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Intramyocellular Lipid is Increased in the Skeletal Muscle of Patients with Dilated Cardiomyopathy with Lowered Exercise Capacity

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Exercise capacity is lowered in patients with heart failure (HF), which is closely related to the prognosis [1]. The abnormalities in skeletal muscle (SKM) have been shown to play a central role in lowered exercise capacity in HF [2]. We previously demonstrated that SKM phosphocreatine (PCr) and intramuscular pH levels were significantly lower during exercise in HF using ^{31}P magnetic resonance spectroscopy (^{31}P-MRS) and these changes were closely correlated with exercise capacity [3]. SKM energy metabolism depends largely on mitochondrial function. Recently, the accumulation of intramyocellular lipid (IMCL) in SKM due to the impairment of fatty acid metabolism in the presence of excessive free fatty acids has been shown to be involved in the pathogenesis of insulin resistance [4]. IMCL content is determined by the balance between fatty acid uptake into SKM cells and β-oxidation of fatty acids within their mitochondria [5]. Therefore, IMCL accumulation may be noted in SKM in HF, in which SKM mitochondrial function is abnormal. We determined whether IMCL accumulation was increased and associated with abnormal SKM energy metabolism and exercise capacity in HF due to idiopathic dilated cardiomyopathy (DCM).

Eighteen male patients with DCM (50±13years, left ventricular ejection fraction (LVEF) 32.3±10.4%) and 12 age- and sex-matched healthy subjects as controls (46±7years, LVEF 74.6±6.8%) were studied. Patients who had the implanted mechanical devices and New York Heart Association functional class IV were excluded. Patients with diabetes mellitus, peripheral artery disease, pulmonary disease, stroke, and who could not perform exercise testing were also excluded. Informed consent was obtained from all subjects and the study protocol, conformed to the ethical guidelines of the Declaration of Helsinki, was

approved by the ethics committee of Hokkaido University Hospital. Body weight (BW), fasting blood glucose, insulin, HbA1c, lipid profiles were measured. Body mass index (BMI) and HOMA-IR were calculated. Peak oxygen uptake ($\dot{V}O_2$) and anaerobic threshold (AT) were determined by respiratory gas analysis using bicycle ergometer with a ramp protocol. Muscle strength by the one repetition maximum (1-RM) method and the calf flexor muscle cross-sectional area at the level of the muscle belly were measured, as described previously [6,7]. The measurements of ^{31}P-MRS were performed at rest and every 30 sec during supine planter flexion exercise with a constant load of 20%1-RM, and spectra were analyzed, as described previously [6,7]. PCr was standardized as [PCr]/([PCr]+[Pi]), where Pi indicates inorganic phosphate. The degree of PCr change (PCr loss) during exercise was calculated as: PCr loss (%) = $(PCr_{rest}-PCr_{peak})/PCr_{rest}$x100. IMCL content was measured in the resting tibialis anterior muscle at the level of the muscle belly of calf using ^{1}H-MRS, and quantified relative to muscle water by using units of mmol/kg wet weight as described previously [6,7]. Based on previous study [6], sample sizes of study subjects were needed to be 18 for DCM and 12 for control (α=0.05, β=0.2 and allocation ratio=1.5 (DCM/control). Data were expressed as means±SD. Student's *t*-test was performed to compare means between groups. Correlations were examined by linear regression analysis using the least-squares method. Statistical significance was defined as $P<0.05$.

Characteristics of the study subjects were shown in **Table 1**. Anthropometry and blood chemistry data except HDL cholesterol were comparable between control and DCM. Peak $\dot{V}O_2$, AT, 1-RM and muscle cross-sectional area were significantly lower in DCM than control. The

standardized PCr at rest was comparable between control and DCM. In contrast, the standardized PCr at peak exercise was significantly decreased, and the rate of PCr loss was significantly increased in DCM compared with control. In consistent with our previous paper, PCr loss was inversely correlated with peak $\dot{V}O_2$ and AT. **Figure 1A** shows the representative spectra of ^{1}H-MRS. IMCL content was significantly greater in DCM than control (**Figure 1B**). IMCL content was inversely correlated with peak $\dot{V}O_2$, AT, and positively correlated with PCr loss among all study subjects (**Figures 1C-E**) and also in the subgroup of DCM.

