Protective effects of low-intensity exercise on metabolic oxidative capacity and capillarization in skeletal muscle of non-obese diabetic rats

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ABSTRACT
Diabetes mellitus induces skeletal muscle dysfunction, such as decreased metabolic activity and capillarization. This study aimed to investigate the effects of aerobic low intensity exercise training on metabolic oxidative capacity and capillarization in skeletal muscle of non-obese diabetic rats. Eleven to twenty-five week-old male non-obese Spontaneous Diabetic Torii (SDT) rats (n = 11) and age-matched healthy male Sprague-Dawley SD rats (n = 11) were randomly assigned to either exercise or sedentary groups. The exercise training was performed on a low-speed motorized treadmill (15 m min\(^{-1}\)) for 60 min per session, 5 sessions per week for 14 weeks in exercised groups. Sedentary SDT rats resulted in hyperglycemia, reduction of metabolic oxidative enzyme, and low percentage of oxidative fibers in the skeletal muscles. The low-intensity exercise training inhibited the growth-related increase in glucose level, and increased the muscle oxidative enzyme in exercised SDT rats compared with sedentary SDT rats. In addition, the exercise program prevented capillary regression in the skeletal muscle of diabetic rats. These results suggest that low-intensity exercise training may be an effective treatment to counter the detrimental effects of type 2 diabetes mellitus on the oxidative capacity and the capillary network of skeletal muscles.

INTRODUCTION
It is well established that skeletal muscles are considered as the main site of insulin-stimulated glucose consumption and regulation (Yki-Jarvinen et al. 1987; Barnard and Youngren 1992). Recent studies have argued that patients with type 2 diabetes are associated with inappropriate metabolic potentials, compromised mitochondrial function, and diverse patterns of fiber type distribution in the skeletal muscle (Kelley et al. 2002; Boushel et al. 2007; Phielix et al. 2010; Jheng et al. 2012). The patterns include increased fast-glycolytic muscle fibers (type IIB) proportion (Lillioja et al. 1987; Marin et al. 1994; Hickey et al. 1995), or reduced slow-oxidative muscle fibers (type I) proportion (Nyholt et al. 1997; Mogensen et al. 2007). Moreover, diabetes has shown to induce remodeling of the skeletal muscle capillary networks resulting in smaller capillary diameter, lower capillary-to-fiber ratio, and reduced capillary diffusion (Sexton et al. 1994; Mathieu-Costello et al. 2003; Kivela et al. 2006). Exercise training was shown as an effective method to reduce blood glucose and HbA1c, and an increase of insulin sensitivity in the diabetic individuals (Devlin et al. 1987; Boule et al. 2001; Snowling and Hopkins 2006). In addition, it is well established that exercise training increases the capillary-to-muscle fiber ratio, particularly in the most oxidative regions of the skeletal muscle in healthy animals and individuals (Gute et al. 1996).
Recently, animal models for metabolic syndrome and life style-related diseases such as diabetes have been developed, aiming to gain better understanding of the disease (Angelova and Boyadjiev 2013). However, the characteristics and sensitivity of skeletal muscle to physical training have not been investigated in the recent models such as Spontaneous Diabetic Torii (SDT) rats. The SDT rats have been proven as a resemble model of non-obese diabetes because it shares similar clinical characteristics with type 2 diabetic patients with hypoinsulinemia (Shinohara et al. 2000; Masuyama et al. 2004; Ohta et al. 2007), and have hyperglycemia and glucose intolerance, resulting from decreased insulin secretion accompanying β-cell degeneration (Masuyama et al. 2004; Sasase et al. 2006). Besides the marked hyperglycemia, the SDT rats are characterized with renal (Ohta et al. 2007) and ocular (Shinohara et al. 2003, 2004; Ohta et al. 2007) complications. However, the complications in skeletal muscles of SDT rats have not been investigated, which includes microvasculature. In addition, the effects of exercise training on the metabolic activity and capillarization in the skeletal muscle of SDT rats have not been explored yet.

