

**ABSTRACT:** The effects of hyperbaric exposure with high oxygen concentration on glucose and insulin levels and skeletal muscle-fiber properties were investigated in type 2 diabetic Goto-Kakizaki rats. Five-week-old rats were exposed to a hyperbaric environment (1.25 atmospheric pressure) with a high oxygen concentration (36%) for 6 h daily. Glucose and insulin levels and properties including fiber-type distribution, cross-sectional area, and oxidative enzyme activity in the soleus muscle were examined after hyperbaric exposure for 4 weeks. The growth-related increase in glucose level was inhibited by hyperbaric exposure, and insulin also showed lower levels compared with control rats. The percentage of low-oxidative type I fibers in the muscle decreased and high-oxidative type IIA and type IIC fibers, which were not detected in the muscle of control rats, were observed after hyperbaric exposure. The oxidative enzyme activity of type I fibers in the muscle increased after hyperbaric exposure. Hyperbaric exposure with high oxygen concentration might therefore provide a new approach to improve the glucose tolerance, insulin resistance, and altered skeletal muscle metabolism that are caused by diabetes mellitus.

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## EFFECTS OF HYPERBARIC EXPOSURE WITH HIGH OXYGEN CONCENTRATION ON GLUCOSE AND INSULIN LEVELS AND SKELETAL MUSCLE-FIBER PROPERTIES IN DIABETIC RATS

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**S**keletal muscles are comprised of heterogeneous types of fibers that have different functional, morphological, and metabolic properties.<sup>7,20</sup> Patients with type 2 diabetes mellitus have altered patterns of fiber types in the skeletal muscles, i.e., a decreased percentage of high-oxidative fibers in the skeletal muscles.<sup>5,7,22</sup> Our previous studies<sup>29,30</sup> revealed that Otsuka Long–Evans Tokushima Fatty (OLETF) and Goto–Kakizaki (GK) rats, animal models of spontaneous type 2 diabetes mellitus that have hyperglyce-

mia and insulin resistance, have a lower percentage of high-oxidative fibers in the skeletal muscles than age-matched nondiabetic rats. Skeletal muscle is a major target of insulin-stimulated glucose uptake. Therefore, altered patterns of fiber types in the skeletal muscles of patients and animal models with type 2 diabetes mellitus may be linked to glucose tolerance and insulin resistance.

We have designed a hyperbaric chamber for animal experiments, which is an oxygen tank with an oxygen concentrator and an air compressor<sup>9</sup> that automatically maintain the elevated atmospheric pressure and oxygen concentration. Increased atmospheric pressure enhances the partial pressure of oxygen and causes more oxygen to dissolve into the blood and plasma.

Our recent study<sup>28</sup> observed that hyperbaric exposure with high oxygen concentration inhibited a growth-related increase in the glucose level of GK

**Abbreviations:** ATPase, adenosine triphosphatase; GK, Goto–Kakizaki; GLUT, glucose transporter; IRI, immunoreactive insulin; OD, optical density; SDH, succinate dehydrogenase

**Key words:** glucose; high oxygen concentration; hyperbaric exposure; muscle oxidative capacity; type 2 diabetes mellitus

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rats. We postulated that the increased availability of oxygen induced by hyperbaric exposure might have a beneficial impact on the metabolism of skeletal muscles, for example, on oxidative enzyme activity, which might be related to improvements in glucose tolerance and insulin resistance. In the present study we tested this hypothesis by exposing GK rats to a hyperbaric environment with high oxygen concentration for a period of 4 weeks, and then determined glucose and insulin levels and closely examined the fiber-type distribution, cross-sectional area, and oxidative enzyme activity of fibers in the soleus muscle.

## MATERIALS AND METHODS

**Animals and Treatments.** All procedures were approved by our institutional review committee and followed US national guidelines.

