

Nutrition Research

High-fat diet-induced reduction of PGC-1 α mRNA levels and oxidative capacity in the soleus muscle of rats with metabolic syndrome

Fumiko Nagatomo^a, Hidemi Fujino^b, Hiroyo Kondo^c, Isao Takeda^d,
Kinsuke Tsuda^e, Akihiko Ishihara^{a,*}

^aLaboratory of Cell Biology and Life Science, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

^bDepartment of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Kobe 654-0142, Japan

^cDepartment of Food Sciences and Nutrition, Nagoya Women's University, Nagoya 467-8610, Japan

^dDepartment of Physical Therapy, Takarazuka University of Medical and Health Care, Takarazuka 666-0162, Japan

^eLaboratory of Metabolism, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan

Abstract

Animal models of type 2 diabetes exhibit reduced peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) mRNA levels, which are associated with decreased oxidative capacity, in skeletal muscles. In contrast, animal models with metabolic syndrome show normal PGC-1 α mRNA levels. We hypothesized that a high-fat diet decreases PGC-1 α mRNA levels in skeletal muscles of rats with metabolic syndrome, reducing muscle oxidative capacity and accelerating metabolic syndrome or inducing type 2 diabetes. We examined mRNA levels and fiber profiles in the soleus muscles of rats with metabolic syndrome (SHR/NDmcr-cp [*cp/cp*], CP) fed a high-fat diet. Five-week-old CP rats were assigned to a sedentary group (CP-N) that was fed a standard diet (3.60 kcal/g, 23.6% protein, 5.3% fat, and 54.4% carbohydrates) or a sedentary group (CP-H) that was fed a high-fat diet (5.16 kcal/g, 23.6% protein, 34.9% fat, and 25.9% carbohydrates) and were housed for 10 weeks. Body weight, caloric intake, and systolic blood pressure were higher in the CP-H group than in the CP-N group. Nonfasting glucose, triglyceride, total cholesterol, and leptin levels were higher in the CP-H group than in the CP-N group. There was no difference in insulin levels between the CP-N and CP-H groups. Muscle PGC-1 α mRNA levels and succinate dehydrogenase activity were lower in the CP-H group than in the CP-N group. We concluded that a high-fat diet reduces PGC-1 α mRNA levels and oxidative capacity in skeletal muscles and accelerates metabolic syndrome.

Keywords: Muscle fiber type; Muscle oxidative enzyme activity; Obesity; Skeletal muscle

1. Introduction

Metabolic syndrome is characterized by obesity, high blood pressure, increased glucose levels, and dyslipidemia [1]. Skeletal muscle is the major site of insulin action and glucose metabolism. Reduced oxidative capacity in the skeletal muscles of humans and animal models with metabolic syndrome is known to impair glucose metabolism and increase the risk of the development of lifestyle-related disease, for example, type 2 diabetes and its complications [2]. The skeletal muscles of patients with type 2 diabetes exhibit a lower percentage of high-oxidative fibers than those of healthy individuals [3–6]. Similarly, the skeletal muscles of rats with type 2 diabetes have a lower oxidative capacity and contain a lower percentage of high-oxidative fibers than those of nondiabetic rats [7–10]. These results indicate that type 2 diabetes is associated with reduced oxidative capacity in skeletal muscles.

Previous studies [11–16] have established that nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) and estrogen-related receptors (ERRs), along with the coregulator PPAR γ coactivator-1 α (PGC-1 α), control diverse aspects of aerobic metabolism, including fatty acid oxidation, oxidative phosphorylation, and mitochondrial biogenesis, in skeletal muscles. The properties of skeletal muscle fibers, including type distribution and oxidative enzyme activity, are regulated by PGC-1 α [11–13]. Our previous studies [14,15] showed that rats with type 2 diabetes have lower PGC-1 α mRNA levels in the slow soleus and fast plantaris muscles than nondiabetic rats. Therefore, we concluded that reduced PGC-1 α mRNA levels in the skeletal muscles of rats with type 2 diabetes are associated with a low percentage of high-oxidative fibers. ERR γ , which is a constitutively active orphan nuclear receptor, is highly expressed in tissues with high metabolic activity, for example, heart, kidney, brown adipose tissue, and skeletal muscle, especially the slow muscle [16]. ERR γ regulates mitochondria, increasing the number of mitochondria and their oxidative capacity in skeletal muscles by mediating the action of PGC-1 α .