The aim of this study was to evaluate effectiveness of low-intensity exercise training on non-obese SDT rats, regarding the changes in fiber type distribution, metabolic activity, and capillarization of the skeletal muscle. In the present study, the gastrocnemius muscle has been chosen carefully because it contains both high oxidative and low oxidative muscle fibers in both deep and superficial layers, and is considered as one of the most important locomotor muscles that get affected in subjects with type 2 diabetes. Furthermore, we adopted low-intensity exercise program because type 2 diabetic patients generally suffer from muscle weakness (Volpato et al. 2002; Andersen et al. 2004; Sayer et al. 2005), cardiovascular comorbidities (Spijkerman et al. 2003; Wackers et al. 2004), reduced exercise tolerance (Fang et al. 2005), and are consequently more challenging to involve in intense exercise program (Dunstan et al. 2005).

MATERIALS AND METHODS

Experimental animals. The experiments were performed with 11- to 25-week-old male non-obese SDT rats (n = 11) and aged-matched healthy male Sprague-Dawley (SD) rats (n = 11) were used in this study. Animals were divided randomly to exercise (n = 10) and sedentary (n = 12) groups, respectively. Exercise groups included SDT diabetes with exercise (DB+Ex, n = 5) and SD control with exercise (Con+Ex, n = 5) rats, while sedentary groups included sedentary SDT diabetic (DB, n = 6) and SD control (Con, n = 6) rats. All rats were housed individually in standard 26.5 × 34.5 cm cages at a temperature-controlled room at 22 ± 2°C with 12 hours light-dark cycle. Animals were adjusted to equal the feed dosage, standard rat chow, and were maintained water ad libitum for 14 weeks. This study was approved by the Institutional Animal Care and Use Committee and carried out according to Kobe University Animal Experiments regulation (Kobe, Japan). All experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Exercise protocol. Animals in exercise groups (DB+Ex and Con+Ex) were familiarized with running program for 10–15 min per day on a motor-driven treadmill for one week before starting the study, thereafter animals ran at 15 m min⁻¹ for 60 min per session, 5 sessions per week for 14 weeks. Exercise protocol had been considered as aerobic low-intensity exercise training, because non-fasting circulating blood lactate concentration, which was determined via tail vein blood samples, did not elicit significant changes prior to and after exercise sessions, remaining below 2 mmol L⁻¹.

Plasma collection and biochemical analyses. At the age of 25 weeks, rats were deeply anesthetized by sodium pentobarbital (50 mg kg⁻¹, i.p.), and placed on a heated surgical table. Blood samples were obtained from the abdominal vena cava after 9 h fasting and subsequently placed on ice. Glycosylated hemoglobin (HbA1c) levels were measured after collecting in tubes approximately 1 μL of whole blood by DCA Vantage Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). The remaining blood samples were centrifuged (3000 ×g for 10 min) at room temperature, and then the plasma was stored at ~80°C until analysis. Serum glucose levels were measured by using Glucose CII-Test Wako kit (Wako Pure Chemical Industries, Osaka, Japan).

Muscle sample extraction and preparation. Gastrocnemius muscle was excised and weighed. Removed muscle samples were immediately frozen in isopentane, pre-cooled in liquid nitrogen and stored at −80°C until further analysis. Serial transverse sec-
Table 1  Characteristics of experimental groups

|                | Body mass (g) | Muscle wet mass (mg) | Relative muscle mass (mg/g) | HbA1c (%) | Blood glucose (mg/dL) |
|----------------|---------------|----------------------|-----------------------------|-----------|-----------------------|
| Con            | 483.2 ± 6.2   | 2475.2 ± 40          | 5.13 ± 0.09                 | 3.13 ± 0.08 | 191.7 ± 18.6          |
| Con+Ex         | 454.4 ± 8.6   | 2325.4 ± 69.4        | 5.12 ± 0.13                 | 3.14 ± 0.16 | 175.1 ± 9.1           |
| DB             | 436.7 ± 14.4* | 1877.3 ± 111.5**†    | 4.3 ± 0.22**†               | 7.2 ± 0.16*† | 432.6 ± 10.2**†       |
| DB+Ex          | 477.8 ± 8.2‡  | 2366.4 ± 47.2‡       | 4.95 ± 0.05‡                | 3.32 ± 0.07‡ | 244.9 ± 32‡           |

Experimental groups: Control: Con (n = 6), Control with exercise: Con+Ex (n = 5), Diabetic: DB (n = 6) and Diabetic with exercise: DB+Ex (n = 5). Values are the mean ± SEM. *, † and ‡ denote a significance difference from the Con, Con+Ex and DB groups, respectively.

