Hemodynamic response during hyperbaric treatment on skeletal muscle in a type 2 diabetes rat model

Natsuki GOTO1, Naoto FUJITA1, Wataru NINO1, Kazuyoshi HISATSUNE1, Ryosuke OCHI1, Hisao NISHIO2, and Susumu URAKAWA1

1 Department of Musculoskeletal Functional Research and Regeneration, Graduate School of Biomedicine and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan and 2 System Emotional Science, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan

(Received 13 November 2019; and accepted 28 November 2019)

ABSTRACT

Mild hyperbaric treatment prevents type 2 diabetes progression due to increased oxygen concentration and blood flow in skeletal muscle. However, it remains unknown whether this treatment is effective during all stages of type 2 diabetes. This study aimed to investigate the influences of hyperbaric treatment at 1.3 atmospheres absolute (ATA) on hemodynamic response in various stages of type 2 diabetes. Otsuka Long-Evans Tokushima fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO) rats were used as models of type 2 diabetes and healthy controls, respectively. Glucose levels were significantly higher in OLETF rats than in LETO rats at all ages. Glucose intolerance gradually increased with age in OLETF rats. Insulin levels in OLETF rats were significantly higher at 20-week-old, however, were significantly lower at 60-week-old than in LETO rats. Oxy-Hb, total Hb, and StO2 in skeletal muscle were increased during hyperbaric treatment in both rats. The hemodynamic changes were significantly higher in OLETF rats than LETO rats, and those changes were also pronounced at 8-week-old compared with other age in OLETF rats. These results suggest that hyperbaric treatment at 1.3 ATA acts on pathophysiological factors and the efficacy could be found only in the early stage of type 2 diabetes.

Hyperbaric oxygen therapy is usually performed at 2–3 atmospheres absolute (ATA) with 100% oxygen, which compresses blood gas and increases tissue oxygen. Tissue oxygenation by the hyperbaric oxygen treatment is effective for various disorders in which hypoxia and edema occur (22). For example, in terms of the disorder with hypoxia and edema, Horie et al. reported that hyperbaric oxygen therapy at 3 ATA with 100% oxygen enhances healing and functional recovery after skeletal muscle injury (8).

Healing of skeletal muscle injury is also reported when the intervention is performed at less than 2 ATA, known as “mild hyperbaric oxygen treatment” (5), suggesting that hyperbaric oxygen therapy at 3 ATA and mild hyperbaric treatment under 2 ATA have a common mechanism. The effectiveness of mild hyperbaric oxygen treatment has also been shown in metabolic diseases such as diabetes (11).

Mild hyperbaric oxygen treatment at 1.25 ATA with 36% oxygen for 4 weeks had a preventive effect on hyperglycemia in a rat model with type 2 diabetes (17, 23). The prevention of hyperglycemia was also shown in a rat model with metabolic syndrome (4, 20). Mild hyperbaric oxygen treatment could increase oxygen concentration and blood flow during the treatment (11), and reduction of hyperglycemia resulted from enhanced glucose metabolism in the skeletal muscle (4, 17, 20, 23). Although
effectiveness of mild hyperbaric oxygen treatment on diabetes was demonstrated by previous several studies (4, 17, 20, 23), only early stage diabetes was investigated. Therefore, it remains unknown whether hyperbaric oxygen treatment is effective during all stages of diabetes. Importantly, the pathological manifestations relating to disease progression are different for each stage of diabetes. It is well known that approaches to hyperglycemia management vary according to stage of disease; these include improving insulin resistance, promoting insulin secretion, and blocking glucose reabsorption (1). In addition to glucose metabolism, factors for vascular dysfunction in type 2 diabetes are different for each stage. Vascular dysfunction is induced mainly by declining vascular endothelial function and increased vasoconstrictors in early type 2 diabetes (2). With progression of hyperglycemia, the main factors shift to decreased vasodilators, which result in further degradation of vascular endothelial function (18, 19). Vascular dysfunction then induces microcirculatory disturbance that affects glucose metabolism in skeletal muscle (16). Therefore, appropriate treatment for vascular dysfunction associated with type 2 diabetes depends on the stage of disease. Indeed, previous studies have shown both positive (5, 8) and negative (6, 7) effects of hyperbaric treatment on healing of skeletal muscle injury, suggesting that the effect could depend on the pathology even though the disorders are same. There is a possibility that the influences of mild hyperbaric treatment differ for the stages of type 2 diabetes.

