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Author(s)	Yokota, Takashi; Kinugawa, Shintaro; Okita, Koichi; Hirabayashi, Kagami; Suga, Tadashi; Hattori, Masaaki; Nakagawa, Yoshinao; Oyama-Manabe, Noriko; Shirato, Hiroki; Tsutsui, Hiroyuki
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Lower Aerobic Capacity was Associated with Abnormal Intramuscular Energetics in Patients with Metabolic Syndrome

Takashi YOKOTA¹, Shintaro KINUGAWA¹, Koichi OKITA², Kagami HIRABAYASHI¹, Tadashi SUGA¹, Masaaki HATTORI³, Yoshinao NAKAGAWA⁴, Noriko OYAMA-MANABE⁵, Hiroki SHIRATO⁵, and Hiroyuki TSUTSUI¹

¹Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo; ²Graduate School of Program in Lifelong Learning Studies, Hokusho University, Ebetsu; ³Department of Community Development, Tokai University, Sapporo; ⁴Department of Health Science, Otaru University, Otaru; and ⁵Department of Diagnostic and Interventional Radiology, Hokkaido University Hospital, Sapporo, Japan

Running title: Skeletal Muscle Dysfunction in Metabolic Syndrome

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Address corresponding: Shintaro Kinugawa, M.D., Ph.D.

Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan

Phone number: +81-11-706-6973, Fax number: +81-11-706-7874

E-mail address: tuckahoe@med.hokudai.ac.jp

Abstract

Lower aerobic capacity is a strong and independent predictor of cardiovascular morbidity and mortality in patients with metabolic syndrome (MetS). However, the mechanisms are not fully elucidated. We tested the hypothesis that skeletal muscle dysfunction could contribute to the lower aerobic capacity in MetS patients. The incremental exercise tests with cycle ergometer were performed in 12 male patients with MetS with no habitual exercise and 11 age-, sex-, and activity-matched control subjects to assess the aerobic capacity. We performed ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS) to assess the high-energy phosphate metabolism in skeletal muscle during aerobic exercise. Proton (^1H)-MRS was also performed to measure intramyocellular lipid (IMCL) content. Peak oxygen uptake (peak $\dot{V}\text{O}_2$; 34.1 ± 6.2 vs. 41.4 ± 8.4 mL/kg/min, $p < 0.05$) and anaerobic threshold (AT; 18.0 ± 2.4 vs. 23.1 ± 3.7 mL/kg/min, $p < 0.01$) adjusted by lean body mass were lower in MetS patients than control subjects. Phosphocreatine (PCr) loss during exercise was 1.5-fold greater in MetS, suggesting reduced intramuscular oxidative capacity. PCr loss was inversely correlated with peak $\dot{V}\text{O}_2$ ($r = -0.64$) and AT ($r = -0.60$), respectively. IMCL content was 3-fold higher in MetS and was inversely correlated with peak $\dot{V}\text{O}_2$ ($r = -0.47$) and AT ($r = -0.52$), respectively. Moreover, there was a positive correlation between IMCL content and PCr loss ($r = 0.64$). These results suggested that lean-body aerobic capacity in MetS patients was lower compared with activity-matched healthy subjects, which might be due to the reduced intramuscular fatty acid oxidative metabolism.

Key words: energy metabolism, exercise, metabolic syndrome, muscles

Introduction

The drastic increase in the number of obese patients with insulin resistance has become a medical and public health crisis in industrialized countries. Metabolic syndrome (MetS) characterized by insulin resistance and obesity contributes to the enhanced risk of developing atherosclerotic cardiovascular disease and type 2 diabetes (1, 2).

Lower aerobic capacity is an independent predictor of all-cause mortality in patients with insulin resistance and type 2 diabetes (3). Moreover, aerobic capacity is more powerful predictor of mortality than other established risk factors of cardiovascular diseases (4). Therefore, improving the aerobic capacity is of great importance in MetS patients. In general, the aerobic capacity is adjusted by body weight and tends to be low in obese subject such as MetS patients because of weight gain primarily due to increased fat mass. However, it has not been fully clarified whether lean-body aerobic capacity is impaired in patients with MetS.