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...tions of 12-μm-thickness were sliced from the middle part of the medial gastrocnemius muscle belly by a cryostat microtome (CM-1510S; Leica Microsystems, Mannheim, Germany) at −25°C, and mounted on glass slides in preparation for several histochemical analyses. The muscle sample sections were dried at room temperature for 30 min. Then sections were stained for myofibrillar adenosine triphosphate (mATPase) to categorize the muscle fibers as types I, IIA, IID, or IIB on the basis of previous study (Punkt et al. 2004). For ATPase staining, the sections were preincubated in barbital acetate buffer (pH 4.5) for 5 min at room temperature. Then slides were washed with 0.1 M barbital buffer containing 0.18 M CaCl₂ (pH 9.4) for 30 s, followed by 45 min incubation at room temperature in similar 0.1 M barbital buffer containing, in addition to 0.18 M CaCl₂, 4 mM ATP at similar pH. Afterwards, sections were washed in 1% CaCl₂ on three different changes for cumulative 10 min and in 2% CoCl₂ for 5 min in one change. Hereafter, slides were washed by 0.01 M sodium barbital for 8 turns and one change with distilled water. Subsequently, they were inserted in 1% ammonium sulphide for 30 s. The sections were observed with a light microscope (BX51; Olympus, Tokyo, Japan), and 2 images for each of the superficial and deep muscle layers were obtained with a CCD camera (VB-7000; Keyence, Osaka, Japan). The images of stained sections with ATPase were used to measure the changes of muscle fiber cross-sectional area (CSA) of each muscle fiber type and the changes in the composition of muscle fiber types through Image J software program (NIH, Maryland, State, USA).

Similarly, muscle sample sections were also stained for succinate dehydrogenase (SDH) activity to measure the levels of mitochondrial activity and oxidative capacity. Sections were incubated for 45 min at 37°C in 0.05% nitroblue tetrazolium and 0.05 M sodium succinate in 0.05 M phosphate buffer (pH 7.5). The images of tissues were visualized with a light microscope, and images were taken with a CCD camera. Likewise, ATPase staining in SDH staining, two microscopic images were taken from each section of deep layer, as well as for superficial layer, that matched with the ATPase images. All muscle fibers in all images were analyzed to determine the SDH activity in every fiber type of the medial gastrocnemius muscle. SDH activity was calculated as the mean optical density (O.D.) by using the ImageJ software program.

Moreover, muscle sample sections were stained to find out the levels of alkaline phosphatase (AP) in capillaries in the skeletal muscle. Sections were incubated for 45 min at 37°C in 0.1% 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt and 0.1% nitro blue tetrazolium in 0.2 M borate buffer and fixed with 4% paraformaldehyde. As well as ATPase and SDH staining, sections in AP staining were observed with a light microscope, and images were obtained with a CCD camera. The capillary-to-fiber ratio (C/F ratio) was measured in 3 microscopic images selected randomly from AP stained sections of the deep layers so as for the superficial layers of gastrocnemius muscle. All measurements were subsequently calculated using the Image J software program. The C/F ratio was determined by counting all capillaries and fibers in microscopic images.

**Statistical analyses.** All data were expressed as the mean ± SEM. Overall comparisons between the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed with post-hoc Tukey’s test for comparisons. For all analyses, the *P* < 0.05 level was considered significant.

**RESULTS**

**Body and muscle wet weights**

The body and muscle wet mass, and the ratio of muscle to body mass (relative muscle mass) were shown in Table 1. The mean values of body, muscle...
lower than DB group by 57% although that in DB+Ex group was higher than control group. The level of HbA1c in DB+Ex group was significantly lower than DB group. Exercise maintained the level of HbA1c to near control levels.