We hypothesized that a high-fat diet decreases PGC-1 α mRNA levels in the skeletal muscles of humans and animal models with metabolic syndrome, reducing muscle oxidative capacity. If this is true, the risk of inducing lifestyle-related diseases would be higher when humans and animal models with metabolic syndrome continuously consume a high-fat diet. In the present study, we examined the mRNA levels of genes related to glucose and lipid metabolism and fiber profiles, including type distribution, cross-sectional area, and oxidative enzyme activity, in the soleus muscles of rats with metabolic syndrome that were fed a

high-fat diet. We used SHR/NDmcr-cp [*cp/cp*] (CP) rats as an animal model of metabolic syndrome. CP rats have a nonsense mutation in the leptin receptor gene [17,18] and develop obesity, high blood pressure, increased glucose levels, hyperinsulinemia, and dyslipidemia as adults.

2. Methods and materials

2.1. Animals and experimental procedures

All experimental procedures and animal care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health. This study was approved by the Institutional Animal Care and Experiment Committee of Kyoto University (Kyoto, Japan).

Five-week-old male CP rats were divided into 2 groups: sedentary, fed a standard diet (CP-N; n = 6) and sedentary, fed a high-fat diet (CP-H; n = 6). Age-matched male Wistar-Kyoto rats fed a standard diet were used as nonobese controls (WKY; n = 6). All rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). The nonpurified standard diet comprised 23.6% protein, 5.3% fat, and 54.4% carbohydrates and provided 3.6 kcal/g (MF; Oriental Yeast Co. Ltd., Tokyo, Japan), while the high-fat diet comprised 23.6% protein, 34.9% fat, and 25.9% carbohydrates and provided 5.16 kcal/g (Test Diet 58Y1, rodent purified diet with 60% energy from fat; PMI Nutrition International, Richmond, IN). Food and water were provided *ad libitum*, and the food and caloric intakes of each rat were measured daily (every 24 h). All rats were housed in individual, uniformly sized standard cages. The room was maintained under a controlled 12-h light/dark cycle (lights were on from 08:00 to 20:00 h) at $22 \pm 2^\circ\text{C}$ with 45–55% relative humidity. Body weight and systolic and diastolic blood pressures of rats in all groups were measured at 5, 7, 9, 11, 13, and 15 weeks of age. Systolic and diastolic blood pressures were determined automatically in conscious rats using the indirect tail-cuff method with a BP-98A sphygmomanometer (Softron Inc., Tokyo, Japan).

2.2. Blood glucose analysis

Fasting glucose levels were measured at 5, 7, 9, 11, 13, and 15 weeks of age after a 15-h fast. Blood samples were obtained from the tail veins of fully conscious rats and were analyzed using a portable glucose meter (GT-1650; Arkray Inc., Kyoto, Japan). Nonfasting

glucose levels were also measured at 15 weeks of age using the same glucose meter.

2.3. Serum biochemical measurements

All rats at 15 weeks of age were fasted for 15 h before blood sampling. Blood samples were obtained from the abdominal aorta under pentobarbital sodium anesthesia (5 mg/100 g body weight, *i.p.*). Serum triglyceride and total cholesterol levels were measured by routine laboratory methods. Serum insulin, leptin, and high molecular-weight adiponectin levels were measured using enzyme-linked immunosorbent assay (ELISA) kits for rats (Shibayagi Co. Ltd., Shibukawa, Japan).

2.4. Biochemical analysis of the muscle

The soleus muscles of both legs were dissected, and the muscle wet weight was measured. After muscle dissection, the rats were killed by overdose of pentobarbital sodium. The right soleus muscle was divided into distal and proximal parts for biochemical and histochemical analyses, respectively. The distal part of the right muscle was immediately frozen and homogenized in 5 volumes of ice-cold 0.3 M phosphate buffer (pH 7.4) using a glass tissue homogenizer. Sodium succinate was added to yield a final concentration of 17 mM. The final concentrations of the components in the reaction mixture were as follows: sodium succinate, 17 mM; sodium cyanide (NaCN), 1 mM; aluminum chloride (AlCl₃), 0.4 mM; and calcium chloride (CaCl₂), 0.4 mM. The reduction of cytochrome *c* in the reaction mixture was analyzed spectrophotometrically by observing an increase in extinction at 550 nm. Succinate dehydrogenase (SDH) activity, an indicator of mitochondrial oxidative capacity, was calculated from ferricytochrome *c* concentrations and protein content [19].