The determinants of aerobic capacity are multifactorial, but aerobic capacity is generally believed to be impaired in the presence of abnormalities in skeletal muscle energy metabolism (5) and energy metabolism largely depends on mitochondrial function (6). Indeed, it has been shown that mitochondrial ATP production in skeletal muscle is impaired in insulin-resistant offspring of patients with type 2 diabetes (7), which raises the possibility that mitochondrial oxidative phosphorylation in skeletal muscle might be impaired in MetS. However, it has not been determined whether lower aerobic capacity is associated with skeletal muscle dysfunction in these patients.

Insulin resistance is characterized not only by abnormal glucose metabolism but also by abnormal fatty acid metabolism, which leads to the ectopic fat accumulation (8). It has been reported that intramyocellular lipid (IMCL) content is inversely correlated with insulin sensitivity in humans (9). IMCL content is determined by the balance between the uptake of free fatty acid into skeletal muscle cells and fatty acid β -oxidation within the mitochondria (10). Thus, abnormal fatty acid metabolism within the mitochondria can reduce the production of energy from fatty acid, which in turn might impair aerobic capacity in MetS. However, the significance of IMCL in the skeletal muscle energy metabolism and aerobic capacity in MetS is not fully elucidated.

Therefore, the purpose of this study was to determine 1) whether lean-body aerobic capacity, skeletal muscle energy metabolism, and IMCL content are abnormal in MetS patients, and 2) whether these abnormalities are related to each other.

Methods

Subjects

Twelve sedentary Japanese male patients with MetS, diagnosed by physical checkups at Hokkaido University Hospital or neighboring hospitals on the basis of International Diabetes Federation (IDF) criteria, were studied. All subjects underwent a physical examination and assessment of medical history. They also underwent electrocardiograms and cardiac ultrasounds. Patients with cardiovascular disease, peripheral artery disease, pulmonary disease, stroke, and orthopedic disease who had difficulty performing exercise testing were excluded. Patients receiving insulin or antidiabetic drugs were also excluded. Six patients were treated with antihypertensive drugs, including calcium antagonists in 4 patients, β -blockers in 3 patients, angiotensin receptor blockers in 3 patients, and diuretics in one patient. One hypercholesterolemic patient was taking atorvastatin. Eleven age-, sex-, and activity-matched healthy subjects were also studied as control subjects. The protocol was approved by the medical ethics committee of Hokkaido University Hospital, and written informed consent was obtained from all participating subjects.

Clinical and anthropometric measurements

Body weight, height, waist circumference, blood pressure, and heart rate were measured, and body mass index (body weight/[height]², kg/m²) was calculated. Whole-body fat mass and lean body mass (LBM) were measured by an air displacement plethysmograph (the BOD POD[®] Body Composition System: Life Measurement Instruments, Concord, CA, USA).

Daily physical activity

To monitor the level of physical activity during daily life, movement-related calorie consumption and steps were measured for 1 week using a pedometer equipped with an accelerometer (Lifecorder Plus, Suzuken, Nagoya, Japan), as described previously (11).

Blood biochemistry

Peripheral blood samples were collected after 10 h of fasting. Blood glucose, plasma insulin, glycohemoglobin A1c (HbA1c), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and free fatty acid (FFA) were measured. The homeostasis assessment model of insulin resistance (HOMA-IR) was also calculated (12).

Aerobic capacity

All subjects exercised on an upright electromechanical ergometric bicycle (Aerobike 75XLII, Combi Wellness, Tokyo, Japan) using a ramp protocol (25 watts/min). As an index of perceived effort, the rating of perceived exertion (RPE) was evaluated with the 10-point Borg scale immediately after the exercise was finished. Respiratory gas analysis was performed with a breath-by-breath apparatus (Aeromonitor AE-300S, Minato Medical Science, Osaka, Japan). The anaerobic threshold (AT) was determined by the V-slope method, as described previously (13). AT could not be measured in one patient with MetS due to technical difficulties. To eliminate the influence of the differences in body composition between groups, the absolute values of peak oxygen uptake (peak

$\dot{V}O_2$) and AT were adjusted by LBM as well as body weight.