The purpose of this study was to investigate histological differences in capillaries within skeletal muscle and the influences of hyperbaric treatment at 1.3 ATA with normal air on hemodynamic response in various stages of type 2 diabetes. The present study might therefore be able to contribute directly to knowledge regarding the appropriate application of hyperbaric oxygen treatment for each stage of type 2 diabetes.

MATERIALS AND METHODS

Experimental design. This study was approved by the International Animal Care and Use Committee of Hiroshima University (A16-5) and was carried out according to the Hiroshima University Regulations for Animal Experimentation. All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Otsuka Long-Evans Tokushima fatty (OLETF, \(n = 24\)) and Long-Evans Tokushima Otsuka (LETO, \(n = 22\)) rats were used as models of type 2 diabetes and healthy controls, respectively. The analysis was performed at 8 (OLETF \(n = 6\), LETO \(n = 6\)), 20 (OLETF \(n = 6\), LETO \(n = 5\)), 30 (OLETF \(n = 6\), LETO \(n = 6\)), and 60-week-old (OLETF \(n = 6\), LETO \(n = 5\)). The animals were housed in a controlled room with a fixed 12 h light and dark cycle and a constant temperature of \(22 \pm 2^\circ C\). Food and water were provided without restriction.

Hemodynamic response during hyperbaric treatment in the calf muscle. Hemodynamic responses during hyperbaric treatment in the calf muscle were measured using near-infrared spectroscopy (NIRS; BOM-L1TRSF, Omegawave, Tokyo, Japan). The rats were restrained with a plastic holder under mild anesthesia with an intraperitoneal injection of sodium pentobarbital (25 mg/kg). The NIRS probe was placed over the muscle belly in the gastrocnemius lateralis muscle, and the hemodynamics were measured in the calf muscle 3–5 mm beneath the probe. Forty min after the light anesthesia was administered, measurement was started after body movements had cleared. Oxygenated hemoglobin (Oxy-Hb) and deoxygenated hemoglobin (Deoxy-Hb) were measured at 1.0 ATA (1013.25 hPa) for 5 min and then at 1.3 ATA (1317.225 hPa) with normal air for 10 min. Total hemoglobin (Total-Hb) defined as blood flow was calculated from the sum of Oxy-Hb and Deoxy-Hb. Oxygen saturation (StO2) was calculated from the percentage of Oxy-Hb in Total-Hb. For Oxy-Hb, Deoxy-Hb, Total-Hb, and StO2, the changes from 1.0 ATA to 1.3 ATA in each rat were calculated by the mean value at 1.3 ATA relative to the mean value at 1.0 ATA. Interference caused by urination and body movement was excluded from the measurement. Sampling frequency was 100 Hz.

Glucose and insulin levels. Oral glucose tolerance test (OGTT) was performed to determine the glucose tolerance of the rats at 48 h after the measurement of the hemodynamic response. The rats were fasted for 12 h and blood samples were obtained from the lateral caudal vein before and at 30, 60, and 120 min after glucose administration. Glucose (2 g/kg body weight) was administered via an esophageal feeding tube. The blood samples were centrifuged at 3000 rpm for 10 min at room temperature and the plasma was stored at \(-80^\circ C\) until analysis. Glucose concentration was measured with a mutant Q–GDH enzyme-based method (ACCU-CHEK ST meter; Roche, Tokyo, Japan). Insulin concentration
was measured with enzyme-linked immunosorbent assay kits (M1101; Morinaga, Yokohama, Japan) according to the manufacturer’s instruction. Area under the curve (AUC) was calculated for glucose and insulin levels during the OGTT.

**Histological analysis of the capillaries within the gastrocnemius lateralis muscle.** At 48 h after the OGTT, the animals were fasted for 12 h and euthanized with an overdose of sodium pentobarbital. The gastrocnemius lateralis muscles were immediately removed, frozen in liquid nitrogen, and stored at −80°C. Using cryostat, transverse sections with a thickness of 10 μm were obtained and mounted on amino-silane-coated slides. The sections were stained for alkaline phosphatase activity in order to visualize the capillaries within the skeletal muscle, then incubated for 45 min at 23°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. The cross-sectional area of muscle fiber and the number of capillaries around the muscle fiber were measured; quantifications were performed using ImageJ (NIH, Bethesda, MD, USA). Two random fields were chosen in the superficial layer of the muscle and >100 muscle fibers were used in the measurements.

