they refrained from exercise. Exercise was planned at 6 a.m. on the first day of the experiment with 5 min warm-up followed by a 60 min treadmill exercise at $80\% \dot{V}O_{2\max}$ intensity. Water consumption was allowed during the experiment. The changes of internal and external triglycerides levels in SOL and TA were measured by ^1H -MRS before exercise, 1 h after exercise, and 2 h after exercise. The detailed study protocol was shown in Figure 1.

Maximum Oxygen Uptake

The gas analyzer (Vmax Spectra, SensorMedics, Yorba Linda, CA) was calibrated for volume and different concentrations of standard gas before the experiment. Subjects arrived at the laboratory and their height and body weights were measured. Changes in heart rate (HR) were monitored during

treadmill exercise by Sport Tester (PE 3000, Polar Electro, Kempele, Finland). Participants were initially jogged on a treadmill (Vision T8600, Johnson, Lake Mills, WI) for 5 min as a warm-up. While measuring the maximum oxygen uptake, the running speed was set at 8 km/h, with a 0% slope for 0–3 min, after which the slope was slightly increased by 3% every 3 min until the subject was exhausted and stopped running. Data were analyzed using a computer to determine the oxygen uptake at 1 min before slope ascension and 1 min before exhaustion. The maximum oxygen uptake values obtained in the test referred as the maximum oxygen uptake.

$80\% \dot{V}O_{2\max}$ Exercise Loading Confirmation Test

$\dot{V}O_{2\max}$ was obtained from the previously mentioned measurement, and $80\% \dot{V}O_{2\max}$ was calculated from the regression equation of loading intensity. Exercise was performed with this intensity loading for 10 min, with measurements of oxygen uptake for 5–6 min and the last minute in order to confirm the accuracy of $80\% \dot{V}O_{2\max}$ loading.

In vivo ^1H MRS Measurement

This study applied ^1H -MRS and the 3T Medspec S300 NMR systems (Bruker, Billerica, MA) with a standard birdcage knee coil for crural muscle measurements, under the following conditions: repetition time (TR) of 2 s, echo time (TE) of 135 ms, 32 scans (No. of scans, NS), and voxel of interest (VOI) set as $20 \times 20 \times 20$ mm. However, individuals have different sizes of muscle groups, so the actual VOI depends on the particular conditions. Therefore, volume normalization on data was necessary when performing statistical analysis.

The measurement sites were the thickest parts of the left crural muscle of the subject: the SOL and the

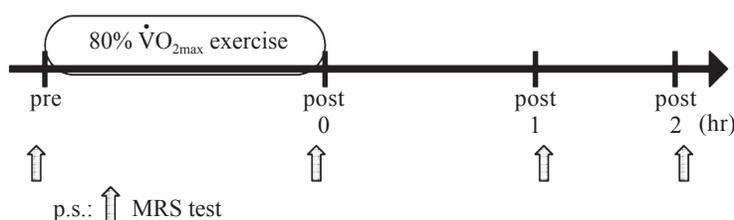


Figure 1. The study protocol.

Note: MRS: magnetic resonance spectroscopy.

TA. Clinically, the transverse view and the coronal view were usually selected for positioning, and the magnetic resonance spectrum was scanned after the VOI had been selected. After scanning had been completed, the original obtained spectrum was subjected to post-processing via an installed software package.

Statistical Analyses

In this study, SPSS version 18.0 was used for the statistical analysis. Data were presented as mean \pm standard error (*SE*). Differences in muscles' IMCL and EMCL levels were analyzed by one-way analysis of variance (ANOVA) before and after exercise and during the recovery period; the levels at different time points were compared via Fisher's least-significant difference (LSD) method. $p < .05$ indicates a significant difference.