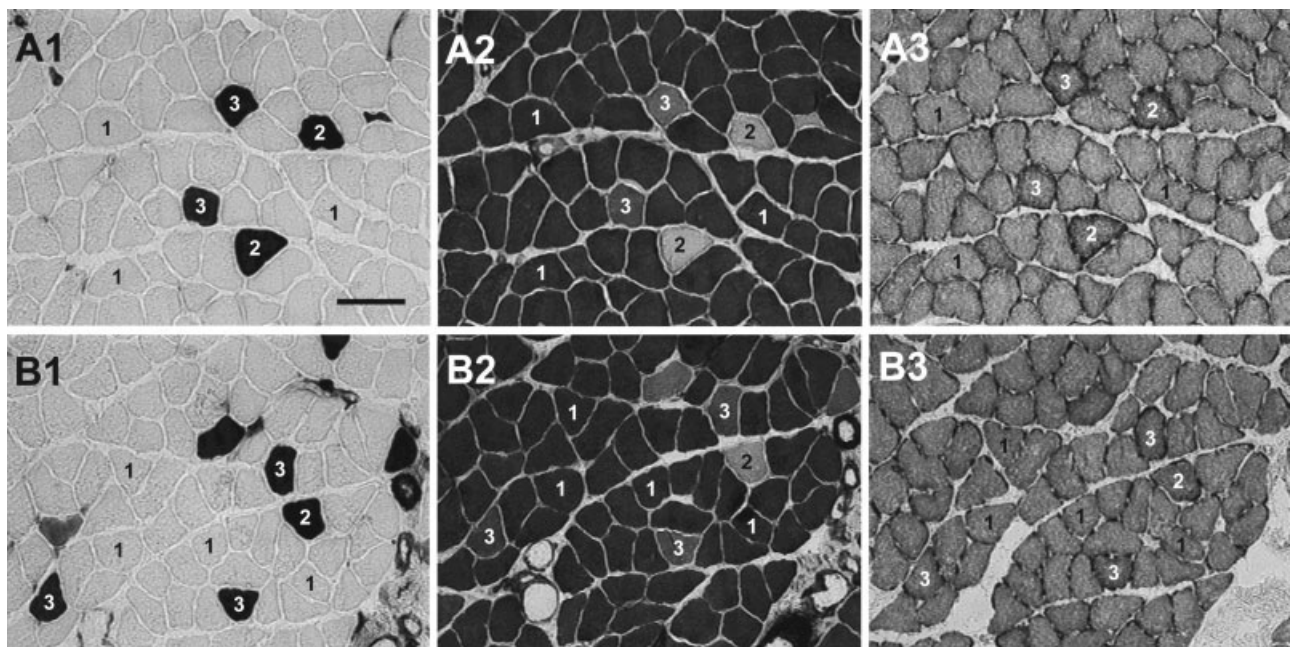
GK rats are animal models of type 2 diabetes mellitus, developed by selective breeding of an outbred colony of Wistar rats with high glucose levels as measured by the oral glucose tolerance test.<sup>4</sup> They were selected for the present study because they have elevated levels of glucose, but not of insulin, and they do not become obese.<sup>1,11,23,24</sup>

Five-week-old male Wistar ( $n = 10$ ) or GK ( $n = 10$ ) rats were randomly assigned to control ( $n = 5$ ) or hyperbaric ( $n = 5$ ) groups. All rats were individually