Changes in cross-sectional area (CSA) & mitochondrial/metabolic activity (SDH Activity)
Representative images of ATPase and SDH activity staining in the medial gastrocnemius muscles of the Con, Con+Ex, DB, and DB+Ex groups are shown in Fig. 1. The images of the superficial layer of the medial gastrocnemius muscle are represented in Fig. 1A–H, and the images of the deep layer are represented in Fig. 1I–P. The changes of the CSA and SDH activity for the four experimental groups after 14 weeks are shown in Fig. 2. The exercise program

and relative muscle mass of the DB group were significantly lower than those of the Con group, whereas the body, muscle and relative muscle mass had been counteracted significantly by exercise in the DB+Ex group compared to DB group.

Glucose and HbA1c levels
The levels of blood glucose and HbA1c are shown in Table 1. The blood glucose level in DB group was about 2.3 and 2.5 folds higher than Con and Con+Ex groups, respectively. The level of HbA1c in the DB group was significantly higher than those in other groups, with about 2.3 folds increase compared to Con and Con+Ex groups. The exercise program resulted in reductions of the levels of blood glucose and HbA1c in the DB+Ex group. The level of blood glucose in DB+Ex group was significantly lower than DB group by 57% although that in DB+Ex group was higher than control group. The level of HbA1c in DB+Ex group was significantly lower than DB group. Exercise maintained the level of HbA1c to near control levels.

Fig. 1 Light microscope images of superficial (A–H) and deep (I–P) layers of the medial gastrocnemius muscle for the Con (A, E, I, M), Con+Ex (B, F, J, N), DB (C, G, K, O), and DB+Ex (D, H, L, P). Transverse sections were assayed for myofibular adenosine triphosphatase (mATP-ase) at pH 4.5 preincubation (superficial A–D and deep I–L), and for succinate dehydrogenase (SDH) activity (superficial E–H and deep M–P). Scale bar on P = 50 μm.
Exercise protects capillary activity significantly compared to other groups in type IID fibers, while a significant decrease was shown in the DB group compared to Con+Ex group (Fig. 2C). Finally, the exercise program preserved the CSA of the type IIB fiber in the DB+Ex group, which was significantly larger than the DB group, keeping the CSA of type IIB fibers in the DB+Ex group near control level (Fig. 2D). Interestingly, the low-intensity exercise training counteracted the reduced level of SDH activity in diabetic rats for type IIB muscle fibers. These results showed a significant increase in the SDH activity level in the DB+Ex group compared to other groups for the fast-glycolytic type IIB fibers (Fig. 2D).

Changes in muscle fiber distribution
The distribution of type IIA fiber in the DB group was significantly lower compared with that of the Con group in superficial layer of the gastrocnemius muscle (Fig. 3A). The distribution of type IIB fiber in the DB group was significantly higher compared with that of the Con group in both superficial and induced hypertrophy in the type I fibers in the Con+Ex group (Fig. 2A). The level of SDH activity in type I fiber was a significant increase in the exercised group compared to non-exercise (Con and DB) groups (Fig. 2A). In the type IIA fibers, the CSA in DB group was significantly smaller compared to Con group, and the exercise program for diabetic rats managed to preserve the CSA of type IIA fibers in DB+Ex group near control levels (Fig. 2B). The level of SDH activity for type IIA fibers was significantly higher in Con+Ex group compared to other groups (Fig. 2B). Although the SDH activity of type IIA fibers in DB+Ex group was significantly lower than Con+Ex group, no significant difference in SDH activity was shown between Con and DB+Ex groups (Fig. 2B). The CSA of type IID fibers in DB group was significantly smaller compared to other groups (Fig. 2C). The CSA of type IID in the DB+Ex group was significantly smaller than that in Con+Ex group but managed to preserve against significant atrophy compared to Con group (Fig. 2C). The low intensity exercise training managed to raise the level of SDH activity significantly compared to other groups in type IID fibers, while a significant decrease was shown in the DB group compared to Con+Ex group (Fig. 2C). Finally, the exercise program preserved the CSA of the type IIB fiber in the DB+Ex group, which was significantly larger than the DB group, keeping the CSA of type IIB fibers in the DB+Ex group near control level (Fig. 2D). Interestingly, the low-intensity exercise training counteracted the reduced level of SDH activity in diabetic rats for type IIB muscle fibers. These results showed a significant increase in the SDH activity level in the DB+Ex group compared to other groups for the fast-glycolytic type IIB fibers (Fig. 2D).