Results

The 8 male subjects had an average age of 20.3

± 0.4 years, height of 173.75 ± 1.28 cm, body weight of 77.50 ± 3.77 kg, body mass index of 25.59 ± 0.91 kg/m², maximum oxygen uptake of 53.80 ± 2.37 ml/kg/min, and 80% $\dot{V}O_{2\max}$ exercise exhaustive time of 31 min 4 s ± 3 min 10 s (Table 1).

The measurements by ¹H-MRS, as shown in Figure 2, were performed on the SOL. The IMCL and EMCL of the SOL were measured after spectral processing, and the results were shown in Figure 3. All of the data were homogenized, statistically analyzed, and compared.

Table 2 shows the changes of IMCL and EMCL levels in crural muscles before and after exercise. The results revealed that the IMCL of SOL were significantly decreased from 7.71 ± 1.28 – 5.46 ± 1.12 arbitrary unit (A.U.) after high-intensity exercise, which indicates IMCL were used as a source of energy in high-intensity exhaustive exercise. During post-exercise recovery period, IMCL level was 7.17 ± 1.31 A.U. at 1 h post-exercise and 6.55 ± 1.01 A.U. at 2 h post-exercise, with no significant differences from the

Table 1. Information of Subjects

Item	Mean \pm SE
Age (years)	20.3 \pm 0.4
Height (cm)	173.75 \pm 1.28
Weight (kg)	77.50 \pm 3.77
Body mass index (kg/m ²)	25.59 \pm 0.91
Maximum oxygen uptake (ml/kg/min)	53.80 \pm 2.37
Time to exhaustion (min:s)	31'04" \pm 3'10"

Note: $n = 8$, values are expressed as mean \pm standard error (*SE*).

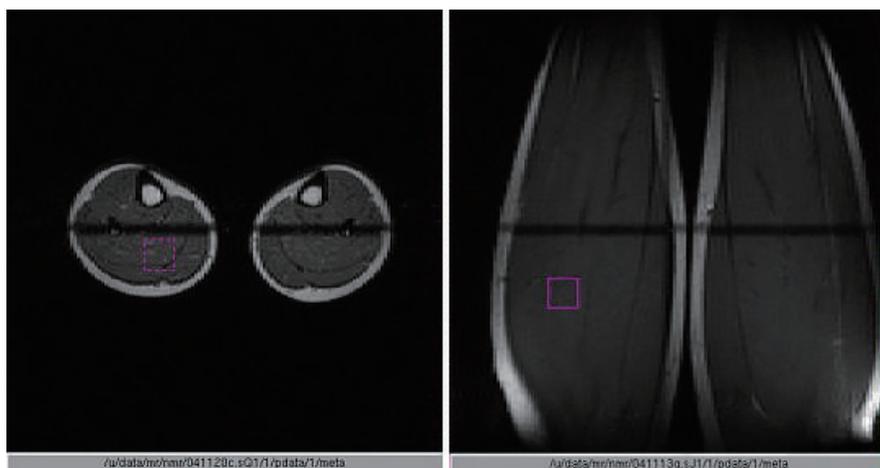


Figure 2. Position for measuring soleus muscle.

Note: An appropriate position was selected from transverse and coronal views.

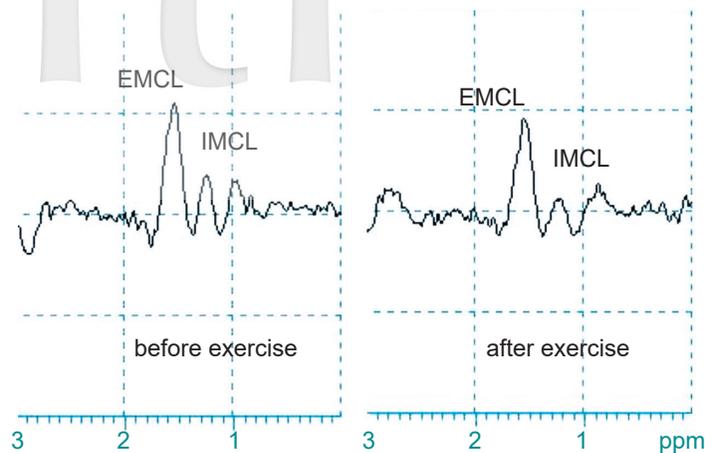


Figure 3. Magnetic resonance spectrum after post-processing.