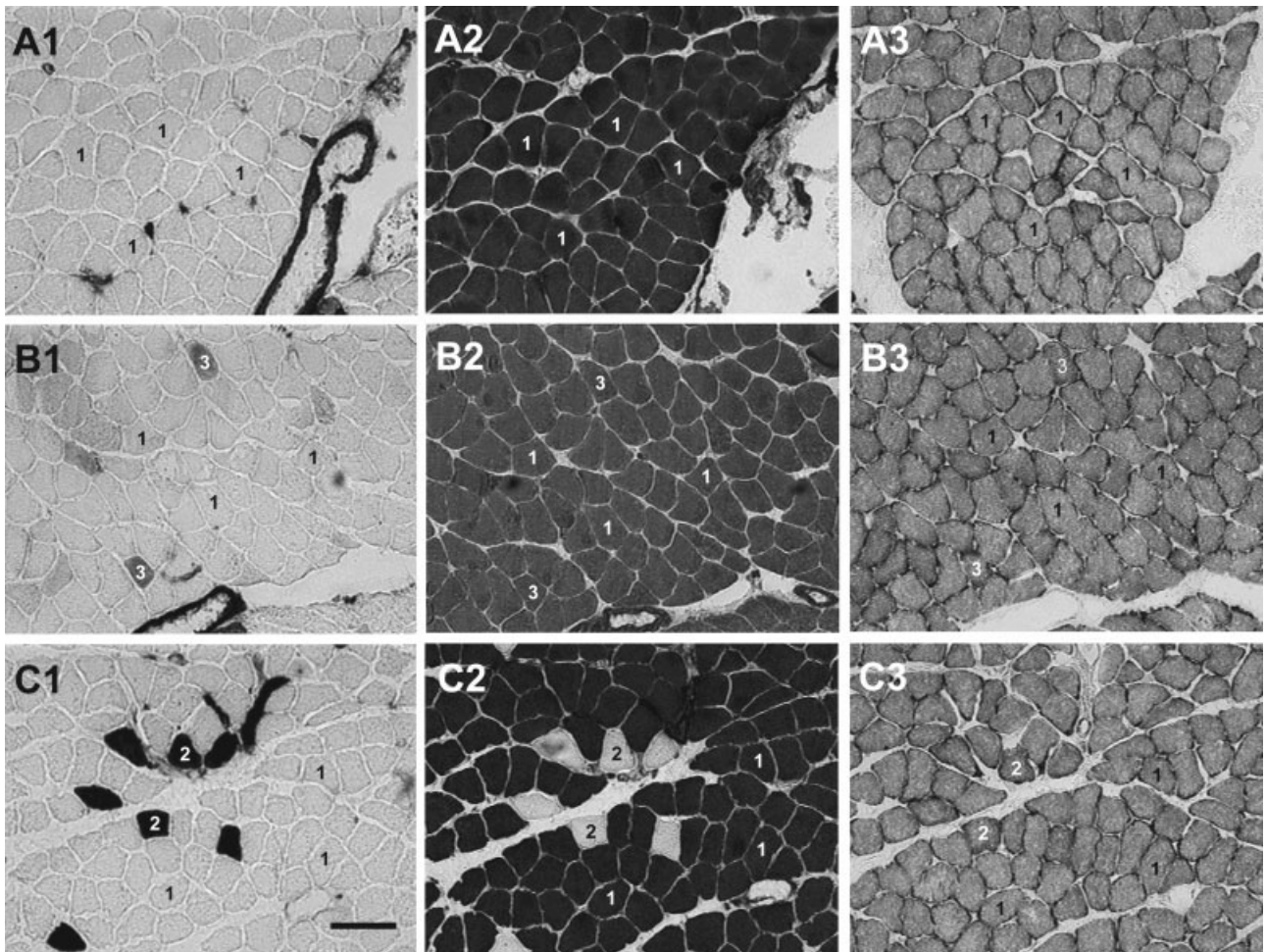
housed in cages of the same size. The rats in the hyperbaric group were exposed to an atmospheric pressure of 1.25 with an oxygen concentration of 36% automatically maintained by a computer-assisted system. The chamber was 180 cm long and 70 cm in diameter, making it large enough to house a number of rats (up to 20 cages) simultaneously.<sup>9</sup> Rats in the hyperbaric group were exposed to the hyperbaric environment for 6 h (10:00 to 16:00) daily for 4 weeks. Food and water were provided ad libitum for both groups. All rats were kept in a controlled environment with fixed 12:12-h light:dark cycles (lights off from 19:00 to 07:00) and room temperature maintained at  $22 \pm 2^\circ\text{C}$ . Food intake in a 24-h period was measured.

**Tissue Preparation.** The rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The soleus muscle was removed, cleaned of excess fat and connective tissue, and wet-weighed. White adipose tissue including epididymal, omental, and retroperitoneal fat was surgically removed and weighed. The total weight of these three types of tissues was taken to be the white adipose tissue weight.

**Measurements of Fasting Plasma Glucose and Insulin.** Plasma obtained by centrifugation was used for measurements of glucose and immunoreactive insulin



**FIGURE 1.** Transverse sections of the soleus muscle in nondiabetic Wistar rats under normobaric (A1–A3) and hyperbaric (B1–B3) conditions. (A1,B1) Stained for adenosine triphosphatase activity following preincubation at pH 10.4; (A2,B2) stained for adenosine triphosphatase activity following preincubation at pH 4.5; (A3,B3) stained for succinate dehydrogenase activity. 1, type I; 2, type IIA; 3, type IIC. Scale bar, 100  $\mu\text{m}$ .