2.5. Analysis of mRNA expression in the muscle

Total RNA was extracted from the left soleus muscle using TRIzol Reagent (Invitrogen, Carlsbad, CA) and then treating samples with deoxyribonuclease I (Invitrogen). The first strand of cDNA was synthesized from 1.0 µg of total RNA using the PrimeScript RT reagent kit (Takara Bio Inc., Otsu, Japan). We analyzed gene expression by real-time polymerase chain reaction (RT-PCR) using a LightCycler DX400 system (Roche Diagnostics, Mannheim, Germany) with SYBR Premix Ex Taq II (Takara Bio Inc.). The mRNA levels of PPAR δ/β , PGC-1 α , stearoyl-CoA desaturase-1 (SCD-1), and glucose transporter 4 (GLUT4) were

normalized to hypoxanthine phosphoribosyltransferase (HPRT) mRNA expression. The primer sets for PPAR δ/β , PGC-1 α , and SCD-1 were described previously [14,15]. GLUT4 primers were as follows: forward, 5'-CAACTGGACCTGTAACCTTCATCGT-3' and reverse: 5'-ACGGCAAATAGAAGGAAGACGTA-3'.

2.6. Histochemical analysis of muscle tissues

The proximal part of the right soleus muscle was pinned to a corkboard and rapidly frozen in isopentane cooled with a mixture of dry ice and acetone. The muscle was mounted on a specimen chuck with Tissue-Tek OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan). Serial transverse 10- μ m thick sections were cut using a cryostat at -20°C . Different fiber types were classified on the basis of ATPase activity [20]. The sections were allowed to reach room temperature, air dried for 30 min, and pre-incubated in alkaline (pH 10.4) or acidic (pH 4.5) solution for subsequent assessment of ATPase activity. The muscle fibers in each section were classified as type I (ATPase-positive with pre-incubation at pH 4.5 and ATPase-negative with pre-incubation at pH 10.4), type IIA (ATPase-negative with pre-incubation at pH 4.5 and ATPase-positive with pre-incubation at pH 10.4), and type IIC (ATPase-positive with pre-incubation at pH 4.5 and 10.4). A single common area was selected from each section and digitized as gray level images by using a computer-assisted image processing system (Neuroimaging System Inc., Kyoto, Japan). The fiber cross-sectional area was measured by tracing the outline of each fiber in the section. Fiber type distribution and cross-sectional area were determined for approximately 500 fibers located in the central region of the muscle section.

The sections were also stained for 10 min to quantify SDH intensity [20]. SDH intensity was quantified for approximately 500 fibers described above using a computer-assisted image processing system (Neuroimaging System Inc.). Sectional images were digitized as gray scale images. Each pixel was quantified as one of 256 gray levels: gray level 0 was equivalent to 100% light transmission and gray level 255 was equivalent to 0% light transmission. The mean optical density (OD) of all pixels (which were converted to gray level values) within a fiber was determined using a calibration photographic tablet with 21 steps of gradient-density ranges and corresponding diffused density values.

2.7. Statistical analyses

All measured values are presented as mean and standard deviation. One-way analysis of variance (ANOVA) was used to evaluate mean differences among the age-matched groups. When the differences were found to be significant by ANOVA, further comparisons were made using Scheffé's *post hoc* test. A probability level of 0.05 was accepted as significant.

3. Results

3.1. Body weight

Body weight at 11–15 weeks was higher in the CP-N group than in the age-matched WKY group (Fig. 1A). Body weight at 7 weeks was higher in the CP-H group than in the age-matched WKY group. Furthermore, body weight at 9–15 weeks was higher in the CP-H group than in the age-matched WKY and CP-N groups.

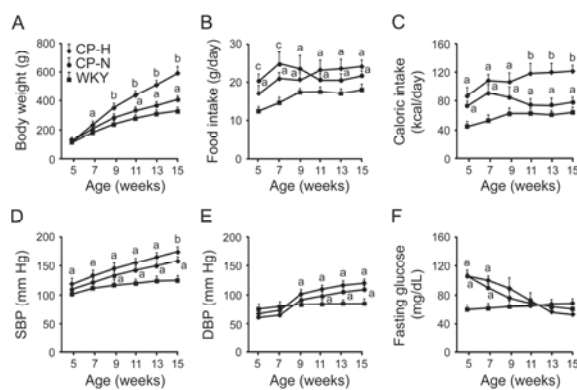


Fig. 1. Body weights (A), food (B) and caloric (C) intakes, systolic (D) and diastolic (E) blood pressures, and fasting glucose levels (F) in the WKY, CP-N, and CP-H groups. Data are presented as the mean \pm standard deviation ($n = 6$). SBP, systolic blood pressure; DBP, diastolic blood pressure. ^a $P < 0.05$, compared to the age-matched WKY group; ^b $P < 0.05$, compared to the age-matched WKY and CP-N groups; ^c $P < 0.05$, compared to the age-matched WKY and CP-H groups.

3.2. Food and caloric intake

Food intake at 5–7 weeks was higher in the CP-N group than in the age-matched WKY and CP-H groups (Fig. 1B). Food intake at 9–15 weeks was higher in the CP-N group than in the age-matched WKY group. Food intake at 5–15 weeks was higher in the CP-H group than in the age-matched WKY group.