**Statistical analysis.** Data are expressed as means ± standard deviation. The main effect of animal strain, week-old, and interaction between animal strain and week-old were evaluated using two-way analysis of variance (ANOVA). In the glucose and insulin level during OGTT, the main effect of animal strain, time after glucose administration, and interaction between animal strain and time after glucose administration were evaluated using two-way ANOVA. Significant differences were analyzed using two-way ANOVA followed by Bonferroni post-hoc test. The correlation was evaluated using Spearman’s rank correlation coefficient. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical analysis software (IBM SPSS Statistics version 19.0; IBM Japan, Tokyo, Japan).

**RESULTS**

**Body weight**

Body weight significantly increased in LETO rats throughout the experimental period (Fig. 1). At 8, 20, and 30-week-old, the body weight was significantly higher in OLETF rats than in LETO rats. Conversely, the body weight was significantly lower in OLETF rats than in LETO rats at 60-week-old. Although the food intake was higher in OLETF rats than in LETO rats during the experimental period (data not shown), the body weights of OLETF rats gradually decreased after 30-week-old.

**Glucose and insulin levels**

Changes of glucose level during OGTT in LETO rats were similar among all ages. The glucose level was slightly increased at 30 and 60 min after glucose administration; at 120 min, the value was almost the same as fasting (Fig. 2a–d). Although the glucose level at fasting in LETO rats was slightly increased with aging (Fig. 2e), there was no significant difference in the glucose AUC for LETO rats of 8, 20, 30, and 60-week-old (Fig. 2f). Conversely, there were noticeable changes in glucose level during OGTT in OLETF rats with aging. The glucose level during OGTT was significantly higher in OLETF rats than in LETO rats at all ages (Fig. 2a–d). The glucose level at fasting in OLETF rats was increased with age (Fig. 2e). Also, the glucose AUC in OLETF rats was significantly increased with age (Fig. 2f). The relation between glucose (Fig. 2) and insulin (Fig. 3) levels at each age are shown below.

At 8-week-old, the glucose levels were significantly higher in OLETF rats than in LETO rats at fasting and at 30 and 60 min after glucose administration.
OLETF rats was significantly higher for 20-week-old rats than those for 8-week-old (Fig. 2f). At 20-week-old, in contrast with 8-week-old, the insulin levels were significantly higher in OLETF rats than in LETO rats at fasting and at 30, 60, and 120 min after glucose administration (Fig. 3b). The insulin level at fasting and the insulin AUC in OLETF rats was significantly higher for 20-week-old rats than those for 8-week-old (Fig. 2f). At 20-week-old, in contrast with 8-week-old, the insulin levels were significantly higher in OLETF rats than in LETO rats at fasting and at 30, 60, and 120 min after glucose administration (Fig. 3b). The insulin level at fasting and the insulin AUC in OLETF rats was significantly higher in 20-week-old than in 8-week-old (Fig. 3e and 3f).

At 30-week-old, consecutively, the glucose levels were significantly higher in OLETF rats than in LETO rats at fasting and at all times after glucose administration (Fig. 2b). The glucose AUC in OLETF rats was significantly higher for 20-week-old rats than those for 8-week-old (Fig. 2f). At 20-week-old, in contrast with 8-week-old, the insulin levels were significantly higher in OLETF rats than in LETO rats at fasting and at 30, 60, and 120 min after glucose administration (Fig. 3b). The insulin level at fasting and the insulin AUC in OLETF rats was significantly higher in 20-week-old than in 8-week-old (Fig. 3e and 3f).

At 30-week-old, consecutively, the glucose levels were significantly higher in OLETF rats than in
LETO rats at fasting and at all times after glucose administration (Fig. 2c). However, in contrast with the 20-week-old values, there were no significant differences in the insulin levels during OGTT between LETO and OLETF rats (Fig. 3c). In fact, there were two subgroups in OLETF rats at 30-week-old: although higher insulin levels were shown for four in OLETF rats at 30-week-old, lower insulin levels were shown for two. The mean values of the glucose AUC and insulin AUC were 49313 ± 3004 and 601 ± 203 in the subgroup with the higher insulin level, respectively. There were 57743 ± 286 and 188 ± 108 in the subgroup that had the lower insulin level, respectively.