Fig. 2 The CSA and SDH activity. Combined graph of cross-sectional area (CSA) and succinate dehydrogenase (SDH) activity of four types of muscle fibers in the medial gastrocnemius muscle. Graphs were divided according to the muscle fiber types (type I, type IIA, type IID, and type IIB). The values represent the data after 14 weeks in the Con (white empty circle), Con+Ex (black filled circle), DB (white empty triangle) and DB+Ex (black filled triangle). Values are the mean ± SEM. *, † and ‡ denote a significance difference from the Con, Con+Ex and DB groups, respectively.
Fig. 3 Muscle-fiber distribution. The values of muscle fiber distribution in superficial (A–C) and deep (D–G) layers of the medial gastrocnemius muscle. Graphs were divided according to the muscle fiber types. The values represent the data after 14 weeks in the Con (white empty bar), SD+Ex (vertical lines), DB (black bar), and DB+Ex (horizontal lines). Values are the mean ± SEM. *, † and ‡ denote a significance difference from the Con, Con+Ex and DB groups, respectively.

Fig. 4A–H. Capillaries around the muscle fibers were visualized as dark spots. The mean C/F ratios in the four experimental groups are shown in Fig. 4I and J. The mean value of the DB group was significantly lower compared to that of the Con, Con+Ex and DB+Ex groups in both superficial (Fig. 4I) and deep muscle layers (Fig. 4J). In contrast, the C/F ratios in the deep and superficial layers of the DB+Ex muscle were significantly higher compared to the DB group. However, the C/F ratio in superficial layer of the Con+Ex muscle was significantly higher than that of the Con group (Fig. 4I). There was no statistical difference between the Con and Con+Ex groups in deep muscle layer (Fig. 4J). Thus, the exercise program resulted in increased capillary-to-fiber ratio in both superficial (Fig. 4I) and deep layers (Fig. 4J) in the diabetic muscle.

DISCUSSION
To the best of our knowledge, this is the first study to report the effects of low-intensity exercise train-
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ing on the characteristics and capillary supply of skeletal muscle in non-obese diabetic model with high level of plasma glucose. This study shows that non-obese diabetic rats are epimorized with prominent hyperglycemia associated with the diminution in general body and muscle mass, and reduced cross sectional area of muscle fiber. In addition, non-obese diabetic rats had reduced metabolic oxidative capacity and oxidative myofiber, and capillary regression in the skeletal muscle. Furthermore, we have shown that low-intensity exercise training can obtain favorable results preventing low metabolic oxidative capacity and hyperglycemia, subsequent to enhanced capillarization in the skeletal muscle (Fig. 5).

It is well known that skeletal muscle is the main target of insulin-stimulated glucose uptake and regulation (Yki-Jarvinen et al. 1987; Barnard and Youngren 1992; Ishihara et al. 2012). Previous studies showed that the skeletal muscle of sedentary type 2 diabetic individuals (Marin et al. 1994; Hickey et al. 1995; Nyholm et al. 1997; Gaster et al. 2001) and sedentary diabetic rats such as GK rats (Yasuda et al. 2002; Nagatomo et al. 2011) and OLETF rats (Yasuda et al. 2001) exhibit a lower percentage of high-oxidative skeletal muscle fibers such as type I and IIA, and higher percentage of low-oxidative skeletal muscle fiber such as type IIB and IID associated with escalated levels of blood glucose and HbA1c. This alteration in fiber types was linked to hyperglycemia, hence the growth-related increase in the glucose levels in diabetic animals is parallel to the increased number of low-oxidative fibers in skeletal muscles (Yasuda et al. 2001, 2002, 2006). In addition, decreased oxidative capacity of type 2 diabetes was related proportionally to hyperglycemia, insulin resistance and impaired glucose metabolism (Simoneau and Kelley 1997; He et al. 2001; Kelley et al. 2002; Yasuda et al. 2006; Nagatomo et al. 2011). Similarly, the results from the present study showed that sedentary non-obese SDT diabetic rats had a lower percentage of high oxidative muscle fiber, type IIA, and a higher percentage of low oxidative muscle fiber, type IIB, in the superficial layer of the medial gastrocnemius muscle compared with the control