Caloric intake at 5–15 weeks was higher in the CP-N group than in the age-matched WKY group (Fig. 1C). Caloric intake at 5–9 weeks was higher in the CP-H group than in the age-matched WKY group. Furthermore, caloric intake at 11–15 weeks was higher in the CP-H group than in the age-matched WKY and CP-N groups.

3.3. Blood pressure

Systolic blood pressure at 9–15 weeks was higher in the CP-N group than in the age-matched WKY group (Fig. 1D). Systolic blood pressure at 5–13 weeks was higher in the CP-H group than in the age-matched WKY group. Furthermore, systolic blood pressure at 15 weeks was higher in the CP-H group than in the age-matched WKY and CP-N groups.

Diastolic blood pressure at 11–15 weeks was higher in the CP-N group than in the age-matched WKY group (Fig. 1E). Diastolic blood pressure at 9–15 weeks was higher in the CP-H group than in the age-matched WKY group.

3.4. Blood glucose levels

Fasting glucose levels at 5–7 weeks were higher in the CP-N and CP-H groups than in the age-matched WKY group (Fig. 1F). Nonfasting glucose levels at 15 weeks were higher in the CP-N group than in the age-matched WKY group (Fig. 2A). Nonfasting glucose levels at 15 weeks were higher in the CP-H group than in the age-matched WKY and CP-N groups.

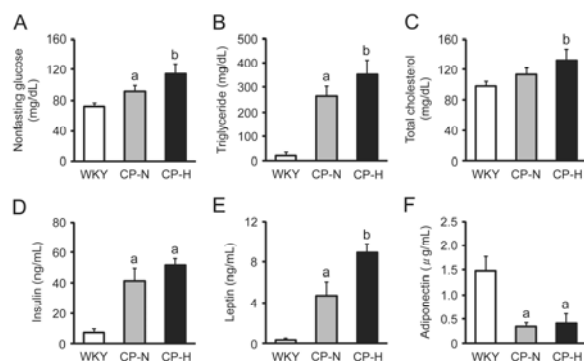


Fig. 2. Nonfasting glucose (A), triglyceride (B), total cholesterol (C), insulin (D), leptin (E), and adiponectin (F) levels in the WKY, CP-N, and CP-H groups. Data are presented as the mean \pm standard deviation ($n = 6$). ^a $P < 0.05$, compared to the WKY group; ^b $P < 0.05$, compared to the WKY and CP-N groups.

3.5. Serum biochemical parameters

Triglyceride (Fig. 2B) and leptin (Fig. 2E) levels were higher in the CP-N group than in the WKY group. Triglyceride and leptin levels were higher in the CP-H group than in the WKY and CP-N groups. Total cholesterol levels were higher in the CP-H group than in the WKY and CP-N groups (Fig. 2C). Insulin levels were higher (Fig. 2D) and adiponectin levels were lower (Fig. 2F) in the CP-N and CP-H groups than in the WKY group.

3.6. Soleus muscle weight and SDH activity

Muscle weight was lower in the CP-N and CP-H groups than in the WKY group (Fig. 3A).

Muscle SDH activity was lower in the CP-N group than in the WKY group (Fig. 3B). Muscle SDH activity was lower in the CP-H group than in the WKY and CP-N groups.

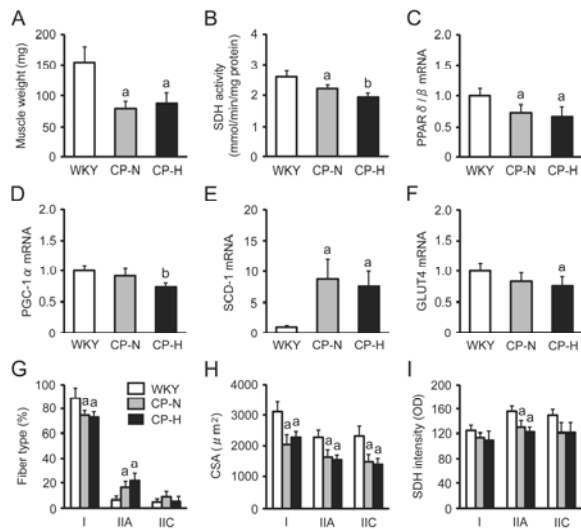


Fig. 3. Soleus muscle weights (A), SDH activities (B), PPAR δ/β (C), PGC-1 α (D), SCD-1 (E), and GLUT4 (F) mRNA levels, fiber type percentages (G), fiber cross-sectional areas (H), and fiber SDH intensities (I) in the WKY, CP-N, and CP-H groups. Data are presented as the mean \pm standard deviation (n = 6). SDH, succinate dehydrogenase; PPAR δ/β , peroxisome proliferator-activated receptor δ/β ; PGC-1 α , PPAR γ coactivator-1 α ; SCD-1, stearoyl-CoA desaturase-1; GLUT4, glucose transporter 4; CSA, cross-sectional area. ^a $P < 0.05$, compared to the WKY group; ^b $P < 0.05$, compared to the WKY and CP-N groups.