Skeletal muscle energy metabolism by ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS)

Muscle strength was initially determined by the one repetition maximum (1-RM) measurement, which measured the maximum weight that could be lifted 5 cm above ground, as described previously (14). The 1-RM was determined by a successful plantar flexion without any assistance from other body parts (e.g. thigh). The 1-RM measurement was designed using increments of 10 kg until 60-80% of the perceived maximum. Then, the load was gradually increased by 1-5 kg weight until the subject was not able to maintain proper form or to completely lift the weight. The final acceptable weight was determined as 1-RM. The calf flexor muscle cross-sectional area (MCA) at the level of the muscle belly was also measured using magnetic resonance imaging.

Measurements of ^{31}P -MRS in the calf flexor muscle were performed by a 1.5-Tesla superconducting magnet (Magnetom Vision VB33G, Siemens, Erlangen, Germany), as described previously (14). A unilateral plantar flexion exercise with a constant load of 20% 1-RM was performed for 4 min with 0.67 Hz on the original apparatus. The spectra of high-energy phosphate metabolites were acquired at rest and every 30 s during exercise at an echo time (TE) of 1 ms and repetition time (TR) of 2000 ms. Phosphocreatine (PCr) was standardized as $[\text{PCr}]/([\text{PCr}]+[\text{Pi}])$, where $[\text{PCr}]$ indicates the concentration of PCr and $[\text{Pi}]$ indicates the concentration of inorganic phosphate (Pi). The maximal degree of PCr change (PCr loss) during exercise was calculated as: $\text{PCr loss} = (\text{PCr}_{\text{rest}} - \text{PCr}_{\text{peak}}) / \text{PCr}_{\text{rest}}$

The intramuscular pH was calculated from changes in the chemical shifts of Pi relative to PCr, as described previously (15).

IMCL content by proton (¹H)-MRS

IMCL content in the resting tibialis anterior muscle at the level of the muscle belly of the calf was measured after the blood correction at fasting state using ¹H-MRS, as described previously (16). Magnetic resonance (MR) images were acquired using a clinical 1.5-Tesla whole body scanner system (Signa Horizon LX, GE Medical systems, Milwaukee, WI, USA) and a standard head coil (28 cm diameter) was used for detection. Transverse T₁-weighed MR images (TE/TR = 8.5/400 ms) were acquired to determine the placement of the ¹H-MRS voxels. The voxel volume was 10 × 10 × 10 mm³. Localized proton spectra were obtained by a point-resolved spectroscopy (PRESS) sequence with TE/TR = 30/3000 ms and 64 averages with water suppression. Unsuppressed water spectra were also acquired as an internal standard. Spectra were processed using the SAGE software package (GE Medical systems, Milwaukee, WI, USA). Quantification of IMCL and extramyocellular lipid (EMCL) was carried out to compare the intensity of (CH₂)_n at 1.3 ppm and 1.5 ppm resonance with the water resonance intensity at 4.7 ppm. IMCL and EMCL were quantified relative to muscle water, as described previously (16).

Statistical analysis

Data are expressed as means ± SD. Student's unpaired t-tests were performed to compare means between patients with MetS and control subjects. Correlations were examined by linear regression analysis using the least-squares

method. Statistical significance was defined as $p < 0.05$.

Results

Characteristics of the study subjects (Table 1)

Age of control subjects and MetS patients were similar. Body weight, body mass index, percent fat, and waist circumference were significantly higher in patients with MetS compared with control subjects; however, there was no significant difference in LBM between groups. There was no significant difference in blood pressure between groups, however, some patients with MetS were treated with antihypertensive drugs. The daily physical activity, assessed by movement-related calorie consumption and steps, was comparable between groups.

Blood biochemistry (Table 2)

As expected, fasting blood glucose, plasma insulin, HOMA-IR, HbA1c, and triglycerides were significantly higher in MetS. By contrast, no significant difference was found in HDL cholesterol, LDL cholesterol, and FFA.

Aerobic capacity (Table 3)

The RPE and peak respiratory exchange ratio were comparable between groups. Peak $\dot{V}O_2$ and AT adjusted by body weight were significantly lower in MetS patients, even when normalized to LBM, they were significantly lower in patients with MetS, suggesting that lean-body aerobic capacity was impaired in MetS. No significant difference was found in peak workload between groups.