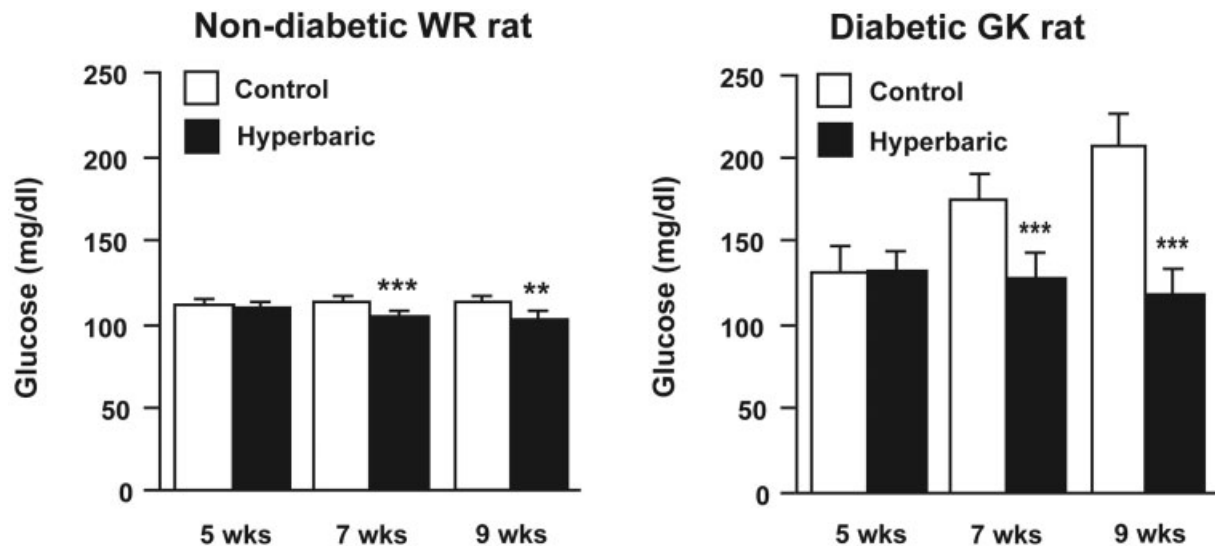
**FIGURE 2.** Transverse sections of the soleus muscle in diabetic Goto-Kakizaki rats under normobaric (**A1–A3**) and hyperbaric (**B1–B3,C1–C3**) conditions. (**A1,B1,C1**) Stained for adenosine triphosphatase activity following preincubation at pH 10.4; (**A2,B2,C2**) stained for adenosine triphosphatase activity following preincubation at pH 4.5; (**A3,B3,C3**) stained for succinate dehydrogenase activity. 1, type I; 2, type IIA, 3, type IIC. Scale bar, 100  $\mu$ m. All fibers in the muscle of GK rats under normobaric conditions were type I (**A1–A3**). Two of five GK rats under hyperbaric conditions had only type I fibers in the muscle, whereas two GK rats had type I and type IIC fibers (**B1–B3**). One rat under hyperbaric conditions had type I and type IIA fibers in the muscle (**C1–C3**).

(IRI) levels. Plasma glucose was determined by a glucose oxidative method<sup>17</sup> on blood samples obtained from the tail veins at 5, 7, and 9 weeks of age. Plasma IRI was determined by a radioimmunoassay using a polyethylene glycol method with rat plasma insulin as the standard<sup>27</sup> on blood samples obtained from the abdominal aorta at 9 weeks of age.

**Histochemical Procedures.** The muscle was placed on cork, stretched to its *in vivo* length, and immediately frozen in isopentane cooled in a mixture of dry ice and acetone. Serial 10- $\mu$ m thick transverse sections of the muscle were cut in a cryostat set at  $-20^{\circ}\text{C}$ . The sections were brought to room temperature, air-dried for 30 min, then stained for adenosine triphosphatase (ATPase) activity following acid (pH 4.3 and 4.5) and alkaline (pH 10.4) preincuba-

tion (Figs. 1, 2).<sup>6,18,19</sup> The muscle fibers were classified into type I (positive at preincubation pH 4.3 and 4.5, and negative at preincubation pH 10.4), type IIA (negative at preincubation pH 4.3 and 4.5, and positive at preincubation pH 10.4), and type IIC (positive at preincubation pH 4.3, 4.5, and 10.4). The fiber-type distribution of the muscle was determined from the entire transverse section of the muscle.