3.7. Soleus muscle mRNA levels

PPAR δ/β mRNA levels were lower (Fig. 3C) and the SCD-1 mRNA levels were higher (Fig. 3E) in the CP-N and CP-H groups than in the WKY group. PGC-1 α mRNA levels were lower in the CP-H group than in the WKY and CP-N groups (Fig. 3D). GLUT4 mRNA levels were lower in the CP-H group than in the WKY group (Fig. 3F).

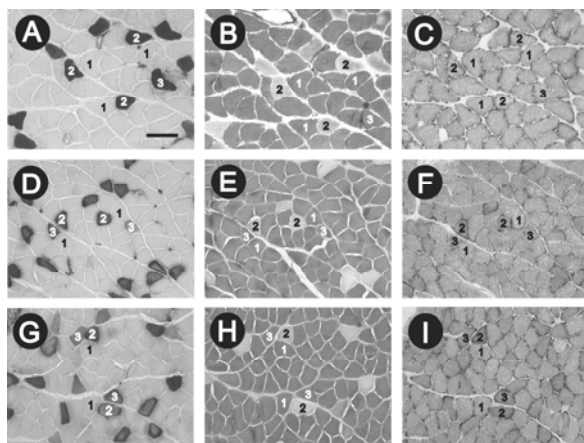


Fig. 4. Serial transverse sections of the soleus muscles in WKY (A–C), CP-N (D–F), and CP-H (G–I) rats stained for ATPase activity after pre-incubation at pH 10.4 (A, D, and G) or pH 4.5 (B, E, and H) and for SDH activity (C, F, and I). 1, type I; 2, type IIA; 3, type IIC. Scale bar on A = 100 μ m.

3.8. Soleus muscle fiber profiles

Muscles in the WKY, CP-N, and CP-H groups contained 3 types of fibers: type I, IIA, and IIC (Fig. 4). Percentage of type I fibers was lower while percentage of type IIA fibers

was higher in the CP-N and CP-H groups than in the WKY group (Fig. 3G). Cross-sectional areas of type I, IIA, and IIC fibers were lower in the CP-N and CP-H groups than in the WKY group (Fig. 3H). SDH intensity of type IIA fibers was lower in the CP-N and CP-H groups than in the WKY group (Fig. 3I).

4. Discussion

4.1. Animal model of metabolic syndrome

Metabolic syndrome is characterized by obesity, high blood pressure, increased glucose levels, and dyslipidemia [1]. In the present study, CP rats with metabolic syndrome (CP-N and CP-H) showed greater body weight (Fig. 1A), higher systolic (Fig. 1D) and diastolic (Fig. 1E) blood pressures, and increased nonfasting glucose (Fig. 2A) and triglyceride (Fig. 2B) levels than normal rats (WKY). Furthermore, CP rats exhibited a lower soleus muscle weight than WKY rats (Fig. 3A). It is likely that the decreased soleus muscle weight of CP rats was due to atrophy of all types of muscle fibers (Fig. 3H). Although we did not measure the spontaneous motor activities of individual rats in the present study, this type of atrophy is known to be associated with the lower motor activity levels induced by obesity. The soleus muscle is an antigravity muscle that is persistently active; therefore, the fiber sizes are more susceptible to decreased muscle activity and loading levels. Our previous study [14] observed that CP rats were inactive in their cages all day long, except when they ate, probably due to their excess body weight.

PPAR δ/β is the main transcription factor that regulates fatty acid oxidation in skeletal muscle and adipose tissue and activates lipid metabolism to prevent obesity [21]. The reduced PPAR δ/β mRNA levels in the skeletal muscles of CP (CP-N and CP-H) rats (Fig. 3C) may have induced a decreased capacity for fatty acid oxidation. A previous study [22] showed that hypertriglyceridemia and hyperinsulinemia were associated with increased SCD-1 activity. Therefore, the hypertriglyceridemia (Fig. 2B) and hyperinsulinemia (Fig. 2D) observed in CP rats may be associated with the increase in SCD-1 mRNA levels (Fig. 3E), which were 8.7- and 7.5-fold higher in CP-N and CP-H rats than in WKY rats, respectively.

4.2. Effects of a high-fat diet on metabolic syndrome

In rats, a high-fat diet stimulates tyrosine-kinase activity and reduces autophosphorylation of the β -subunit of the insulin receptor in the liver [23–25].