High-energy phosphate metabolism in skeletal muscle

There was no significant difference in the muscle strength (1-RM; 40.5 ± 6.9 kg for control vs. 43.2 ± 6.0 kg for MetS) or muscle mass (MCA; 53.4 ± 7.4 cm² for control vs. 56.4 ± 8.8 cm² for MetS) between groups. The representative spectra of ³¹P-MRS are shown in **Fig. 1A and B**. Spectra of ³¹P-MRS at rest were similar in the two groups. The PCr level was lower and the Pi level was higher in a MetS patient than in a control subject at peak exercise. By contrast, no alteration in ATP level during exercise was found in either group. The summary data are shown in **Fig.1C-E**. There was no significant difference in the standardized PCr at rest between groups (0.88 ± 0.03 for control vs. 0.89 ± 0.02 for MetS) (**Fig.1C**), whereas the standardized PCr at peak exercise was significantly lower in patients with MetS compared with control subjects (0.60 ± 0.09 vs. 0.69 ± 0.08 , $p < 0.05$) (**Fig.1D**). Accordingly, PCr loss, difference in standardized PCr between resting and peak exercise, was significantly greater in MetS patients than in control subjects (0.20 ± 0.08 vs. 0.29 ± 0.08 , $p < 0.01$) (**Fig. 1E**). There was no decrease in the intramuscular pH during plantar flexion exercise in either group.

To examine whether lean-body aerobic capacity is related to high-energy phosphate metabolism in skeletal muscle, the indices of lean-body aerobic capacity were plotted against PCr loss within the same individuals. Peak $\dot{V}O_2$ and AT normalized to LBM were inversely correlated with PCr loss (**Fig. 2A and B**).

IMCL content

Fig. 3A shows the representative spectra of ¹H-MRS. IMCL content was significantly greater in MetS patients than in control subjects (5.1 ± 1.5

mmol/kg wet weight vs. 1.7 ± 1.0 mmol/kg wet weight, $p < 0.01$) (**Fig. 3B**).

IMCL was significantly correlated with body weight ($r = 0.67$, $p < 0.01$), body mass index ($r = 0.74$, $p < 0.01$), percent fat ($r = 0.71$, $p < 0.01$), waist circumference ($r = 0.78$, $p < 0.01$), fasting blood glucose ($r = 0.44$, $p < 0.05$), plasma insulin ($r = 0.62$, $p < 0.01$), HOMA-IR ($r = 0.61$, $p < 0.01$), and triglyceride ($r = 0.57$, $p < 0.01$).

To examine whether IMCL content is related to lean-body aerobic capacity or high-energy phosphate metabolism in skeletal muscle, IMCL content was plotted against peak $\dot{V}O_2$ and AT adjusted by LBM or PCr loss within the same individuals. Peak $\dot{V}O_2$ and AT adjusted by LBM were inversely correlated with IMCL content (**Fig. 3C and D**). Moreover, PCr loss was positively correlated with IMCL content (**Fig. 3E**).

Discussion

The present study demonstrated for the first time that the lean-body aerobic capacity in MetS patients with no habitual exercise was lower compared with activity-matched control subjects and was inversely correlated with high-energy phosphate metabolism in skeletal muscle. Furthermore, IMCL content was increased in MetS patients, and importantly, was inversely correlated with the aerobic capacity as well as insulin sensitivity. The correlation between IMCL content and impairment of high-energy phosphate metabolism in skeletal muscle might reflect the impaired fatty acid oxidation in skeletal muscle of MetS patients. Therefore, our data suggest that the impaired intramuscular fatty acid oxidative metabolism might contribute to the lower lean-body aerobic capacity in MetS patients.