![Fig. 4 Light microscope images of superficial (A–D) and deep (E–H) layers in the gastrocnemius muscle of the Con (A, E) Con+Ex (B, F), DB (C, G), and DB+Ex (D, H) for alkaline phosphatase (AP) level. The capillary-to-fiber ratio (C/F ratio) in superficial (I) and deep (J) layers in the medial gastrocnemius muscle of the Con (white empty bar), Con+Ex (vertical lines), DB (black bar), and DB+Ex (horizontal lines). Values are the mean ± SEM. *, † and ‡ denote significance differences from the Con, Con+ Ex and DB groups, respectively. Scale bar on H = 50 μm.](image-url)
group (Fig. 3A, C). In addition, the higher percentage of low oxidative muscle fibers, type IID and type IIB, was observed in deep layer of the gastrocnemius muscle (Fig. 3E, F). Moreover, these results were predominantly associated with reduced mitochondrial enzyme activity levels in both high and low oxidative muscle fibers (Fig. 2), and elevated glucose levels (Table 1). Reduced mitochondrial enzyme activity can contribute to the metabolic dysfunction in skeletal muscle (Padrao et al. 2012) and also explain the changes in muscle fiber type distribution.

It is well known that increased physical activity increases the demands for oxygen levels. In addition, a chronic imbalance between the perfusion capabilities of the blood vessels and the metabolic requirements of the skeletal tissue lead to modifications in the vasculature to accommodate the tissue needs (Adair et al. 1990), which motivates the angiogenesis in skeletal muscle. Low-intensity exercise training increased blood flow particularly in the most oxidative muscle fiber of unloaded hindlimb in rats (Laughlin and Ripberger 1987), as well as C/F ratio and capillary density in all muscle fibers of healthy animals and individuals (Gute et al. 1996; Poole and Mathieu-Costello 1996; Andersen et al. 2004). In addition, low-intensity exercise training increased the level of SDH activity, which is an indicator of mitochondrial oxidative enzyme activity (Nakatani et al. 1999; Wust et al. 2009). Also, several studies on non-diabetic individuals and rats reported that exercise training led to an increase in oxidative enzyme activity prior to the increase in capillary supply in skeletal muscles (Andersen and Henriksson 1977; Poole and Mathieu-Costello 1996). However, the effects of low-intensity exercise on the skeletal muscle of this non-obese type 2 diabetic model have not been investigated. In the present study, the increased demand for oxygen levels in skeletal muscle fibers due to low-intensity exercise training increased the C/F ratio (Fig. 4I, J) as well as SDH enzyme activity levels (Fig. 2). The exercise training did not only inhibit low metabolic oxidative capacity but also inhibited the regression of the capillary network in the skeletal muscle of non-obese diabetic rats. It also stimulated the transformation of muscle fiber type from glycolic fiber to oxidative fibers through the augmentation of mitochondrial oxidative enzyme activity in muscle fibers prior to increased C/F ratio. Interestingly, our data suggest that the exercise training suppresses diabetes mellitus associated the hyperglycemia and bounding glycosylated hemoglobin A1c. This could be justified as the increased oxidative capacity and muscle fiber transformation of skeletal muscle through exercise training substantially decrease hyperglycemia levels (Yasuda et al. 2001, 2002, 2006; Ishihara et al. 2012). Thus, increased oxidative capacity in the skeletal muscles may be a key factor that influences the prevention of type 2 diabetes.

In conclusion, this study demonstrates the main differences between diabetic and control rats, which is characterized mainly by hyperglycemia and high levels of HbA1c associated with reduced metabolic function and oxidative myofiber type prior to reduced capillarization abundance. More importantly, these results indicate that low-intensity exercise training can reduce hyperglycemia and HbA1c levels in association with enhanced metabolic oxidative function and prevent reduced oxidative myofiber...
Exercise protects capillary regression in skeletal muscle.

CONFLICT OF INTERESTS
The authors have nothing to disclose financially, and there is no conflict of interest.

