Systemic Oxidative Stress Is Associated With Lower Aerobic Capacity and Impaired Skeletal Muscle Energy Metabolism in Patients With Metabolic Syndrome

Takashi Yokota, md, phd[1,2]
Shintaro Kinugawa, md, phd[1]
Mayumi Yamato, phd[3]
Kagami Hirabayashi, md, phd[1]
Tadashi Suga, phd[1,4]
Shingo Takada, ms[1,4]
Kuniaki Harada, phd[5]
Noriteru Morita, phd[6]
Noriko Oyama-Manabe, md, phd[7]
Yasuka Kikuchi, md[7]
Koichi Okita, md, phd[8]
Hiroyuki Tsutsui, md, phd[1]

OBJECTIVE—Systemic oxidative stress is associated with insulin resistance and obesity. We tested the hypothesis that systemic oxidative stress is linked to lower aerobic capacity and skeletal muscle dysfunction in metabolic syndrome (MetS).

RESEARCH DESIGN AND METHODS—The incremental exercise testing with cycle ergometer was performed in 14 male patients with MetS and 13 age-, sex-, and activity-matched healthy subjects. Systemic lipid peroxidation was assessed by serum thiobarbituric acid reactive substances (TBARS), and systemic antioxidant defense capacity was assessed by serum total thiols and enzymatic activity of superoxide dismutase (SOD). To assess skeletal muscle energy metabolism, we measured high-energy phosphates in the calf muscle during plantar flexion exercise and intramyocellular lipid (IMCL) in the resting leg muscle, using ^{31}P- and ^{1}proton-magnetic resonance spectroscopy, respectively.

RESULTS—Serum TBARS were elevated (12.4 ± 7.1 vs. 3.7 ± 1.1 μmol/L; $P < 0.01$), and serum total thiols and SOD activity were decreased (290.8 ± 51.2 vs. 398.7 ± 105.2 μmol/L, $P < 0.01$; and 22.2 ± 8.4 vs. 31.5 ± 8.5 units/L, $P < 0.05$, respectively) in patients with MetS compared with healthy subjects. Peak VO_2 and anaerobic threshold normalized to body weight were significantly lower in MetS patients by 25 and 31%, respectively, and inversely correlated with serum TBARS ($r = -0.49$ and $r = -0.50$, respectively). Moreover, muscle phosphocreatine loss during exercise was 1.4-fold greater in patients with MetS ($P < 0.05$), and IMCL content was 2.9-fold higher in patients with MetS ($P < 0.01$), indicating impaired skeletal muscle energy metabolism, and these indices positively correlated with serum TBARS ($r = 0.45$ and $r = 0.63$, respectively).

CONCLUSIONS—Systemic oxidative stress was associated with lower aerobic capacity and impaired skeletal muscle energy metabolism in patients with MetS.

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From the [1]Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan; the [2]Department of Biomedical Sciences, Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark; the [3]Innovation Center for Medical Redox Navigation, Kyusyu University, Fukuoka, Japan; [4]Research Fellow of the Japan Society for the Promotion of Science, Tokyo, Japan; the [5]Division of Radiology, Sapporo Medical University, Sapporo, Japan; the [6]Department of Sports Education, Hokkaido University of Education, Iwamizawa, Japan; the [7]Department of Diagnostic and Interventional Radiology, Hokkaido University Hospital, Sapporo, Japan; and the [8]Graduate School of Program in Lifelong Learning Studies, Hokusho University, Ebetsu, Japan.
Corresponding author: Shintaro Kinugawa, tuckahoe@med.hokudai.ac.jp.
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Metabolic syndrome (MetS) is mainly characterized by insulin resistance and obesity, which increases not only the risk of developing type 2 diabetes and cardiovascular diseases but also all-cause mortality (1). The drastic increase in patients with MetS has been now recognized as a medical and public health crisis in the world.

Lower aerobic capacity is one of the strongest and independent predictors of all-cause mortality in subjects with obesity and insulin resistance (2,3), and it is essential to improve lower aerobic capacity in the treatment of MetS. Skeletal muscle energy metabolism is a major determinant of aerobic capacity, and its metabolic regulation is largely dependent on mitochondrial function, which plays a pivotal role in energy homeostasis, including the metabolism of nutrients and production of ATP (4). Recent studies have shown that impaired mitochondrial function in the skeletal muscle may be involved in the pathogenesis of insulin resistance (5–7). Moreover, it has been reported that intramyocellular lipid (IMCL) is accumulated in patients with MetS, which is mainly due to impaired fatty acid oxidation in the skeletal muscle rather than increased uptake of fatty acid into the skeletal muscle (8,9). Recently, we demonstrated that increased IMCL was associated with lower aerobic capacity and impaired intramuscular high-energy phosphate metabolism in patients with MetS (10). However, the mechanisms by which aerobic capacity and skeletal muscle energy metabolism are impaired in MetS remain fully unexplored.