The aerobic capacity was lower in patients with MetS, which was supported by the reduced peak $\dot{V}O_2$ and AT (**Table 3**). The lower aerobic capacity in MetS was not merely due to the increased body weight and fat mass, because peak $\dot{V}O_2$ was significantly reduced in these patients even after adjusted by LBM (**Table 3**). Moreover, all subjects enrolled in the present study had usual physical activity, and their daily physical activity was comparable between the two groups (**Table 1**). Therefore, physical activity did not affect the difference in aerobic capacity between groups in this setting, although physical activity is one of the most important factors for aerobic capacity. Previous studies have demonstrated that peak $\dot{V}O_2$ was decreased in patients with type 2 diabetes (17). However, these studies were not designed to strictly match the physical activity between control subjects and patients with type 2 diabetes. The present study

clearly demonstrated that the lean-body aerobic capacity in patients with MetS was lower than that in activity-matched healthy subjects.

In the present study, PCr loss was greater in MetS patients than that in control subjects (**Fig. 1E**). PCr always works as an energy buffer, which can be converted to ATP to compensate for impaired oxidative phosphorylation or glycolysis and maintain the ATP level constant during exercise. The intramuscular pH in the calf flexor muscle did not significantly fall during plantar flexion exercise in either group because of low-intensity exercise, indicating that oxidative metabolism was mainly observed in our ^{31}P -MRS study, although glycolysis was also a source of ATP production. Therefore, the greater PCr loss in MetS patients (**Fig.1E**) suggests the impairment of intramuscular high-energy phosphate metabolism assessed by, at least in part, oxidative phosphorylation in mitochondria. This finding was consistent with the previous studies that skeletal muscle biopsy samples from patients with insulin resistance and type 2 diabetes demonstrated mitochondrial dysfunction or reduced gene expression involved in mitochondrial oxidative phosphorylation (18, 19). Moreover, the present study demonstrated for the first time that peak $\dot{V}\text{O}_2$ and AT normalized to LBM were closely correlated with PCr loss (**Fig. 2A and B**). In contrast, muscle strength and muscle mass were comparable between groups. Therefore, skeletal muscle energy metabolism is a major determinant of aerobic capacity in MetS patients.

IMCL content was increased in MetS patients compared with control subjects (**Fig. 3B**). An imbalance of uptake and oxidation of fatty acid could lead to lipid accumulation within skeletal muscle in the setting of insulin resistance (20). Insulin resistance has been characterized by the reduced

capacity of fatty acid oxidation in skeletal muscle rather than by the rate of fatty acid uptake into skeletal muscle in obese subjects with insulin resistance (21). Moreover, a state of metabolic inflexibility in skeletal muscle, which is characterized by lower rate of fatty acid oxidation during fasting conditions and impaired glucose oxidation on insulin stimulation, could contribute to the accumulation of IMCL (22). Taken together, the increased IMCL content in patients with MetS may directly reflect the impaired fatty acid oxidation in skeletal muscle, which is consistent with our finding that IMCL content was correlated with impairment of high-energy phosphate metabolism (**Fig. 3E**). Interestingly, IMCL content was inversely correlated with the lean-body aerobic capacity (**Fig.3C and D**). These findings suggest that the energy production within the mitochondria and the energy substrate supply to the mitochondria are decreased in skeletal muscle, and that this decrease might lead to the lower aerobic capacity in patients with MetS.

In the present study, IMCL content was correlated with insulin resistance, such as fasting blood glucose, insulin, and HOMA-IR, as previously described (9). Blaak et al. showed that the fatty acid oxidation was impaired in skeletal muscle from patients with type 2 diabetes (23). They concluded that the impairment of fatty acid oxidation could be a cause of insulin resistance and type 2 diabetes, and not merely a consequence. Importantly, the impaired fatty acid oxidation can lead to the accumulation of specific IMCL intermediates including long-chain fatty acyl-CoAs, diacylglycerol, and ceramide as well as IMCL (24). Recent studies have revealed that the accumulation of IMCL intermediates might impair insulin signaling (25) and the mitochondrial function

(26). Therefore, IMCL might play a major role in the pathogenesis of insulin resistance.