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REFERENCES
Adair TH, Gay WJ and Montani JP (1990) Growth regulation of the vascular system: evidence for a metabolic hypothesis. Am J Physiol 259, R393–404.
Andersen H, Nielsen S, Mogensen CE and Jakobsen J (2004) Muscle strength in type 2 diabetes. Diabetes 53, 1543–1548.
Andersen P and Henriksen J (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270, 677–690.
Angelova P and Boyadjiev N (2013) A review on the models of obesity and metabolic syndrome in rats. Trakia J Sci 1, 5–12.
Barnard RJ and Youngren JF (1992) Regulation of glucose transport in skeletal muscle. FASEB J 6, 3238–3244.
Boule NG, Haddad E, Kenny GP, Wells GA and Sigal RJ (2001) Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA 286, 1218–1227.
Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraaenoo R, et al. (2007) Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia 50, 790–796.
Devlin JT, Hirshman M, Horton ED and Horton ES (1987) Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes 36, 434–439.
Dunstan DW, Daly RM, Owen N, Jolley D, Vullikh E, et al. (2005) Home-based resistance training is not sufficient to maintain improved glycemic control following supervised training in older individuals with type 2 diabetes. Diabetes Care 28, 3–9.
Fang ZY, Sharman J, Prins JB and Marwick TH (2005) Determinants of exercise capacity in patients with type 2 diabetes. Diabetes Care 28, 1643–1648.
Gaster M, Staeher P, Beck-Nielsen H, Schroder HD and Handberg A (2001) 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 50, 1324–1329.
Gute D, Fraga C, Laughlin MH and Amann JF (1996) Regional changes in capillary supply in skeletal muscle of high-intensity endurance-trained rats. J Appl Physiol (1985) 81, 619–626.
Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, et al. (1995) Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. Am J Physiol 268, E453–457.
Ishihara A, Nagatomo F, Fujino H, Kondo H and Tsuda K (2012) Lifestyle-related disease and skeletal muscle: A review. J Phys Fitness Sports Med 1, 17–27.
Ishihara A, Nagatomo F, Fujino H, Kondo H and Tsuda K (2012) Skeletal muscle capillary density and fiber type are possible determinants of insulin resistance in man. J Clin Invest 120, 415–424.
Kivelä R, Silvennoinen M, Touva AM, Lehti TM, Kainulainen H, et al. (2007) Effects of experimental type 1 diabetes and exercise training on angiogenic gene expression and capillarization in skeletal muscle. FASEB J 21, 1570–1572.
Laughlin MH and Ripperger J (1987) Vascular transport capacity of hindlimb muscles of exercise-trained rats. J Appl Physiol (1985) 62, 438–443.
Lillioja S, Young AA, Cutler CL, Ivy JL, Abbott WG, et al. (1987) Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 80, 1189–1197.
Marin P, Andersson B, Krotkiewski M and Bjorntorp P (1994) Muscle fiber composition and capillary density in women and men with NIDDM. Diabetes Care 17, 382–386.
Masuyama T, Fuse M, Yokoi N, Shinohara M, Tsuji H, et al. (2003) Genetic analysis for diabetes in a new rat model of nonobese type 2 diabetes, Spontaneously Diabetic Torii rat. Biochem Biophys Res Commun 304, 196–206.
Masuyama T, Komeda K, Hara A, Noda M, Shinohara M, et al. (2004) Chronological characterization of diabetes development in male Spontaneously Diabetic Torii rats. Biochem Biophys Res Commun 314, 870–877.
Mathieu-Costello O, Kong A, Caraldi TP, Cui L, Ju Y, et al. (2003) Regulation of skeletal muscle morphology in type 2 diabetic subjects by troglitazone and metformin: relationship to glucose disposal. Metabolism 52, 540–546.
Mogensen M, Sahlin K, Fernstrom M, Glintborg D, Vind BF, et al. (2007) Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 56, 1592–1599.
Nagatomo F, Fujino H, Kondo H, Gu N, Takeda I, et al. (2011) PGC-1alpha mRNA level and oxidative capacity of the plantaris muscle in rats with metabolic syndrome, hypertension, and type 2 diabetes. Acta Histochem Cytochem 44, 73–80.
Nakatani T, Nakashima T, Kita T, Hirofuji C, Itoh K, et al. (1999) Succinate dehydrogenase activities of fibers in the rat extensor digitorum longus, soleus, and cardiac muscles. Arch Histol Cytol 62, 393–399.
Nyholm B, Qu Z, Kaaal A, Pedersen SB, Gravholt CH, et al. (1997) Evidence of an increased number of type Iib muscle fibers in insulin-resistant first-degree relatives of patients with NIDDM. Diabetes 46, 1822–1828.
Ohta T, Matsu K, Miyajima K, Sasase T, Masuyama T, et al. (2007) Effect of insulin therapy on renal changes in spontaneously diabetic Torii rats. Exp Anim 56, 355–362.
Padró AI, Carvalho T, Vitorino R, Alves RM, Caseiro A, et al. (2012) Impaired protein quality control system underlies mitochondrial dysfunction in skeletal muscle of streptozotocin-induced diabetic rats. Biochim Biophys Acta 1822, 1189–1197.
Schrauwen P (2010) Exercise training increases mitochondrial content and ex vivo mitochondrial function similarly in patients with type 2 diabetes and in control individuals. Diabetesologia 53, 1714–1721.