Oxidative stress is generally defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity. An increased amount of evidence suggests that oxidative stress is linked to either primary or secondary pathogenesis of various chronic diseases, such as cancer (11), type 2 diabetes (12), inflammation (13), and neurodegenerative disorders (14). Recently, it has been demonstrated

that systemic oxidative stress is related to insulin resistance and obesity (15,16). Furthermore, it has been reported that oxidative stress can cause mitochondrial dysfunction in the skeletal muscle from diet-induced insulin-resistant mice (17). These reports raise the possibility that systemic oxidative stress may contribute to lower aerobic capacity and skeletal muscle dysfunction in patients with MetS.

In the current study, we tested the hypothesis that systemic oxidative stress is associated with lower aerobic capacity and impaired skeletal muscle energy metabolism in patients with MetS. For a comprehensive investigation of oxidative stress, we measured byproducts of ROS by serum thiobarbituric acid reactive substances (TBARS) as well as serum total thiols, superoxide dismutase (SOD) activity, and glutathione peroxidase (GPx) activity as an antioxidant defense capacity. Moreover, to assess skeletal muscle energy metabolism, high-energy phosphates in the calf muscle during plantar flexion exercise and IMCL content in the resting leg muscle were measured using magnetic resonance spectroscopy (MRS).

RESEARCH DESIGN AND METHODS

Subjects

Fourteen male patients with MetS diagnosed on the basis of International Diabetes Federation criteria and 13 age-, sex-, and activity-matched healthy control subjects were enrolled in the current study. Patients with cardiovascular disease, peripheral artery disease, pulmonary disease, stroke, and orthopedic disease who had difficulty performing exercise testing were excluded. None of the subjects were taking medications to treat type 2 diabetes or dyslipidemia. Among 14 patients with MetS, 7 patients were treated with antihypertensive drugs, including calcium antagonists in 5 patients, β-blockers in 3 patients, angiotensin II receptor blockers in 3 patients, and/or diuretics in 1 patient. 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, and blood pressure were measured. Whole-body fat mass and lean body mass (LBM) were measured by an air displacement plethysmograph (BOD POD Body Composition System; Life Measurement Instruments, Concord, CA), as previously described (10).

Blood analysis

Peripheral blood samples were collected after 10 h of fasting. Blood glucose, plasma insulin, HbA_{1c}, HDL cholesterol, LDL cholesterol, triglyceride, and free fatty acids were measured. The homeostasis assessment model of insulin resistance (HOMA-IR) was calculated (18).

Aerobic capacity

All subjects exercised on an upright electromechanical bicycle ergometer with electrocardiogram using a ramp protocol (25 W/min after a 3-min warm-up), as previously described (10). Respiratory gas analysis was simultaneously performed with a breath-by-breath apparatus (Aeromonitor AE-300S; Minato Medical Science, Osaka, Japan), and peak VO_2 was measured. Anaerobic threshold (AT) was determined by the V-slope method (19), except for one patient.

Daily physical activity

To monitor the level of physical activity during daily life, daily steps and movement-related calorie consumption were measured for at least 1 week by using a pedometer with an accelerometry sensor (Lifecorder Plus; Suzuken, Nagoya, Japan), as previously described (10).

Intramuscular high-energy phosphate metabolism

Before measurements, muscle strength was determined by the one-repetition maximum (1-RM) method, as previously described (10). The calf flexor muscle cross-sectional area was measured at the level of muscle belly using magnetic resonance images. After at least 30 min rest, high-energy phosphate metabolism in the calf muscle was measured during plantar flexion exercise in supine position on the original apparatus equipped with a 1.5-Tesla whole-body scanner system (Magnetom Vision VB33G; Siemens, Erlangen, Germany) using ^{31}P-MRS, as previously described (10). Unilateral plantar-flexion exercise was performed with a constant load of 20% 1-RM for 4 min at the pace of 40 repetitions/min. Phosphocreatine (PCr) was standardized as [PCr]/([PCr] + [Pi]) on the basis of the notion that [PCr] + [Pi] is constant at rest and during exercise, in which [PCr] indicates concentration of PCr, and [Pi] indicates concentration of inorganic phosphate (Pi). Moreover, the degree of PCr change (i.e., PCr loss) during exercise was calculated as PCr loss = standardized PCr_{rest} − standardized PCr_{peak}, in which PCr_{rest} indicates the PCr level at rest, and PCr_{peak} indicates the lowest PCr level during exercise.