The sections were also stained for succinate dehydrogenase (SDH) activity, an indicator of mitochondrial capacity (Figs. 1, 2).<sup>6,18,19</sup> Tissue sections were digitized as gray scale images and the value of the SDH staining intensity was expressed as an optical density (OD) value on a computer-assisted image processing system (Neuroimaging System, Kyoto, Japan).<sup>8,10</sup> Each pixel was quantified as one of 256 gray



**FIGURE 3.** Glucose levels of nondiabetic Wistar (WR) and diabetic Goto–Kakizaki (GK) rats. Values are expressed as mean  $\pm$  standard deviation ( $n = 5$ ). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with control value.

levels. A gray value of zero was equivalent to 100% transmission of light, and that of 255 was equivalent to 0% transmission of light. The OD units of all pixels within the muscle fiber were converted to a mean OD unit using a calibration photographic tablet, which has 21-step gradient density ranges of diffused density values.

**Statistics.** Means and standard deviations were calculated from individual values using standard procedures. Student's *t*-test was used to determine significant differences between the control and hyperbaric groups.

## RESULTS

**Body Weight and Food Intake.** The body weights of Wistar rats at 9 weeks of age in the control and hyperbaric groups were  $365.8 \pm 24.6$  g and  $364.6 \pm 19.2$  g, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were  $217.4 \pm 14.2$  g and  $206.8 \pm 4.5$  g ( $n = 5$  for all groups), respectively. There was no difference in body weight of Wistar or GK rats between the control and hyperbaric groups.

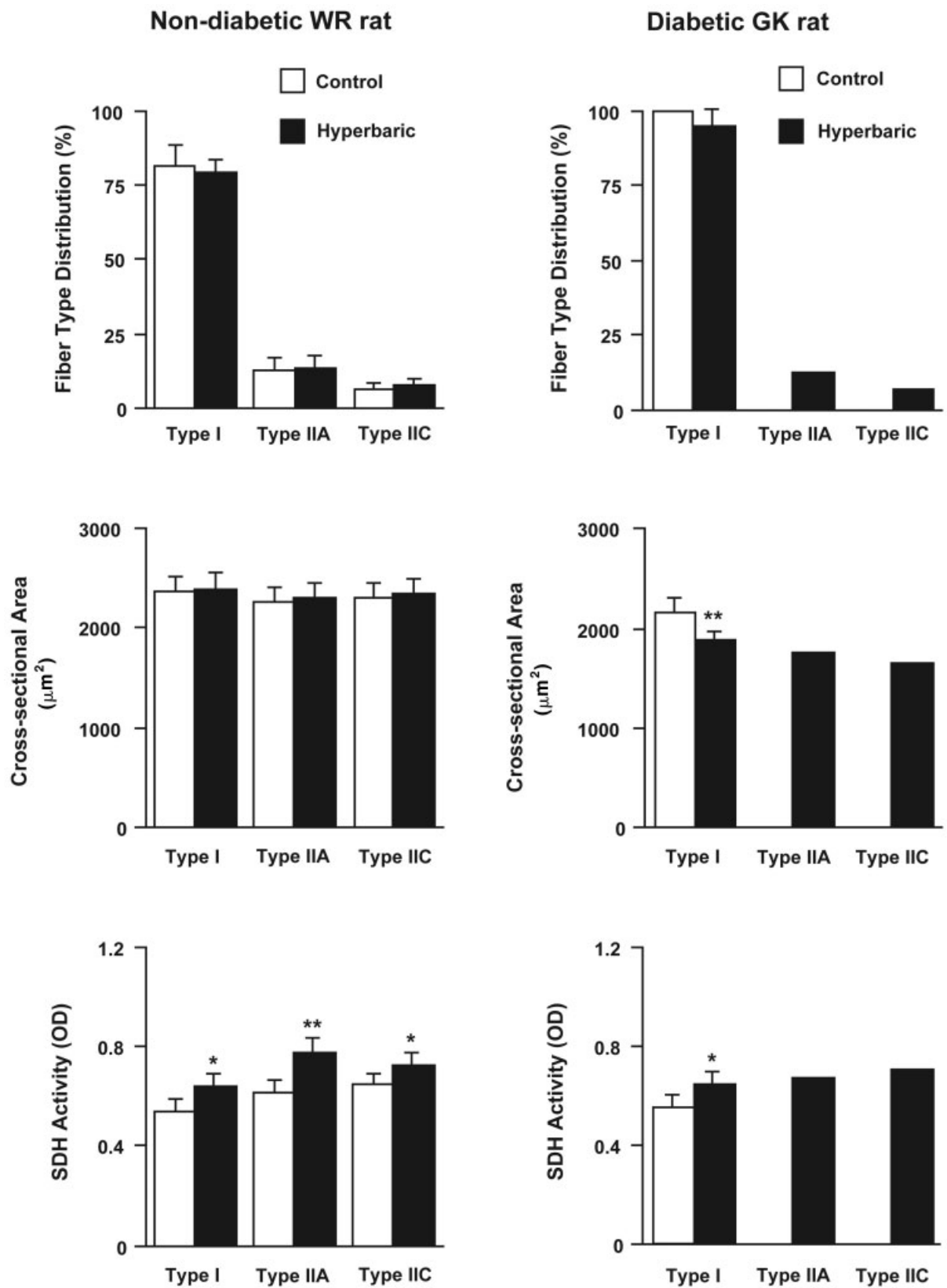
The food intakes of Wistar rats at 9 weeks of age in the control and hyperbaric groups were  $30.8 \pm 3.8$  g/day and  $29.0 \pm 3.3$  g/day, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were  $9.5 \pm 1.1$  g/day and  $9.0 \pm 1.2$  g/day ( $n = 5$  for all groups), respectively. Wistar or GK rats in the control and hyperbaric groups had equivalent levels of food intake.