Furthermore, *in vitro* exposure of isolated hepatocytes to free fatty acids causes a dose-dependent decrease in cell-surface insulin receptor binding along with diminished receptor-mediated internalization and degradation [24,26]. A reduction in insulin action in the liver leads to decreased inhibition of hepatic glucose output. On the other hand, the oxidative stress pathway responsible for the production of reactive oxygen species is upregulated in both liver and adipose tissue of mice fed a high-fat diet before the onset of insulin resistance and obesity [27]. These results indicate that liver and adipose tissue are important in the maintenance of glucose and lipid metabolism and that a condition of excessive nutrient intake induces and accelerates insulin resistance and enhances oxidative stress levels. Insulin resistance has been recognized as the integral feature of metabolic syndrome, which includes glucose intolerance, hypertriglyceridemia, low HDL-cholesterol, and hypertension. In the present study, we investigated PGC-1 α mRNA levels and oxidative capacity of the soleus muscles in rats with metabolic syndrome because a high-fat diet may have significant effects on the skeletal muscles of rats with metabolic syndrome as well as on liver and adipose tissue.

In CP (CP-N and CP-H) rats, CP-H rats exhibited a greater body weight (Fig. 1A), higher systolic blood pressure (Fig. 1D), and increased nonfasting glucose (Fig. 2A) and triglyceride (Fig. 2B) levels than CP-N rats, while there was no difference in insulin levels between CP-N and CP-H rats (Fig. 2D). The insulin signaling pathway may be downregulated in CP-H rats, and decreased phosphorylation of the insulin receptor and insulin receptor substrate (IRS)-1 may be present, although this was not investigated in the present study. These results indicate that a high-fat diet accelerates metabolic syndrome. Insulin and leptin stimulate the sympathetic nervous system, and this contributes to increased blood pressure [28]. CP-H rats had higher systolic blood pressure (Fig. 1D) and leptin levels (Fig. 2E) than CP-N rats, suggesting that the increased leptin levels in CP-H rats may contribute to higher blood pressure.

In the present study, CP-H rats showed higher insulin levels than WKY rats (Fig. 2D), suggesting that these rats were still able to produce insulin at this stage. However, continuation of increased caloric intake under the influence of metabolic syndrome may have resulted in decreased insulin levels because the pancreas may have exhausted its ability to produce the large amounts of insulin required for continued hyperinsulinemia. This decrease in insulin levels would eventually lead to the development of type 2 diabetes.

ERR γ is exclusively expressed in the slow muscle [16], indicating that there is an ERR γ pathway that promotes and coordinates vascular supply and metabolic demand in the slow muscle. In a previous study [29], mice that transgenically overexpressed ERR γ in the fast muscle exhibited high running endurance, resistance to diet-induced weight gain, increased oxygen consumption, enhanced oxidative capacity, dense vasculature, and increased expression of genes promoting fat metabolism, mitochondrial respiration, and high-oxidative fiber specification. These results indicate that transgenic expression of ERR γ in the fast muscle triggers an anaerobic to aerobic transformation and vascular remodeling to enhance aerobic capacity. In addition, the intrinsic effects of ERR γ do not depend on PGC-1 α induction, but rather are associated with ERR γ -directed adenosine monophosphate-activated protein kinase (AMPK) activation in skeletal muscles [29].

Multiple diseases, including metabolic syndrome and type 2 diabetes, are commonly linked to deregulation of both oxidative metabolism and vascularity. In skeletal muscles, oxidative capacity is an important factor influencing the progression or prevention of lifestyle-related diseases, for example, type 2 diabetes [14,15]. In the present study, we focused on PGC-1 α mRNA levels and oxidative capacity in the skeletal muscles of rats with metabolic syndrome fed a high-fat diet. A previous study [11] observed that PGC-1 α mRNA levels in skeletal muscles were associated with oxidative capacity; enhanced PGC-1 α mRNA levels induced an increased percentage of high-oxidative fibers in skeletal muscles, while reduced PGC-1 α mRNA levels induced a decreased percentage of high-oxidative fibers. In our previous studies [14,15], we observed that the slow soleus and fast plantaris muscles of rats with type 2 diabetes exhibited lower PGC-1 α mRNA levels than normal rats and rats with metabolic syndrome, whereas there were no differences in PGC-1 α mRNA levels in the skeletal muscles of normal rats and rats with metabolic syndrome. These results indicated that rats with metabolic syndrome maintain normal PGC-1 α mRNA levels, while those with type 2 diabetes show reduced PGC-1 α mRNA levels, inducing decreased muscle oxidative capacity. In the present study, we hypothesized that a high-fat diet induces the decrease in PGC-1 α mRNA levels in the skeletal muscles of animal models with metabolic syndrome, leading to reduced muscle oxidative capacity. Our present data support this hypothesis; decreased PGC-1 α mRNA levels (Fig. 3D) and reduced oxidative enzyme activity (Fig. 3B) were observed in the soleus muscles of CP-H rats compared with those of CP-N rats.