IMCL content

IMCL content in the resting tibialis anterior muscle at the level of the muscle belly of calf was measured by proton (^{1}H)-MRS on a 1.5-Tesla whole-body scanner system (Signa Horizon LX; GE Medical Systems, Milwaukee, WI), as previously described (10).

Systemic oxidative stress

Serum TBARS were measured by fluorometric analysis using a GENios Pro (Tecan Group Ltd., Männedorf, Switzerland), as previously described (20). We also measured serum total thiols and enzymatic activity of SOD and GPx. The amount of total thiols was measured spectrophotometrically with 5,5′-dithiobis (2-nitrobenzoic acid) (Sigma-Aldrich, St. Louis, MO), as previously described (21). Enzymatic activity of SOD and GPx was measured by fluorometric analysis, as previously described (20).

Statistical analysis

Data are expressed as means ± SD. Student unpaired *t* tests were performed to compare means between control subjects and patients with MetS. Correlations were examined by linear regression analysis using the least-squares method. Based on the previous report that compared the marker of oxidative stress between control subjects and patients with MetS (22), a sample size of 13 subjects in each group was needed to detect the effect compared with the threshold change of 0 under the conditions of $\alpha = 0.05$ and $\beta = 0.2$. Moreover, to determine the association between oxidative stress and aerobic capacity of a large effect size (i.e., $r = 0.5$), a sample size of total 27 subjects was needed to yield efficient power ($1-\beta = 0.8$) based on $\alpha = 0.05$. Statistical analysis was performed using StatView software (SAS Institute, Inc., Cary, NC), and $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study subjects

Body weight, BMI, percent fat, and waist circumference were significantly higher in patients with MetS than control subjects (Table 1). Fasting blood glucose,

Table 1—*Characteristics of the study subjects*

	Control (n = 13)	MetS (n = 14)
Age (years)	48 ± 9	48 ± 9
BW (kg)	66.9 ± 8.3	80.0 ± 10.8†
LBM (kg)	52.8 ± 5.6	56.4 ± 8.7
BMI (kg/m^2)	22.8 ± 2.0	26.8 ± 3.4†
Percent fat (%)	20.9 ± 4.2	28.8 ± 4.3†
Waist circumference (cm)	82.3 ± 7.0	95.5 ± 8.0†
Systolic blood pressure (mmHg)	123 ± 12	134 ± 16
Diastolic blood pressure (mmHg)	76 ± 10	81 ± 11
Fasting blood glucose (mg/dL)	91 ± 8	112 ± 17†
Insulin (μIU/mL)	4.4 ± 2.0	13.1 ± 7.5†
HOMA-IR	1.0 ± 0.5	3.6 ± 2.2†
HbA_{1c} (%)	5.1 ± 0.3	5.6 ± 0.5†
HDL cholesterol (mg/dL)	62 ± 14	51 ± 11*
LDL cholesterol (mg/dL)	110 ± 28	130 ± 30
Triglyceride (mg/dL)	102 ± 49	158 ± 66*
Free fatty acids (mEq/L)	0.46 ± 0.22	0.55 ± 0.20
Peak VO_2/BW (mL/kg/min)	32.5 ± 7.3	24.0 ± 4.3†
Peak VO_2/LBM (mL/kg/min)	42.0 ± 10.2	34.2 ± 5.7*
AT/BW (mL/kg/min)	18.0 ± 4.3	12.4 ± 1.3†
AT/LBM (mL/kg/min)	22.1 ± 4.2	17.7 ± 2.4†

Data are means ± SD. BW, body weight; LBM, lean body mass. *$P < 0.05$, †$P < 0.01$ vs. control.

plasma insulin, HOMA-IR, HbA_{1c}, and triglycerides were significantly elevated, and HDL cholesterol was significantly decreased in patients with MetS (Table 1).

Peak VO_2 and AT normalized to both body weight and LBM were significantly lower in patients with MetS (Table 1). There was no significant difference in daily physical activity between patients with MetS and control subjects (steps: 8,367 ± 3,670 vs. 7,172 ± 1,717 steps/day; movement-related calorie consumption: 265 ± 209 vs. 214 ± 62 kcal/day).