There are limitations to the present study that should be acknowledged. First, peripheral blood flow was not measured in the study subjects. Because vasodilation is impaired in patients with insulin resistance and diabetes (27), the impaired aerobic capacity we observed might be due to the decreased blood flow to skeletal muscle. However, Hällsten et al. demonstrated that the peripheral blood flow to skeletal muscle in obese and insulin-resistant subjects was not lower than that in controls, even during exercise (28). Therefore, skeletal muscle blood flow is not likely to have influenced the aerobic capacity in MetS patients in the present study. Second, the correlation between the aerobic capacity and the skeletal muscle energy metabolism was not significant when the analysis was performed only within MetS patients. Because the range of aerobic capacity in MetS patients was small, we could not detect a significant correlation.

In conclusion, the present study demonstrated that the lean-body aerobic capacity was impaired in MetS patients with no habitual exercise compared with activity-matched control subjects. This result is likely due to the impaired intramuscular fatty acid oxidative metabolism. These findings provide new insights into the pathophysiology regarding the lower aerobic capacity in MetS, which might be useful for its therapeutic treatment.

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uptake in obese men. *Obes Res.* 2003;11(2):257-265.

Table 1 Characteristics of the study subjects

	Control	MetS
N	11	12
Age, years	49 ± 10	49 ± 10
Body weight, kg	65.6 ± 8.2	80.7 ± 11.5*
Body mass index, kg/m ²	22.5 ± 2.0	27.2 ± 3.3*
Waist circumference, cm	80.8 ± 6.3	95.6 ± 8.7*
Percent fat, %	21.2 ± 4.5	29.0 ± 4.6*
Lean body mass, kg	51.6 ± 5.1	56.4 ± 9.0
Systolic blood pressure, mmHg	122.9 ± 12.8	135.0 ± 16.5
Diastolic blood pressure, mmHg	77.0 ± 9.1	81.0 ± 11.6
Steps, steps/day	7185 ± 1835	7353 ± 2180
MCC, kcal/day	215 ± 66	238 ± 65

MCC, movement-related calorie consumption. Data are means ± SD. * $p < 0.01$ vs. control subjects.

Table 2 Blood biochemistry

	Control	MetS
N	11	12
Blood glucose, mg/dL	90.4 ± 7.4	110.0 ± 17.2†
Insulin, μIU/mL	4.7 ± 2.1	13.7 ± 7.6†
HOMA-IR	1.0 ± 0.5	3.8 ± 2.2†
HbA1c, %	5.2 ± 0.3	5.6 ± 0.6*
HDL cholesterol, mg/dL	62.3 ± 15.0	52.8 ± 11.6
LDL cholesterol, mg/dL	109.7 ± 29.6	129.7 ± 31.7
Triglyceride, mg/dL	95.6 ± 48.3	160.4 ± 71.8*
Free fatty acid, mEq/L	0.47 ± 0.23	0.53 ± 0.20

HbA1c, glycohemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; LDL, low-density lipoprotein. Data are means ± SD. * $p < 0.05$ and † $p < 0.01$ vs. control subjects.

Table 3 Aerobic capacity

	Control	MetS
N	11	12
RPE	7.6 ± 1.5	7.4 ± 1.5
Peak respiratory exchange ratio	1.27 ± 0.10	1.21 ± 0.09
Peak $\dot{V}O_2$ /BW, mL/kg/min	31.9 ± 5.7	23.9 ± 4.7†
Peak $\dot{V}O_2$ /LBM, mL/kg/min	41.4 ± 8.4	34.1 ± 6.2*
AT/BW, mL/kg/min	18.9 ± 4.0	12.7 ± 1.2†
AT/LBM, mL/kg/min	23.1 ± 3.7	18.0 ± 2.4†
Peak workload, watts	185.8 ± 32.8	163.1 ± 36.2

AT, anaerobic threshold; BW, body weight; LBM, lean body mass; Peak $\dot{V}O_2$, peak oxygen uptake; RPE, rating of perceived exertion. Data are means ± SD. * $p < 0.05$ and † $p < 0.01$ vs. control subjects.

Figures legends

Figure 1 – A and B: Representative ^{31}P -MR spectra at rest (upper panel) and peak plantar flexion exercise (lower panel) in the calf muscle of a control subject (A) and a MetS patient (B). C-E: The summary data of standardized PCr at rest (C), peak exercise (D), and PCr loss (E) from control subjects ($n = 11$) and MetS patients ($n = 12$). Pi, inorganic phosphate; PCr, phosphocreatine. $*p < 0.05$ and $\dagger p < 0.01$ vs. control subjects.