IMCL accumulation seen in DCM was considered to be caused by the impaired fatty acid β-oxidation in SKM mitochondria rather than increased fatty acid uptake. Indeed, IMCL was not significantly correlated with body weight (r=-0.26), body mass index (r=-0.07), waist circumference (r=0.07), fasting blood glucose (r=0.11), plasma insulin (r=0.02), HOMA-IR (r=0.06), HbA1c (r=0.02), triglyceride (r=0.20), and free fatty acid (r=0.10). The impairment of mitochondrial fatty acid β-oxidation may be due to the low activity levels of β-hydroxyacyl coenzyme A dehydrogenase, an enzyme involved in β-oxidation, which has been reported in the biopsied samples of SKM from HF [8]. The most important finding of the present study was that the IMCL level was significantly correlated to SKM energy metabolism and exercise capacity (**Figure 1**), suggesting that impairment of energy substrate supply to the mitochondria as well as mitochondrial energy production plays an important role in lowered exercise capacity in DCM. IMCL accumulation in SKM seems to be caused by impaired β-oxidation in the mitochondria. This impairment may induce not only lipid accumulation but also the accumulation of long-chain fatty acyl-CoA,

diacylglycerol and ceramide, intermediate metabolites of fatty acids [9]. These intermediate metabolites may further impair mitochondrial function in SKM [10]. Therefore, IMCL accumulation may not be merely consequence of mitochondrial dysfunction in SKM but also a cause for further impairment of mitochondrial function, resulting in a vicious cycle between IMCL and mitochondrial dysfunction and leading to lowered exercise capacity. The present study for the first time demonstrated that IMCL content was increased in SKM of patients with DCM. Increased IMCL content was associated with reduced exercise capacity and impaired energy metabolism in SKM. Therefore, lowered exercise capacity might be attributable to intrinsic SKM abnormalities including not only the abnormal energy metabolism but also the impairment of fatty acid metabolism. These findings provide new insights into the pathophysiology regarding impaired exercise capacity in HF.

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Figure Legends

Figure 1: Representative ^{1}H-MR spectra recorded at the resting tibialis anterior muscle from control subject and a patient with DCM in the quantification of IMCL content (A). The summarized data of IMCL content from control subjects (n = 12) and patients with DCM (n = 18) (B). Scatterplot between IMCL content and peak $\dot{V}O_2$ (C), AT (D), or PCr loss (E) in control subjects (○; n = 12) and patients with DCM (●; n = 18 or 17). Each plot represents the individual data of exercise capacity, ^{31}P-MRS, and ^{1}H-MRS obtained from the same subject. IMCL, intramyocellular lipid; EMCL, extramyocellular lipid; TCr, total creatine. Data are expressed as means ± SD. *P<0.01 vs. control.

A

Control

DCM

EMCL

IMCL

TCr

EMCL

IMCL

TCr

3 2 1 0

ppm

B

IMCL content (mmol/kg wet weight)

6 4 2 0

1.6±0.9

3.4±1.2

*

Control DCM

C

○ Control (n=12)
● DCM (n=18)

r=-0.589, p<0.01

Peak VO_2 (mL/kg/min)

40 30 20 10 0

0 2 4 6

IMCL content (mmol/kg wet weight)

D

○ Control (n=12)
● DCM (n=17)

r=-0.602 , p<0.01

AT (mL/kg/min)

30 20 10 0

0 2 4 6

IMCL content (mmol/kg wet weight)

E

○ Control (n=12)
● DCM (n=18)

r=0.630, p<0.01

PCr loss (%)

80 60 40 20 0

0 2 4 6

IMCL content (mmol/kg wet weight)