**White Adipose Tissue Weight.** The white adipose tissue weights of Wistar rats in the control and hyperbaric groups were  $7.07 \pm 2.89$  g and  $7.16 \pm 1.28$  g, respectively, and those of GK rats in the control and hyperbaric groups were  $3.53 \pm 0.69$  g and  $3.85 \pm 0.57$  g ( $n = 5$  for all groups), respectively. There was no difference in white adipose tissue weight of Wistar or GK rats between the control and hyperbaric groups.

**Fasting Plasma Glucose and Insulin Levels.** The fasting plasma glucose levels of Wistar and GK rats were significantly lower in the hyperbaric groups at 7 and 9 weeks of age than in the control groups (Fig. 3).

The fasting plasma IRI levels of Wistar rats at 9 weeks of age in the control and hyperbaric groups were  $1707.6 \pm 526.7$  pg/ml and  $1514.5 \pm 631.6$  pg/ml, respectively, and those of GK rats at 9 weeks of age in the control and hyperbaric groups were  $472.1 \pm 238.0$  pg/ml and  $123.2 \pm 41.3$  pg/ml ( $n = 5$  for all groups), respectively. The fasting plasma IRI levels of GK rats were significantly lower ( $P < 0.05$ ) in the hyperbaric than control group, but there was no difference in fasting plasma IRI level of Wistar rats between the control and hyperbaric groups.

**Soleus Muscle Weight.** The muscle weights of Wistar rats in the control and hyperbaric groups were  $0.13 \pm 0.02$  g and  $0.13 \pm 0.02$  g, and those of GK rats in the control and hyperbaric groups were  $0.08 \pm 0.01$  g and  $0.09 \pm 0.01$  g ( $n = 5$  for all groups),



**FIGURE 4.** Fiber-type distributions, cross-sectional areas, and succinate dehydrogenase activities of the soleus muscle in nondiabetic Wistar (WR) and diabetic Goto-Kakizaki (GK) rats. SDH, succinate dehydrogenase; OD, optical density. Values are expressed as mean  $\pm$  standard deviation [ $n = 5$ , except for type IIA ( $n = 1$ ) and type IIC ( $n = 2$ ) in diabetic GK rats of the hyperbaric group]. \* $P < 0.05$ , \*\* $P < 0.01$  compared with control value.

respectively. There was no difference in muscle weight of Wistar or GK rats between the control and hyperbaric groups.