Intramuscular high-energy phosphate metabolism and IMCL content

After the initiation of constant load of 20% 1-RM exercise, PCr level in the calf muscle started to decrease and was finally stabilized within a few minutes in all subjects. The representative spectra of ^{31}P-MRS are shown in Supplementary Fig. 1. In summary, the standardized PCr level at rest was comparable, whereas the lowest level of standardized PCr during exercise was significantly reduced in patients with MetS (Table 2). As a result, muscle PCr loss during exercise was greater in patients with MetS (Table 2). In addition, there was an inverse correlation between muscle PCr loss and peak VO_2 ($r = -0.49$; $P < 0.01$) or AT ($r = -0.51$; $P < 0.01$) normalized to body weight in all subjects by linear regression analysis, indicating that impairment in intramuscular high-energy phosphate metabolism was associated with the lower aerobic capacity in MetS.

Table 2—*Skeletal muscle energy metabolism*

	Control (n = 13)	MetS (n = 14)
1-RM (kg)	39.8 ± 6.6	43.9 ± 5.9
MCA of calf muscle (cm^2)	54.0 ± 7.3	55.9 ± 8.6
^{31}P-MRS		
Standardized PCr_{rest}	0.88 ± 0.03	0.88 ± 0.02
Standardized PCr_{peak}	0.68 ± 0.07	0.61 ± 0.09*
PCr loss	0.19 ± 0.07	0.27 ± 0.08*
^{1}H-MRS		
IMCL content (mmol/kg wet weight)	1.7 ± 1.0	4.9 ± 1.5†

Data are means ± SD. MCA, muscle cross-sectional area; PCr_{rest}, PCr level at rest; PCr_{peak}, lowest PCr level during exercise. *$P < 0.05$, †$P < 0.01$ vs. control.

IMCL content was higher in patients with MetS than control subjects (Table 2). IMCL content also inversely correlated with peak VO_2 ($r = -0.64$; $P < 0.001$) or AT ($r = -0.61$; $P < 0.001$) normalized to body weight. Moreover, IMCL content positively correlated with muscle PCr loss ($r = 0.55$; $P < 0.01$), indicating that the increased IMCL content might be attributable to impaired fatty acid oxidation in the skeletal muscle in MetS.

Systemic oxidative stress

Serum TBARS were significantly increased in patients with MetS (Fig. 1*A*). Moreover, serum total thiols and SOD activity were significantly lower in patients with MetS than control subjects (Fig. 1*B* and *D*). In contrast, there was no significant difference in GPx activity between groups (Fig. 1*C*).

Relationships between systemic oxidative stress and insulin resistance, aerobic capacity, or skeletal muscle energy metabolism

Fasting blood glucose, plasma insulin, free fatty acids, and HOMA-IR positively correlated with serum TBARS (Supplementary Fig. 2). Peak VO_2 normalized to body weight inversely correlated with serum TBARS; however, it did not correlate with systemic antioxidant defense capacity including total thiols and SOD activity (Fig. 2*A*). In contrast, AT normalized to body weight had close relationships with both serum TBARS and systemic antioxidant defense capacity (Fig. 2*B*). There were also relationships between skeletal muscle energy metabolism and some indices of systemic oxidative stress (Fig. 2*C* and *D*).

CONCLUSIONS—Patients with MetS had significantly higher systemic lipid peroxidation products and lower systemic antioxidant defense capacity, including serum total thiols and SOD activity compared with age-, sex-, and activity-matched healthy subjects, indicating enhanced systemic oxidative stress in MetS. Moreover, importantly, the increased systemic lipid peroxidation products and the decreased systemic antioxidant defense capacity correlated with lower aerobic capacity and impaired skeletal muscle energy metabolism. Therefore, we established a new association between systemic oxidative stress and aerobic capacity in patients with MetS.