Note: The signals measured from right to left were intramyocellular lipids (IMCL-CH₂, 1.3 ppm), extramyocellular lipids (EMCL-CH₂, 1.5 ppm). EMCL: extramyocellular lipids; IMCL: intramyocellular lipids.

Table 2. Changes in IMCL and EMCL Levels of Crural Muscle Before and After Exercise

Variable	Pre-Ex	Post-Ex	Post-Ex 1 h	Post-Ex 2 h
TA/IMCL (A.U.)	11.24 ± 2.02	9.41 ± 1.45	12.10 ± 2.73	13.07 ± 2.38
TA/EMCL (A.U.)	90.65 ± 15.71	87.71 ± 19.59	105.55 ± 25.59	102.66 ± 23.42
SOL/IMCL (A.U.)	7.71 ± 1.28	5.46 ± 1.12*	7.17 ± 1.31	6.55 ± 1.01
SOL/EMCL (A.U.)	11.06 ± 0.87	9.73 ± 0.82	10.47 ± 0.97	10.99 ± 1.32

Note: $n = 8$, values are expressed as mean ± standard error (*SE*). * indicates significant difference from pre-exercise ($p < .05$).

IMCL: intramyocellular lipids; EMCL: extramyocellular lipids; Pre-Ex: pre-exercise; Post-Ex: post-exercise; Post-Ex 1 h: post-exercise 1 h; Post-Ex 2 h: post-exercise 2 h; TA: tibialis anterior muscles; A.U.: arbitrary unit; SOL: soleus muscles.

pre-exercise levels. The EMCL levels of SOL were decreased from 11.60 ± 0.87 A.U. pre-exercise to 9.73 ± 0.82 A.U. post-exercise. Although the mean values decreased, statistical significance was not reached. Similarly, EMCL levels of SOL were 10.47 ± 0.97 A.U. at 1 h post-exercise and 10.99 ± 1.32 A.U. at 2 h post-exercise, with no significant differences from the pre-exercise levels.

The IMCL of TA was 11.24 ± 2.02 A.U. before exercise and 9.41 ± 1.45 A.U. after exercise; and no significant difference at 1 h and 2 h after exercise. The pre-exercise and post-exercise EMCL levels of TA were 90.65 ± 15.71 and 87.71 ± 19.59 A.U., respectively. There were no significant differences for each measurement time point in the post-exercise recovery period from the pre-exercise level. The IMCL of SOL was significantly decreased after exercise and returned to the pre-exercise level at 1 and 2 h after exercise. These changes were shown in Figure 4.

Discussion

After an overnight fasting, plasma FFA (mainly from EMCL) are the main source of oxidation in low-intensity exercise, and the rate of IMCL metabolism as a fuel source increases at a high-intensity exercise relative to that of EMCL (Romijn et al., 1993). Previous research focused on fat metabolism in long-term low-intensity exercise, but no investigations were focused on changes in IMCL and EMCL of different muscles after high-intensity exercise. Therefore, in this study, 80% $\dot{V}O_{2max}$ high-intensity exhaustive exercise was performed, and found that IMCL of SOL significantly decreased after exercise. This results indicates that IMCL are also an immediate source of energy during high-intensity exercise, while carbohydrates are not the only main energy source for short-term exercise. The IMCL are located next to mitochondria in the form of droplets, which can be hydrolyzed in mitochondria