Figure 2 – Association between lean-body aerobic capacity and intramuscular high-energy phosphate metabolism. AT, anaerobic threshold; LBM, lean body mass; Peak $\dot{V}\text{O}_2$, peak oxygen uptake; PCr, phosphocreatine.

Figure 3 - A and B: Representative ^1H -MR spectra in the resting tibialis anterior muscle (A) of a control subject (upper panel) and a MetS patient (lower panel), and the summary data of IMCL content (B) from control subjects ($n = 11$) and MetS patients ($n = 12$). C-E: The association between IMCL and lean-body aerobic capacity or high-energy phosphate metabolism in skeletal muscle. EMCL, extramyocellular lipid; IMCL, intramyocellular lipid; TCr, total creatine. $*p < 0.01$ vs. control subjects.

Figure 1

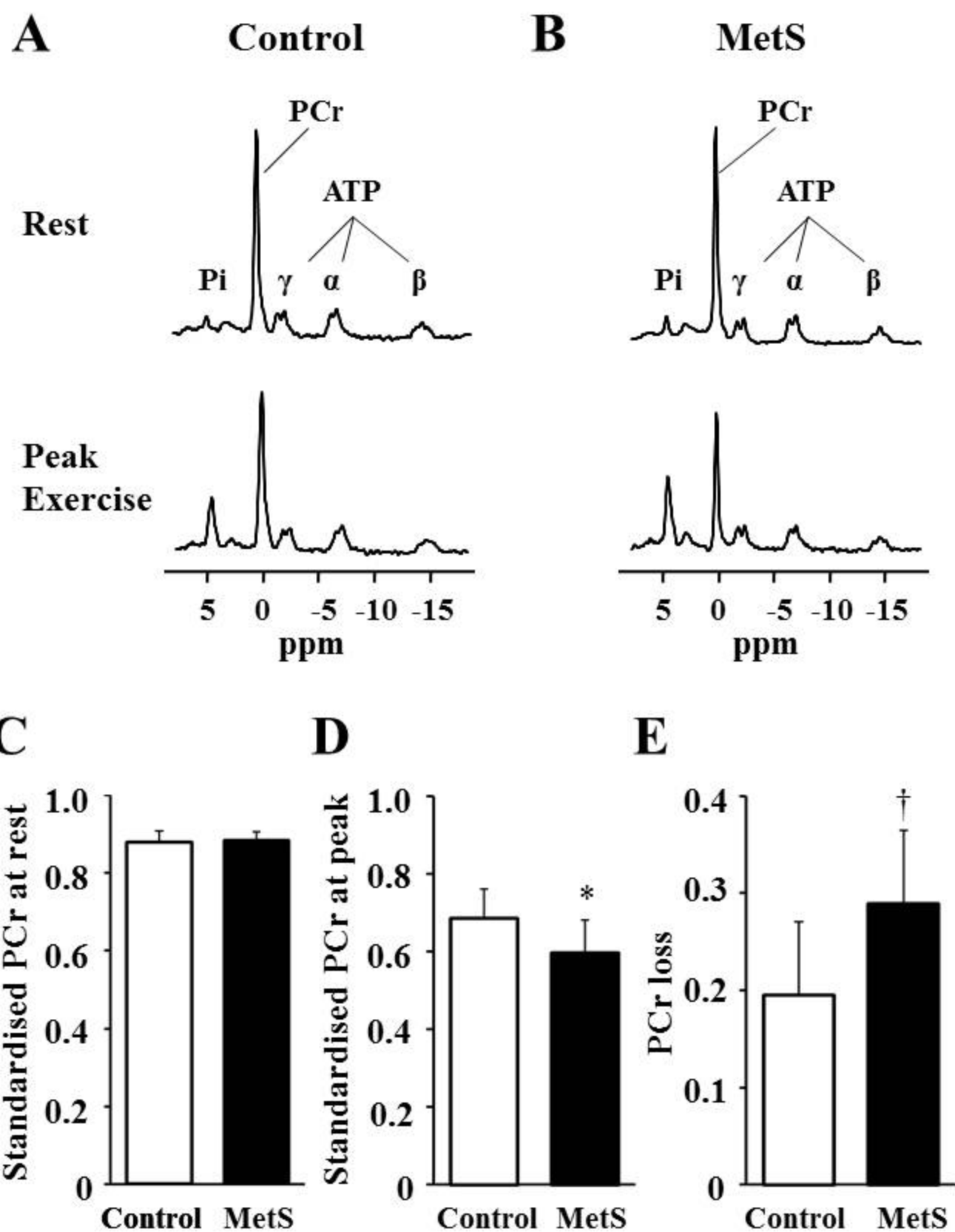
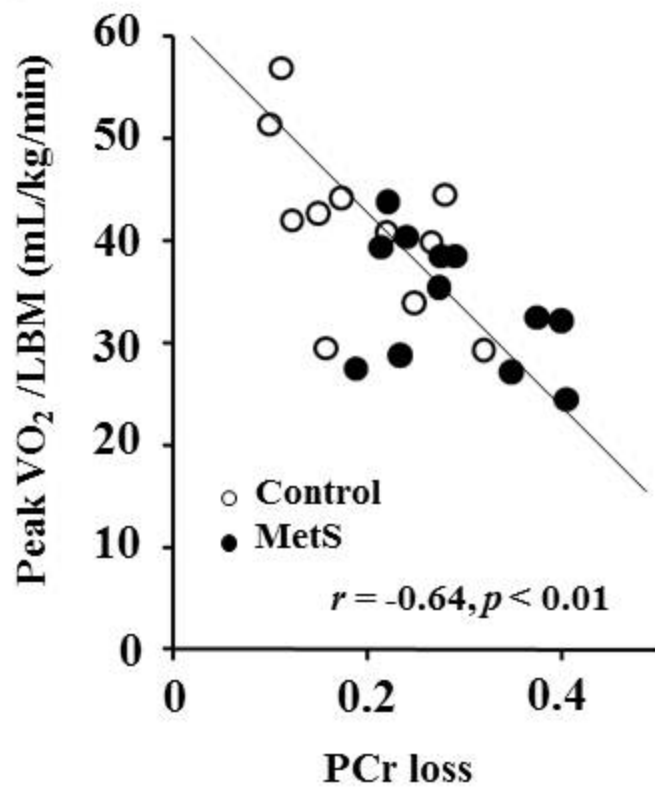