Lipid peroxidation products including TBARS are commonly used as biomarkers

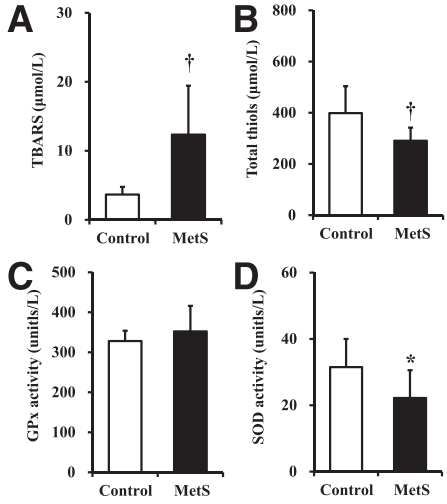

Figure 1—*Systemic lipid peroxidation and antioxidant defense capacity in control subjects (Control) and patients with MetS. TBARS* (A)*, total thiols* (B)*, GPx activity* (C)*, and SOD activity* (D)*. Data are expressed as means ± SD. *P < 0.05, †P < 0.01 vs. control.*

of oxidative stress, as they can contribute to or amplify cellular damage resulting from generation of oxidized products (23). Moreover, thiol compounds, such as glutathione, cysteine, and cysteinyl-glycine, are natural reservoirs of reductive power and act as intracellular and extracellular redox buffers (24). SOD is also an antioxidant which enzymatically converts superoxide (O_2^-) into hydrogen peroxide. Therefore, decreased serum total thiols and SOD activity may contribute to the enhanced systemic oxidative stress in patients with MetS.

There was a close relationship between systemic oxidative stress and impaired skeletal muscle energy metabolism (Fig. 2*C* and *D*), which might indicate that increased ROS production in the skeletal muscle is involved in impairment of skeletal muscle energy metabolism in MetS. Hyperglycemia, hyperinsulinemia, and an increase in free fatty acids can cause an increase in ROS production (25,26). In general, there are several ROS production sources within the cell including NAD(P)H oxidase, xanthine oxidase, uncoupled nitric oxide synthase, and mitochondria. We previously demonstrated that NAD(P)H oxidase–derived O_2^- production was significantly increased in the skeletal muscle from high-fat diet–induced diabetic mice in association with lower aerobic capacity and mitochondrial dysfunction in the skeletal muscle, and inhibition of NAD(P)H oxidase ameliorated these impairments (27). Therefore, ROS derived from NAD(P)H oxidase may play a crucial role in increased oxidative damage in the skeletal muscle in patients with MetS. Moreover, mitochondria can be a primary target for oxidative damage, because O_2^- can easily impair the electron transport chain with an iron-sulfur center. Indeed, an abundance of evidence suggests that mitochondrial function in the skeletal muscle is impaired in insulin-resistant subjects (5,7). The impairment of mitochondrial function in the skeletal muscle results in further production of ROS (4,28), which might, at least in part, contribute to the enhanced systemic oxidative stress in patients with MetS.

Recent large clinical trials have demonstrated that systemic oxidative stress is related to insulin resistance and obesity (15,16), which is consistent with our results that there were tight correlations between serum TBARS and insulin resistance markers such as FBS, insulin level, or free fatty acid level (Supplementary Fig. 2). Excess circulating free fatty acids or triglycerides lead to their overflow into the mitochondria in the skeletal muscle as well as enhanced systemic oxidative stress. Because fatty acids are particularly prone to oxidative damage, lipid peroxidation products are easily formed in the skeletal muscle under the condition of increased ROS production, which can cause mitochondrial damage and subsequently decrease capacity for fatty acid oxidation (29). The increased uptake of fatty acids and decreased fatty acid oxidation in the skeletal muscle may result in accumulation of muscle lipids, which can further deteriorate skeletal muscle energy metabolism, known as lipotoxicity (29,30). Indeed, there was a close relationship between accumulated IMCL and impaired high-energy phosphate metabolism in the skeletal muscle in the current study. Accumulation of IMCL and its intermediates such as diacylglycerol may impair insulin signaling in the skeletal muscle (29,31). Furthermore, oxidative stress can directly impair insulin signaling (28,32). Therefore, an elaborate interdependency on mitochondrial dysfunction