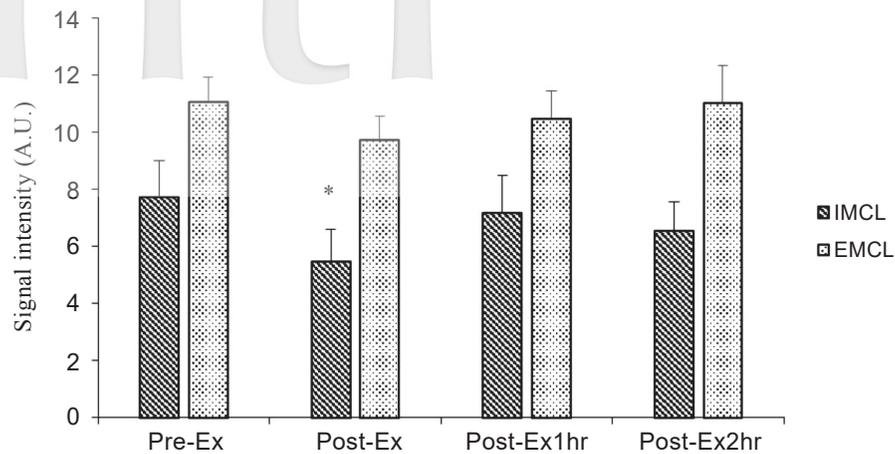


Figure 4. Changes in IMCL and EMCL levels of soleus muscle at each measurement time point before and after exercise.

Note: $n = 8$, * indicates a significant difference from pre-exercise ($p < .05$). A.U.: arbitrary unit; Pre-Ex: pre-exercise; Post-Ex: post-exercise; Post-Ex 1 hr: post-exercise 1 hr; Post-Ex 2 hr: post-exercise 2 hr; IMCL: intramyocellular lipids; EMCL: extramyocellular lipids.

during exercise and subjected to aerobic metabolism to generate energy (Boesch et al., 1997; Sjöros et al., 2019). For the same unit weight, fat provides greater energy (twice) than glycogen (Jeukendrup, Saris, & Wagenmakers, 1998). Previous study focused on the effects of exercise on muscular glycogen, but muscle fat has also been shown to play an important role in exercise (Achten, Gleeson, & Jeukendrup, 2002).

Fatty fuels that can be oxidized in the human body were including IMCL, plasma FFA, and triglycerides in plasma. The fuels derived from fat utilized during exercise can be distinguished from plasma and muscle; triglyceride in plasma is exogenously derived from chylomicrons or endogenously derived from lipoproteins and EMCL (Jeukendrup et al., 1998).

The TA and SOL work at the same time, but the responses of IMCL to these 2 muscles are different. This may be related to their different ratios of the 2 types of muscle fibers. SOL is mainly composed of slow-twitch muscle fibers, while TA is a mixture of a large number of fast-twitch muscle fibers and a small number of slow-twitch ones (Boesch et al., 1997; Saltin et al., 1977). Variation in the proportion of muscle fiber types causes differences in muscles, and the rate of use of the particular energy systems in exercise also differs (Frayn & Maycock, 1980; Guo & Jensen, 1998). Therefore, for the movement of SOL rich in type I fibers, the aerobic system is mainly utilized, while type II-rich TA mainly uses the anaerobic system, with phosphocreatine or glycolysis

as an energy source. When the crural muscle performs high-intensity exercise, SOL fibers utilizes the aerobic system to use IMCL as a fuel source. Therefore, it is more reasonable that SOL utilizes IMCL, which can produce energy more rapidly than TA does (Nakagawa & Hattori, 2017; Vermathen et al., 2012).

