Hyperbaric Exposure with High Oxygen Concentration Improves Altered Fiber Types in the Plantaris Muscle of Diabetic Goto-Kakizaki Rats

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Abstract: Hyperbaric exposure with high oxygen concentration inhibits a growth-related increase in the glucose and insulin of diabetic rats. In this study, 5-week-old diabetic Goto-Kakizaki rats were exposed to a hyperbaric environment (1.25 atmospheric pressure) with a high oxygen concentration (36%) for 6 h daily. Fiber type distributions and oxidative enzyme activities in the fast-twitch plantaris muscle of Goto-Kakizaki rats were examined after hyperbaric exposure for 4 weeks. The percentages of high-oxidative type I and type IIA fibers increased and that of low-oxidative type IIB fibers decreased after hyperbaric exposure. Furthermore, the fiber oxidative enzyme activity increased after hyperbaric exposure, regardless of fiber type. It is concluded that altered patterns of fiber types in the plantaris muscle of diabetic rats shift toward normal, which is observed in nondiabetic rats, following hyperbaric exposure with high oxygen concentration.

Key words: diabetes mellitus, hyperbaric oxygenation, plantaris muscle.

Patients and animal models with type 2 diabetes mellitus exhibit altered patterns of fiber types in skeletal muscles [1–5]. Goto-Kakizaki rats, animal models of spontaneous type 2 diabetes mellitus [6], have a lower percentage of high-oxidative fibers in the slow soleus and fast plantaris muscles than age-matched nondiabetic rats [7]. Therefore, altered patterns of fiber types in skeletal muscles may be linked to impaired glucose tolerance and insulin resistance. Our previous study [8] showed that the decreased percentage of high-oxidative fibers in the slow soleus muscle of Goto-Kakizaki rats shifted toward normal, which is observed in age-matched nondiabetic rats, after hyperbaric exposure with high oxygen concentration. In this study, we examined whether similar effects on the fiber type distribution and oxidative enzyme activity in the fast plantaris muscle of Goto-Kakizaki rats were observed after hyperbaric exposure with high oxygen concentration.

Materials and methods

Five-week-old male Wistar (n = 10) or Goto-Kakizaki (n = 10) rats were randomly assigned to the 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 in a hyperbaric chamber [9]. They were exposed to the hyperbaric environment for 6 hours (10:00 to 16:00) daily for 4 weeks. Food and water were provided ad libitum for all 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 ± 2°C. All procedures were approved by our institutional review committee and followed the guidelines of the Physiological Society of Japan for the care and use of experimental animals.

The rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). After blood sampling, the plantaris muscle was removed, cleaned of excess fat and connective tissue, and immediately frozen in isopentane cooled in a mixture of dry ice and acetone. Serial 10-μm thick transverse sections of the muscle were cut in a cryostat set...
at –20°C. The sections were brought to room temperature, air-dried for 30 min, and then stained for adenosine triphosphatase activity following acid (pH 4.3 and 4.5) and alkaline (pH 10.4) preincubation [12, 13]. The muscle fibers were classified into type I (positive at preincubation pH 4.3 and 4.5, negative at preincubation pH 10.4), type IIA (negative at preincubation pH 4.3 and 4.5, positive at preincubation pH 10.4), and type IIB (negative at preincubation pH 4.3, positive at preincubation pH 4.5 and 10.4).

The sections were also stained for succinate dehydrogenase (SDH) activity, an indicator of mitochondrial oxidative potential [12, 13]. The SDH activity was rendered visible by incubating the sections in 0.1 M phosphate buffer (pH 7.6) containing 0.9 mM sodium azide, 0.9 mM 1-methoxyphenazine methylsulfate, 1.5 mM nitroblue tetrazolium, 5.6 mM EDTA-disodium salt, and 48 mM succinate disodium salt. The reaction was terminated by multiple washings in distilled water, dehydration in graded ethanol, and passing through xylene. For histochemical controls, either the succinate disodium salt or the nitroblue tetrazolium was excluded from the incubation medium. 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 [14, 15]. The fiber type distribution and SDH activity were determined from approximately 100 fibers in the central region of the muscle.

A two-way analysis of variance (ANOVA) was used to evaluate the influence of environment (control vs. hyperbaric) and strain (Wistar vs. Goto-Kakizaki). When the differences were significant based on the ANOVA analyses, further comparisons were made using post hoc tests. The 0.05 level of probability was established for statistical significance.

Results

In the Wistar and Goto-Kakizaki rats, there were no differences in body weight between the control and hyperbaric groups (Fig. 1). The body weights of the control and hyperbaric groups in the Goto-Kakizaki rats were lower than those in the Wistar rats. The body weights of the control and hyperbaric groups in the Goto-Kakizaki rats were lower than those in the Wistar rats. The plantaris muscle weight per body weight of the control group in the Goto-Kakizaki rats was the lowest among the four groups.