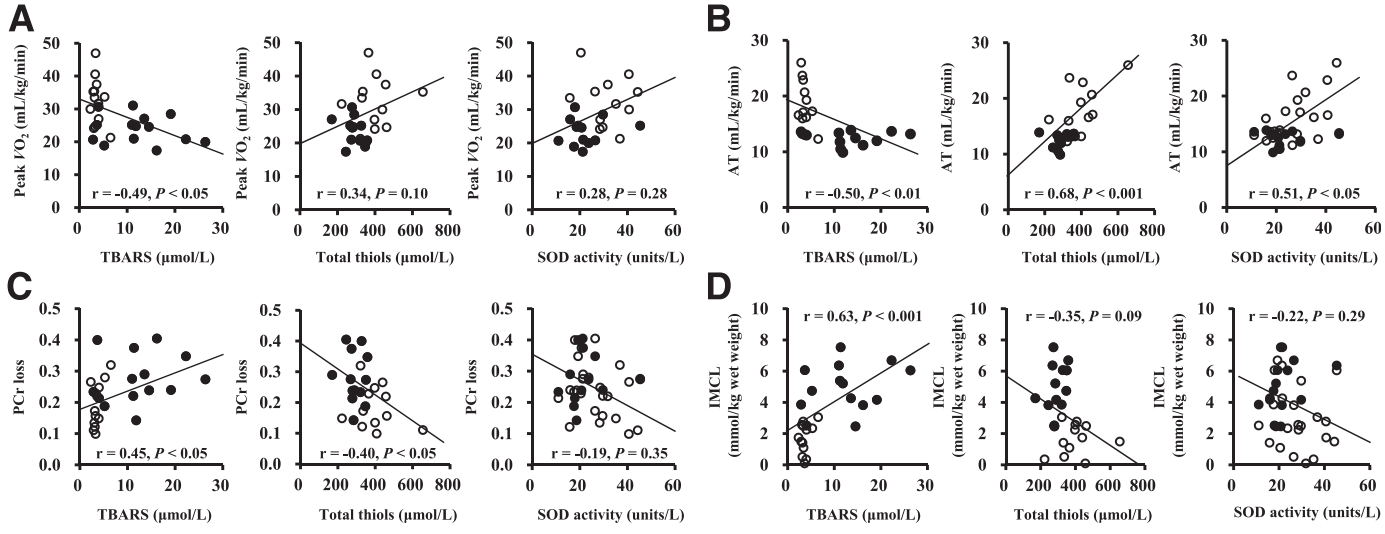

Figure 2—*Linear relation between systemic oxidative stress and aerobic capacity or skeletal muscle energy metabolism in control subjects (white circles) and MetS patients (black circles). Systemic oxidative stress and peak* VO_2 (A)*, systemic oxidative stress and AT* (B)*, systemic oxidative stress and muscle PCr loss* (C)*, and systemic oxidative stress and IMCL content* (D)*.*

and oxidative stress may cause a catastrophic cycle in the skeletal muscle in patients with MetS, which can lead to further deterioration of aerobic capacity as well as insulin resistance. Unfortunately, we could not directly measure oxidative stress in the skeletal muscle and thus could not address whether it was enhanced. However, lipid peroxidation products in the skeletal muscle have been reported to be significantly elevated in obese subjects (33).

Low aerobic capacity is a major determinant of mortality and morbidity in insulin resistance and type 2 diabetes (2,3). The relationship between systemic oxidative stress and lower aerobic capacity may indicate that systemic oxidative stress, at least in part, contributes to a poor prognosis in patients with MetS with low aerobic capacity, as oxidative stress can increase the risk of developing type 2 diabetes and cardiovascular diseases (34). It has been demonstrated that weight loss induced by diet and exercise therapy improves insulin resistance and reduces biomarkers of systemic oxidative stress in patients with MetS (35). Therefore, diet and exercise therapies are beneficial for patients with MetS from the perspective of treatment of enhanced systemic oxidative stress as well as insulin resistance and lower aerobic capacity.

There are some limitations that should be acknowledged. First, we could not completely eliminate the possibility of type II error in some of the correlation analyses. Although we could not necessarily detect the association between aerobic capacity or skeletal muscle energy metabolism and all indices of oxidative stress, our conclusions would not be overestimated. Second, we could not show the causal relationships between the increase in systemic oxidative stress and the decrease in aerobic capacity or the impairment in skeletal muscle energy metabolism. Even though we showed their correlations, further studies are needed to clarify the causal relationships.

In summary, we demonstrated for the first time that systemic oxidative stress including higher levels of lipid peroxidation and lower antioxidant defense capacity was related to lower aerobic capacity and impaired skeletal muscle energy metabolism in patients with MetS. These findings provide new insights into the pathophysiology of MetS and have an implication that to restore the normal balance of systemic redox state might be beneficial in the treatment of MetS.

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T.Y. wrote the manuscript and researched data. S.K. researched data and reviewed and edited the manuscript. M.Y. performed analysis of oxidative stress and reviewed the manuscript. K.Hi., T.S., S.T., N.M., and K.O. researched data and contributed to the discussion. K.Ha., N.O.-M., and Y.K. contributed to the discussion and reviewed the manuscript. H.T. contributed to the discussion and reviewed and edited the manuscript. T.Y. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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