**Soleus Muscle Fiber Properties.** In Wistar rats there was no difference in fiber-type distribution or cross-sectional area between the control and hyperbaric groups (Fig. 4). The oxidative enzyme activities of all types of fibers were significantly higher in the hyperbaric than control group (Fig. 4).

In GK rats all fibers in the muscles of the control group were type I (Figs. 2, 4). The muscles of two rats in the hyperbaric group were composed of only type I fibers (Fig. 4). The muscles of two other rats in the hyperbaric group were composed of type I (94.0% and 92.5%) and type IIC (6.0% and 7.5%) fibers, whereas that of the other rat in this group contained type I (87.7%) and type IIA (12.3%) fibers. The cross-sectional area of type I fibers in the muscle was significantly smaller in the hyperbaric than control group, whereas the oxidative enzyme activity of type I fibers was significantly higher in the hyperbaric than control group (Fig. 4).

## DISCUSSION

Skeletal muscle plays an important role in the regulation of blood glucose because it is the site with the highest level of insulin-stimulated glucose uptake and disposal.<sup>25</sup> It is largely accepted that type 2 diabetes mellitus is associated with impaired insulin-stimulated glucose disposal capacity, which is attributed to insulin resistance in skeletal muscle. Patients with type 2 diabetes mellitus have disrupted metabolic potentials and different patterns of fiber types in the skeletal muscles compared with nondiabetic subjects.<sup>5,16,22</sup> Diabetes has been associated with a high percentage of low-oxidative fibers (particularly type IIB fibers) and a low percentage of high-oxidative fibers in the fast skeletal muscles, such as the biceps femoris, vastus lateralis, and rectus abdominis muscles. We observed similar changes in the fiber-type distribution of both the fast plantaris and slow soleus muscles in diabetic rats.<sup>29,30</sup> Previous studies<sup>2,3</sup> suggested that a decreased percentage of high-oxidative fibers in the skeletal muscles combined with a reduction in glucose transporter (GLUT)-4 expression in high-oxidative fibers reduces the insulin-sensitive GLUT-4 pool in patients with type 2 diabetes mellitus and contributes to skeletal muscle insulin resistance. These results strongly indicate that changes in the fiber-type distribution of skeletal muscles in diabetic rats are due to an impairment in insulin sensitivity and glucose metabolism.

Consistent with our hypothesis that the increased availability of oxygen induced by hyperbaric exposure with high oxygen concentration has a beneficial impact on glucose and insulin levels and the metabolism of skeletal muscles, we observed that a growth-related increase in glucose level of GK rats was completely inhibited by hyperbaric exposure (Fig. 3). These findings are consistent with those in our recent study.<sup>28</sup> In addition, the insulin level was significantly lower in the hyperbaric than control group. Hyperbaric exposure with high oxygen concentration might therefore provide a new approach to improve glucose tolerance and insulin resistance.

In the present study, we examined the soleus muscle because it has a higher percentage of high-oxidative fibers, which are more insulin sensitive and responsive than low-oxidative fibers.<sup>12,13,15</sup> In addition, high-oxidative fibers are characterized by increased fatty acid oxidation, low glycolytic capacity, and high triglyceride accumulation compared with low-oxidative fibers.<sup>14,21</sup> A previous study<sup>26</sup> also observed that skeletal muscle insulin resistance in GK rats is associated with high-oxidative fiber-specific defects in the insulin-signal transduction pathway to glucose transport, suggesting that hyperglycemia affects high-oxidative fibers more severely than low-oxidative fibers, and that it selectively reduces GLUT-4 expression in high-oxidative fibers in patients and animal models with type 2 diabetes mellitus. In the present study, hyperbaric exposure with high oxygen concentration prevented diabetes-associated changes in the fiber-type distribution of the soleus muscle in GK rats. In addition, we observed that the type I fiber oxidative enzyme activity of the soleus muscle in GK rats increased after hyperbaric exposure. These results suggest that the increase in oxidative capacity of skeletal muscles is an adaptive response to hyperbaric exposure with high oxygen concentration.

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