The plasma insulin levels of the control and hyperbaric groups in the Goto-Kakizaki rats were lower than those in the Wistar rats. The plasma insulin level of the hyperbaric group was lower than that of the control group.

In the Wistar and Goto-Kakizaki rats, the plasma glucose levels of the hyperbaric groups were lower than those of the control groups (Fig. 3). The plasma glucose level of the control group in the Goto-Kakizaki rats was the highest among the four groups.

In the Wistar rats, the percentage of type IIA fibers was higher and that of type IIB fibers was lower in the hyperbaric group than in the control group (Fig. 4). In the Goto-Kakizaki rats, the percentages of type I and type IIA fibers were higher and that of type IIB fibers was lower in the hyperbaric group than in the control group. The percentages of type I and type IIA fibers in the control group of the Goto-Kakizaki rats were the lowest and that of type IIB fibers was the highest among the four groups.

![Fig. 1.](image1.png) **Fig. 1.** Body weights of nondiabetic Wistar and diabetic Goto-Kakizaki rats. C, control; H, hyperbaric. Values are expressed as mean ± standard deviation (n = 5). a p < 0.05 compared with C and H of Wistar.

![Fig. 2.](image2.png) **Fig. 2.** Absolute (left) and relative (right) plantaris muscle weights of nondiabetic Wistar and diabetic Goto-Kakizaki rats. C, control; H, hyperbaric; BW, body weight. Values are expressed as mean ± standard deviation (n = 5). a p < 0.05 compared with C and H of Wistar, b p < 0.05 compared with C and H of Wistar and H of Goto-Kakizaki.
In the Wistar and Goto-Kakizaki rats, the fiber SDH activity was higher in the hyperbaric group than in the control group, regardless of fiber type (Fig. 4).

Discussion

Type 2 diabetes mellitus is associated with an impaired insulin-stimulated glucose uptake and disposal capacity, which is attributed to insulin resistance in skeletal muscles. Patients with type 2 diabetes mellitus are known to have disrupted metabolic potentials and different patterns of fiber types in skeletal muscles compared with nondiabetic subjects [1–3]. Similar results were obtained in animal models with type 2 diabetes mellitus [4, 5, 7]. Diabetes mellitus has been associated with a high percentage of low-oxidative fibers (particularly type IIB fibers) and a low percentage of high-oxidative fibers in skeletal muscles.

The increase in the pressure and concentration of oxygen by hyperbaric exposure with high oxygen concentration results in more oxygen being dissolved in the blood and plasma, making the oxygen available for diffusion into the tissues and offering an increased oxidative capacity in skeletal muscles [9, 16].

Fig. 3. Plasma insulin (left) and glucose (right) levels of nondiabetic Wistar and diabetic Goto-Kakizaki rats. C, control; H, hyperbaric. Values are expressed as mean ± standard deviation (n = 5). *p < 0.05 compared with C and H of Wistar and H of Goto-Kakizaki, **p < 0.05 compared with C and H of Wistar, †p < 0.05 compared with C of Wistar.

Fig. 4. Fiber type distributions (left) and succinate dehydrogenase activities (right) of the plantaris muscles of nondiabetic Wistar and diabetic Goto-Kakizaki rats. Values are expressed as mean ± standard deviation (n = 5). C, control; H, hyperbaric; SDH, succinate dehydrogenase; OD, optical density. *p < 0.05 compared with C and H of Wistar and H of Goto-Kakizaki, †p < 0.05 compared with C of Wistar, ‡p < 0.05 compared with H of Wistar, ††p < 0.05 compared with C of Wistar and Goto-Kakizaki.
An interesting finding in our previous studies [8, 17] was that the growth-related increase in the glucose level of diabetic rats was completely inhibited by hyperbaric exposure with high oxygen concentration. Furthermore, the insulin level of diabetic rats decreased after hyperbaric exposure with high oxygen concentration [8].

The increased availability of oxygen induced by hyperbaric exposure with high oxygen concentration would have a beneficial impact on the metabolism of skeletal muscles. Our previous study [8] observed that the fiber oxidative enzyme activity of the slow soleus muscle in Goto-Kakizaki rats increased after hyperbaric exposure with high oxygen concentration. In this study, the percentage of high-oxidative fibers in the fast plantaris muscle increased after hyperbaric exposure with high oxygen concentration. Therefore, no difference in the fiber type distribution was observed between the control Wistar and hyperbaric-exposed Goto-Kakizaki rats. Moreover, the fiber oxidative enzyme activity in the muscle increased after hyperbaric exposure with high oxygen concentration, regardless of fiber type. In summary, the present results combined with our previous findings [8, 17] strongly indicate that an increase in the oxidative capacity of skeletal muscles is an adaptive response to hyperbaric exposure with high oxygen concentration, which is related to an improvement in impaired skeletal muscle metabolism of diabetic rats, and shifts their glucose levels toward normal, which is observed in age-matched nondiabetic rats.

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