Brechtel et al. (2001) divided 12 male runners into three groups: 6 in a non-competitive run (NCR) group, 3 in a competitive half-marathon (HM, 21 km) group, and 3 in a competitive marathon (M, 42 km) group. The NCR group was under moderate intensity (60–70% $\dot{V}O_{2max}$) exercise for 105–110 min; the HM group was under high-intensity (83–85% $\dot{V}O_{2max}$) exercise for 80–120 min; and the M group was under 68–70% $\dot{V}O_{2max}$ exercise for 210–240 min. Changes in IMCL levels of TA and SOL were measured by 1H-MRS before and after exercise. In the results, they found that the amount of IMCL in TA and SOL was significantly decreased (10–36%) in NCR group. The IMCL content in TA was significantly decreased by 42–57%, while the IMCL of SOL was decreased by 27–56% in M group. Although the IMCL of two muscles decreased in HM group, this did not reach significance. In our study, high-intensity exercise for 31 min caused decreased levels of IMCL in SOL muscle, but not TA muscle. The differences of the exercise time may lead to the different results of these two studies. Pasanta, Tungjai and Kothan (2018) suggested that leg positioning influences the appearance and quantification of ¹H-MRS in the

muscle. The difference of leg orientation caused the different results. When the exercise intensity was 60–70% $\dot{V}O_{2max}$, IMCL of TA and SOL were significantly decreased. It is worth noting that, in low-intensity exercise, the rate of metabolism is several times higher than in a resting state and the rate of lipid metabolism also increases. As the intensity of exercise increases, plasma fatty acid turnover does not increase (Coyle, 1995).

Biopsy combined with electron microscopy and morphometry is a traditional method for measuring the effects of exercise on IMCL and EMCL; it can also determine the amount of fat droplets in IMCL in different muscle fiber types (Kayar et al., 1986). However, if biopsied muscle tissue contains more adipose tissue, the sample is susceptible to EMCL contamination during analysis, and results in a large increase in variability (Wendling et al., 1996). The 1H -MRS is a novel approach to measure the changes in IMCL and EMCL *in vivo* (Fischer et al., 2018; Schick et al., 1993). Compared with the biopsy method, the systematic error of measuring EMCL with 1H -MRS is very small (~ 6%) (Boesch et al., 1997). Owing to the advantages of non-invasiveness and an ability to achieve continuous measurement of MRS, there have been gradual technological advances in muscle research, and the role of magnetic resonance imaging has become increasingly important, and its application in medicine has become widespread. In this study, these advantages were exploited to measure the changes in IMCL and EMCL before and after exercise.

This study demonstrates that the applications of 1H magnetic resonance spectrum technology, indeed enables continuous monitoring of changes in metabolites in skeletal muscle, and solves the limitations imposed by traditional methods of extracting muscle fibers. It is believed that this approach can substantially boost clinical research and the field of sports science.

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高強度衰竭運動對小腿肌肉之肌細胞內、外脂肪含量的影響蔡佈曦¹、何應志²¹ 臺灣 金門縣 892 國立金門大學運動與休閒學系² 臺灣 嘉義縣 622 南華大學體育教學中心

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摘要

本研究的目的為探討高強度衰竭運動對比目魚肌 (soleus, SOL) 及脛前肌 (tibialis anterior, TA) 肌細胞內脂肪 (intramyocellular lipids, IMCL)、肌肉細胞外脂肪 (extramyocellular lipids, EMCL) 含量的影響。研究對象為 8 位健康男學生，以強度 80% $\dot{V}O_{2max}$ 之跑步機運動至衰竭，於運動前、運動後及運動後 1、2 小時，進行小腿中兩種肌肉群之活體 ¹H- 磁振頻譜掃描。測量 SOL 及 TA 在運動前後 IMCL 及 EMCL 的變化。結果顯示高強度衰竭運動後，SOL 的 IMCL 比運動前顯著下降，EMCL 則運動後沒有差異，而運動後 1、2 小時，不論 IMCL 或 EMCL 都與運動前沒有差異。TA 則運動前與運動後恢復期 IMCL 及 EMCL 都沒有顯著的差異。本研究結論為在高強度運動，SOL 肌肉纖維會使用較多的 IMCL 能量，因此在運動後顯著下降。然而，TA 高強度運動期間沒有使用 IMCL 及 EMCL 能量，可能高強度運動期間 SOL 及 TA 肌纖維利用形態的不同，造成兩者不同的結果。

關鍵詞：比目魚肌、脛前肌、磁振頻譜