4.3. Limitations of the present study

In the present study, we used CP rats having a nonsense mutation in the leptin receptor [17,18]. The results obtained by this animal model cannot be directly applied to humans, although profiles of metabolic syndrome, for example, obesity, high blood pressure, increased glucose levels, and dyslipidemia, are similar in CP rats and humans.

Although previous investigations using several animal models have studied the traditional high-fat diet in animal models of diet-induced obesity, comparison of this high-fat diet to diets consisting of food regularly consumed by humans, including high-salt, high-fat, low-fiber, and energy dense foods, are lacking. In the “cafeteria diet” model [30,31], animal are allowed free access to standard chow and water while highly palatable, energy-dense, and unhealthy human foods are offered concurrently. This diet promotes rapid weight gain, increased fat mass, and enhanced prediabetic parameters, including glucose and insulin intolerance [32–34]. Since diet-induced obesity in animal models is a commonly used tool to support clinical research in humans, further experiments using an alternative dietary model of energy-dense and highly palatable cafeteria-style foods in animal models would be useful.

In conclusion, while the development and progression of metabolic syndrome depends on genetic background, it is also highly influenced by daily lifestyle. Increased energy intake is the major cause of decreased PGC-1 α mRNA levels and oxidative capacity in skeletal muscles, and this accelerates metabolic syndrome and eventually induces type 2 diabetes.

Acknowledgment

The authors have no conflicts of interest to disclose. The present study was supported by a grant from the Japan Aerospace Exploration Agency.

References

- [1] Reaven GM. The metabolic syndrome: Time to get off the merry-go-round? *J Intern Med* 2011;269:127-36.
- [2] Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. *J Biomed Biotechnol* 2010;doi:10.1155/2010/476279.
- [3] Mårin P, Andersson B, Krotkiewski M, Björntorp P. Muscle fiber composition and capillary density in women and men with NIDDM. *Diabetes Care* 1994;17:382-6.
- [4] Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, Israel RG, Dohm GL.

- Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. *Am J Physiol Endocrinol Metab* 1995;268:E453-7.
- [5] Nyholm B, Qu Z, Kaal A, Pedersen SB, Gravholt CH, Andersen JL, Saltin B, Schmitz O. Evidence of an increased number of type IIb muscle fibers in insulin-resistant first-degree relatives of patients with NIDDM. *Diabetes* 1997;46:1822-8.
- [6] Gaster M, Staehr P, Beck-Nielsen H, Schröder HD, Handberg A. GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients: is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? *Diabetes* 2001;50:1324-9.
- [7] Yasuda K, Ishihara A, Adachi T, Shihara N, Seino Y, Tsuda K. Growth-related changes in skeletal muscle fiber type and insulin resistance in Diabetic Otsuka Long-Evans Tokushima fatty rats. *Acta Histochem Cytochem* 2001;34:371-82.
- [8] Yasuda K, Nishikawa W, Iwanaka N, Nakamura E, Seino Y, Tsuda K, Ishihara A. Abnormality in fibre type distribution of soleus and plantaris muscles in non-obese diabetic Goto-Kakizaki rats. *Clin Exp Pharmacol Physiol* 2002;29:1001-8.
- [9] Yasuda K, Adachi T, Gu N, Matsumoto A, Matsunaga T, Tsujimoto G, Tsuda K, Ishihara A. Effects of hyperbaric exposure with high oxygen concentration on glucose and insulin levels and skeletal muscle-fiber properties in diabetic rats. *Muscle Nerve* 2007;35:337-43.
- [10] Gu N, Nagatomo F, Fujino H, Takeda I, Tsuda K, Ishihara A. Hyperbaric oxygen exposure improves blood glucose level and muscle oxidative capacity in rats with type 2 diabetes. *Diabetes Technol Ther* 2010;12:125-33.
- [11] Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. Transcriptional co-activator PGC-1 α drives the formation of slow-twitch muscle fibres. *Nature* 2002;418:797-801.
- [12] Wende AR, Huss JM, Schaeffer PJ, Giguère V, Kelly DP. PGC-1 α coactivates PDK4 gene expression via the orphan nuclear receptor ERR α : a mechanism for transcriptional control of muscle glucose metabolism. *Mol Cell Biol* 2005;25:10684-94.
- [13] Schuler M, Ali F, Chambon C, Duteli D, Borner JM, Tardivel A, Desvergne B, Wahli W, Chambon P, Metzger D. PGC-1 α expression is controlled in skeletal muscles by PPAR β , whose ablation results in fiber-type switching, obesity, and type 2 diabetes. *Cell Metab* 2006;4:407-14.
- [14] Nagatomo F, Gu N, Fujino H, Takeda I, Tsuda K, Ishihara A. Skeletal muscle