Poole DC and Mathieu-Costello O (1996) Relationship between fiber capillarization and mitochondrial volume density in control and trained rat soleus and plantaris muscles. Microcirculation 3, 175–186.

Sasase T, Ohta T, Ogawa N, Miyajima K, Ito M, et al. (2006) Preventive effects of glycaemic control on ocular complications of Spontaneously Diabetic Torii rat. Diabetes Obes Metab 8, 501–507.

Sayer AA, Dennison EM, Syddall HE, Gilbody HJ, Phillips DI, et al. (2005) Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? Diabetes Care 28, 2541–2542.

Sexton WL, Poole DC and Mathieu-Costello O (1994) Microcirculatory structure-function relationships in skeletal muscle of diabetic rats. Am J Physiol 266, H1502–1511.

Shinohara M, Masuyama T, Shoda T, Takahashi T, Katsuda Y, et al. (2000) A new spontaneously diabetic non-obese Torii rat strain with severe ocular complications. Int J Exp Diabetes Res 1, 89–100.

Simoneau JA and Kelley DE (1997) Altered glycolytic and oxidative capacities of skeletal muscle contribute to insulin resistance in NIDDM. J Appl Physiol (1985) 83, 166–171.

Snowling NJ and Hopkins WG (2006) Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: a meta-analysis. Diabetes Care 29, 2518–2527.

Spijkerman AM, Dekker JM, Nijpels G, Adriaanse MC, Kostense PJ, et al. (2003) Microvascular complications at time of diagnosis of type 2 diabetes are similar among diabetic patients detected by targeted screening and patients newly diagnosed in general practice: the Hoorn screening study. Diabetes Care 26, 2604–2608.

Volpato S, Blaum C, Resnick H, Ferrucci L, Fried LP, et al. (2002) Comorbidities and impairments explaining the association between diabetes and lower extremity disability: The Women’s Health and Aging Study. Diabetes Care 25, 678–683.

Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, et al. (2004) Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 27, 1954–1961.

Wust RC, Gibbings SL and Degens H (2009) Fiber capillary supply related to fiber size and oxidative capacity in human and rat skeletal muscle. Adv Exp Med Biol 645, 75–80.

Yasuda K, Adachi T, Kikuchi N, Tsujimoto G, Aoki N, et al. (2006) Effects of running exercise on fibre-type distribution of soleus and plantaris muscles in diabetic Otsuka Long-Evans Tokushima fatty rats. Diabetes Obes Metab 8, 311–321.

Yasuda K, Ishihara A, Adachi T, Shihara N, Seino Y, et al. (2001) Growth-related changes in skeletal muscle fiber type and insulin resistance in Diabetic Otsuka Long-Evans Tokushima Fatty rats. Acta Histochem Cytochem 34, 371–382.

Yasuda K, Nishikawa W, Iwanaka N, Nakamura E, Seino Y, et al. (2002) Abnormality in fibre type distribution of soleus and plantaris muscles in non-obese diabetic Goto-Kakizaki rats. Clin Exp Pharmacol Physiol 29, 1001–1008.

Yki-Jarvinen H, Young AA, Lamkin C and Foley JE (1987) Kinetics of glucose disposal in whole body and across the forearm in man. J Clin Invest 79, 1713–1719.