At 60-week-old, consecutively, the glucose levels were significantly higher in OLETF rats than in LETO rats at fasting and at all times after glucose administration (Fig. 2d). The glucose level at fasting and the glucose AUC in OLETF rats were largest at 60-week-old (Fig. 2e and 2f). The insulin levels were significantly lower in OLETF rats than in LETO rats at fasting and at 30 and 60 min after glucose administration (Fig. 2c). However, in contrast with the 20-week-old values, there were no significant differences in the insulin levels during OGTT between LETO and OLETF rats (Fig. 3c). In fact, there were two subgroups in OLETF rats at 30-week-old: although higher insulin levels were shown for four in OLETF rats at 30-week-old, lower insulin levels were shown for two. The mean values of the glucose AUC and insulin AUC were 49313 ± 3004 and 601 ± 203 in the subgroup with the higher insulin level, respectively. There were 57743 ± 286 and 188 ± 108 in the subgroup that had the lower insulin level, respectively.

**Fig. 3** Insulin level during OGTT at 8 (a), 20 (b), 30 (c), and 60 (d)-week-old, and the changes of fasting insulin level (e), and insulin AUC (f). LETO: healthy controls; OLETF: type 2 diabetes. Time 0 means fasting. The values represent means ± standard deviation. S, T, and S×T denote significant main effects of animal strain, time after glucose administration, and interaction between animal strain and time after glucose administration, respectively. S, W, and S×W denote significant main effects of animal strain, week-old, and interaction between animal strain and week-old, respectively. * is significantly different from LETO rats at the same time after glucose administration (a–d) and for the same week-old (e and f), P < 0.05.
ly, strong and long signals were often observed especially in OLETF rats. The mean number of capillaries around the muscle fiber was $4.7 \pm 0.11$, $5.3 \pm 0.38$, $5.6 \pm 0.53$, and $6.0 \pm 0.69$ in LETO rats at 8, 20, 30, and 60-week-old, respectively. The values in LETO rats gradually increased with age. The value in OLETF rats was $4.3 \pm 0.06$, $5.2 \pm 0.70$, $5.5 \pm 0.51$, and $5.1 \pm 0.27$ at 8, 20, 30, and 60-week-old, respectively. The value was significantly decreased in OLETF rats than in LETO rats at all ages and the differences were most remarkable at administration (Fig. 3d). The insulin level at fasting and the insulin AUC in OLETF rats were significantly lower at 60-week-old than at 20-week-old (Fig. 3e and 3f).

**Histological analysis for the capillaries within the gastrocnemius lateralis muscle**

The representative figures in LETO and OLETF rats at all ages were shown in Fig. 4. Dot-like positive signals that show capillaries were found around muscle fiber in LETO and OLETF rats. Additional-
Effects of hyperbaric treatment for diabetes

Histological analysis also revealed muscle atrophy in OLETF rats at 60-week-old (Fig. 4). The mean cross-sectional area of muscle fiber was $3151 \pm 284$, $4783 \pm 178$, $4537 \pm 375$, and $5019 \pm 295$ in LETO rats at 8, 20, 30, and 60-week-old, respectively. The values in LETO rats gradually increased with age. In OLETF rats, the mean cross-sectional area of muscle fiber was $3284 \pm 206$, $4476 \pm 339$, $4283 \pm 143$, and $3268 \pm 269$ at 8, 20, 30, and 60-week-old, respectively. Although these values in OLETF rats increased at 20 and 30-week-old comparing to 8-week-old, the values were significantly lower at 60-week-old than 20 and 30-week-old. Additionally, the values at 60-week-old were significantly lower in OLETF rats than in LETO rats.

Hemodynamic response

The changes of Oxy-Hb, Deoxy-Hb, Total-Hb, and StO$_2$ from 1.0 ATA to 1.3 ATA were showed in Fig. 5. The hyperbaric treatment increased Oxy-Hb, Total-Hb, and StO$_2$ while decreased Deoxy-Hb in both
and OLETF rats. The changes of Oxy-Hb and Total-Hb were significantly higher in OLETF rats than in LETO rats at all ages (Fig. 5e–h). The changes were notable in OLETF rats at 8-week-old compared with 20, 30, and 60-week-old. There was no significant difference in the change of Deoxy-Hb among animal strains at any weeks old. There was a negative correlation between the changes of Oxy-Hb and the glucose AUC only in OLETF rats (Fig. 6).

DISCUSSION

The present study revealed that tissue oxygenation and increased blood flow in the rat calf muscle are caused during hyperbaric treatment at 1.3 ATA with normal air in both LETO and OLETF rats. The hemodynamic responses were remarkable in OLETF rats compared with LETO rats. The influences of this intervention were also remarkable at the early stage compared with the progressed stage of type 2 diabetes.