Figure 2

A



B

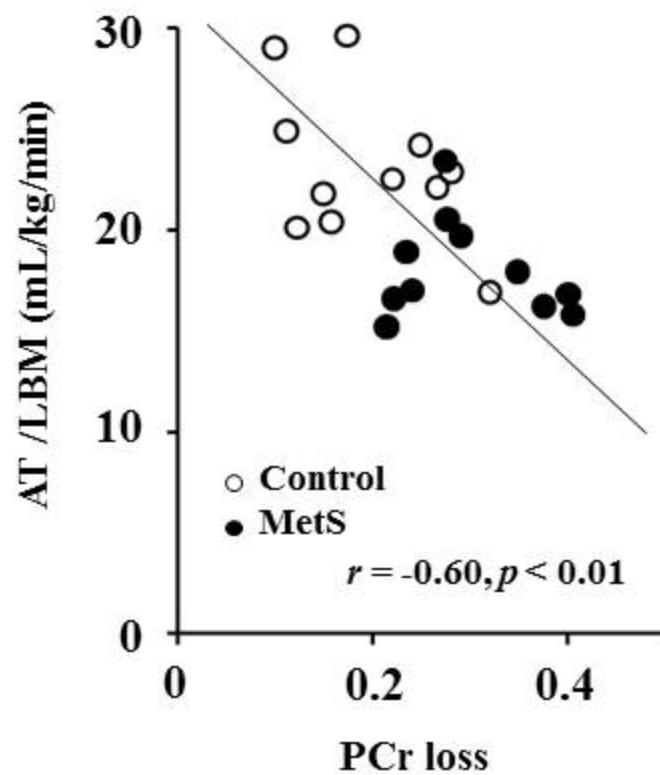


Figure 3