- characteristics of rats with obesity, diabetes, hypertension, and hyperlipidemia. *J Atheroscler Thromb* 2009;16:576-85.
- [15] Nagatomo F, Fujino H, Kondo H, Gu N, Takeda I, Ishioka N, Tsuda K, Ishihara A. PGC-1 α mRNA level and oxidative capacity of the plantaris muscle in rats with metabolic syndrome, hypertension, and type 2 diabetes. *Acta Histochem Cytochem* 2011;44:73-80.
- [16] Alaynick WA. Nuclear receptors, mitochondria and lipid metabolism. *Mitochondrion* 2008;8:329-37.
- [17] Takaya K, Ogawa Y, Hiraoka J, Hosoda J, Yamori Y, Nakao K, Koletsky RJ. Nonsense mutation of leptin receptor in the obese spontaneously hypertensive Koletsky rat. *Nat Genet* 1996;14:130-1.
- [18] Friedman JE, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Kaung HL, Ernsberger P. Reduced insulin receptor signaling in the obese spontaneously hypertensive Koletsky rat. *Am J Physiol Endocrinol Metab* 1997;273:E1014-23.
- [19] Nishizaka T, Nagatomo F, Fujino H, Nomura T, Sano T, Higuchi K, Takeda I, Ishihara A. Hyperbaric oxygen exposure reduces age-related decrease in oxidative capacity of the tibialis anterior muscle in mice. *Enzyme Res* 2010;doi:10.4061/2010/824763.
- [20] Nagatomo F, Fujino H, Kondo H, Suzuki H, Kouzaki M, Takeda I, Ishihara A. PGC-1 α and FOXO1 mRNA levels and fiber characteristics of the soleus and plantaris muscles in rats after hindlimb unloading. *Histol Histopathol* 2011;26:1545-53.
- [21] Wang YX, Lee CH, Tjep S, Yu RT, Ham J, Kang H, Evans RM. Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity. *Cell* 2003;113:159-70.
- [22] Gbaguidi FG, Chinetti G, Milosavljevic D, Teissier E, Chapman J, Olivecrona G, Fruchart JC, Griglio S, Fruchart-Najib J, Staels B. Peroxisome proliferator-activated receptor (PPAR) agonists decrease lipoprotein lipase secretion and glycated LDL uptake by human macrophages. *FEBS Lett* 2002;512:85-90.
- [23] Nagy K, Levy J, Grunberger G. High-fat feeding induces tissue-specific alteration in proportion of activated insulin receptors in rats. *Acta Endocrinol* 1990;122:361-8.
- [24] Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiol Rev* 1994;74:761-811.
- [25] Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral

- adiposity. *Diabetes* 2000;49:883-8.
- [26] Hennes MM, Shrago E, Kissebah AH. Receptor and postreceptor effects of free fatty acids (FFA) on hepatocyte insulin dynamics. *Int J Obes* 1990;14:831-41.
- [27] Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, Ota T, Yokoyama M, Honda M, Miyamoto K, Kaneko S. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. *Metabolism* 2008;57:1071-7.
- [28] Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *J Hypertens* 2001;19:523-8.
- [29] Narkar VA, Fan W, Downes M, Yu RT, Jonker JW, Alaynick WA, Banayo E, Karunasiri MS, Lorca S, Evans RM. Exercise and PGC-1 α -independent synchronization of type I muscle metabolism and vasculature by ERR γ . *Cell Metab* 2011;13:283-93.
- [30] Sclafani A, Springer D. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol Behav* 1976;17:461-71.
- [31] Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, Newgard CB, Makowski L. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity* 2011;19:1109-7.
- [32] Morris MJ, Chen H, Watts R, Shulkes A, Cameron-Smith D. Brain neuropeptide Y and CCK and peripheral adipokine receptors: temporal response in obesity induced by palatable diet. *Int J Obes* 2008;32:249-58.
- [33] Heyne A, Kiesselbach C, Sahún I, McDonald J, Gaiffi M, Dierssen M, Wolffgramm J. An animal model of compulsive food-taking behaviour. *Addict Biol* 2009;14:373-83.
- [34] Caimari A, Oliver P, Keijer J, Palou A. Peripheral blood mononuclear cells as a model to study the response of energy homeostasis-related genes to acute changes in feeding conditions. *OMICS* 2010;14:129-41.