OLETF rats are known to be an animal model that develops diabetes spontaneously due to hyperphagia (14). Obesity with hyperphagia induces insulin resistance, which is usually noticeable in OLETF rats after 20-week-old. The insulin resistance and insulin hypersecretion cause degenerative changes in the pancreatic islet, and then insulin secretion decreases in OLETF rats usually after 40-week-old (13). The hypoinsulinemia caused by the degenerated islet results in more severe hyperglycemia and weight loss in OLETF rats at end stage of type 2 diabetes (3). In the present study, OLETF rats demonstrated hyperglycemia after glucose administration without hyperinsulinemia at 8-week-old. Both hyperglycemia and hyperinsulinemia were observed in OLETF rats at 20-week-old, suggesting that 8-week-old and 20-week-old rats are at onset stage and early stage of type 2 diabetes, respectively. We observed 2 subgroups that demonstrated higher insulin levels or lower insulin levels in OLETF rats at 30-week-old. The subgroup that shows lower insulin levels also demonstrated weight loss. The results suggest that the stage of type 2 diabetes in the subgroup with lower insulin level is more severe compared with the subgroup with higher insulin level. Subsequently, severe hyperglycemia, hypoinsulinemia, and weight loss with skeletal muscle atrophy were observed in OLETF rats at 60-week-old, suggesting to correspond to end stage of type 2 diabetes. In the present study, OLETF rats at 8, 20, 30, and 60-week-old are defined as onset, early, progressive, and end stage of type 2 diabetes, respectively.

Hyperbaric treatment at 1.3 ATA with normal air increased Oxy-Hb, Total-Hb, and StO₂ in both LETO and OLETF rats. However, the increases of Oxy-Hb and Total-Hb were significantly higher in OLETF rats than in LETO rats. Insulin resistance and hyperinsulinemia induce vessel wall thickening, and this thickness is found in OLETF rats at 30-week-old (9, 12). Insulin resistance of vascular endothelial cells and subsequent vessel wall thickening cause dysregulation for blood vessel diameter and substance exchange (2, 24). The dysfunction of endothelial vasodilation also impairs blood flow response in type 2 diabetes (15). The present study revealed that the number of capillaries around the muscle fiber in the gastrocnemius was significantly lower in OLETF rats than in LETO rats at all ages, implying the endothelial dysfunction in the skeletal muscle of OLETF rats. The remarkable hemodynamic responses during hyperbaric treatment at 1.3 ATA with normal air in OLETF rats could be caused by temporary
improvement of the impaired blood flow response in the skeletal muscle with the endothelial dysfunction. In other words, the hyperbaric treatment could have little influence on LETO rats without impaired blood flow response. A previous study reported benefit of hyperbaric oxygen therapy on incisional wounds, especially in ischemia (21), suggesting that hyperbaric treatment affects pathophysiological factors such as endothelial dysfunction and ischemia in OLETF rats. Additionally, the increases of Oxy-Hb and Total-Hb during hyperbaric treatment were remarkable in OLETF rats at 8-week-old compared with 20, 30, and 60-week-old. The glucose AUC gradually increased with age in OLETF rats. The negative correlation was found between the glucose AUC and the changes of Oxy-Hb during the treatment in OLETF rats. In addition, the skeletal muscle atrophy and severe decreasing of capillary numbers were shown in OLETF rats at 60-week-old. These results suggest that the hemodynamic response during hyperbaric treatment at 1.3 ATA with normal air is remarkable at the onset and early stages of type 2 diabetes but inhibited in later stages of disease. The effects of hyperbaric treatment depend on the severity of insulin resistance, endothelial dysfunction, and capillary distribution in skeletal muscle.

In the present study, blood flow at 1.3 ATA was approximately 1.2 times higher compared with 1.0 ATA. Ishihara et al. reported that mild hyperbaric oxygen treatment at 1.25 ATA with 36% oxygen doubles blood flow (10). The difference in results between our study and the previous study by Ishihara et al. could be due to factors such as differences in subjects, oxygen concentration, exposure time, and methodology for measuring hemodynamic response. Additionally, changes in heart rate, respiratory rate, and dissolved oxygen during hyperbaric treatment were not measured in the present study. We speculate that the change of dissolved oxygen is also different between healthy control and diabetes, and between the stages of disease progression. Further research adding measurement of physiological signs is needed to ascertain the true differences among studies that investigate hyperbaric treatment for type 2 diabetes.

In conclusion, mild hyperbaric treatment increased skeletal muscle hemodynamics in both models of normal and type 2 diabetes. The responses were remarkable in type 2 diabetes, especially at the early stage compared with the progressed stage. These results suggest that mild hyperbaric treatment could be prevention for hyperglycemia and hyperinsulinemia in early type 2 diabetes.

Acknowledgments
This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, and Technology [16K01505, 19K11346]. This work was carried out at the Analysis Center of Life Science, Natural Science for Basic Research and Development, Hiroshima University. We would like to thank the staff members of the institution for technical support.

REFERENCES

1. American Diabetes Association (2019) Pharmacologic approaches to glycemic control. Diabetes Care 42, 90–102.
2. Bender SB, Newcomer SC and Harold Laughlin MH (2011) Differential vulnerability of skeletal muscle feed arteries to dysfunction in insulin resistance: Impact of fiber type and daily activity. Am J Physiol - Heart Circ Physiol 300, 1434–1441.
3. Fujita N, Aono S, Karasaki K, Sera F, Kurose T, Fujino H and Urakawa S (2018) Changes in lipid metabolism and capillary density of the skeletal muscle following low-intensity exercise training in a rat model of obesity with hyperinsulinemia. PLoS One 13, e0196895.
4. Fujita N, Nagatomo F, Murakami S, Kondo H, Ishihara A and Fujino H (2012) Effects of hyperbaric oxygen on metabolic capacity of the skeletal muscle in type 2 diabetic rats with obesity. Sci World J 2012, 637978.
5. Fujita N, Omo M, Tomioka T and Deie M (2014) Effects of hyperbaric oxygen at 1.25 atmospheres absolute with normal air on macrophage number and infiltration during rat skeletal muscle regeneration. PLoS One 9, e115685.
6. Germain G, Delaney J, Moore G,Lee P, Lacroix V and Montgomery D (2003) Effect of hyperbaric oxygen therapy on exercise-induced muscle soreness. Undersea Hyperb Med 30, 135–145.
7. Harrison BC, Robinson D, Davison BJ, Foley B, Seda E and Byrnes WC (2001) Treatment of exercise-induced muscle injury via hyperbaric oxygen therapy. Med Sci Sport Exerc 33, 36–42.
8. Horie M, Enomoto M, Shimoda M, Okawa A, Miyakawa S and Yagishita K (2014) Enhancement of satellite cell differentiation and functional recovery in injured skeletal muscle by hyperbaric oxygen treatment. J Appl Physiol 116, 149–155.
9. Hosomi N, Noma T, Ohyama H, Takahashi T and Kohno M (2002) Vascular proliferation and transforming growth factor-β expression in pre- and early stage of diabetes mellitus in Otsuka Long-Evans Tokushima fatty rats. Atherosclerosis 162, 69–76.
10. Ishihara A, Nagatomo F, Fujino H and Kondo H (2014) Exposure to mild hyperbaric oxygen increases blood flow and resting energy expenditure but not oxidative stress. J Sci Res Reports 3, 1886–1896.
11. Ishihara A (2019) Mild hyperbaric oxygen: mechanisms and effects. J Physiol Sci 69, 573–580.
12. Jenkins NT, Padilla J, Martin JS, Crissey JM, Thyfault JP, Rector RS and Laughlin MH (2014) Differential vasomotor effects of insulin on gastrocnemius and soleus feed arteries in the OLETF rat model: role of endothelin-1. Exp Physiol 99, 262–271.
13. Katsuda Y, Ohta T, Miyajima K, Kemmochi Y, Sasase T, Tong B, Shinhara M and Yamada T (2014) Diabetic complications in obese type 2 diabetic rat models. Exp Anim 63, 121–132.

14. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M and Natori T (1992) Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes 41, 1422–1428.

15. Kingwell BA, Formosa M, Muhlmann M, Bradley SJ and McConell GK (2003) Type 2 diabetic individuals have impaired leg blood flow responses to exercise: Role of endothelium-dependent vasodilation. Diabetes Care 26, 899–904.

16. Liu J and Liu Z (2019) Muscle insulin resistance and the inflamed microvasculature. Int J Mol Sci 20, E562.

17. Matsumoto A, Nagatomo F, Yasuda K, Tsuda K and Ishihara A (2007) Hyperbaric exposure with high oxygen concentration improves altered fiber types in the plantaris muscle of diabetic Goto-Kakizaki rats. J Physiol Sci 57, 133–136.

18. Mikus CR, Rector RS, Arce-Esquivel AA, Libla JL, Booth FW, Ibdah JA, Laughlin MH and Thyfault JP (2010) Daily physical activity enhances reactivity to insulin in skeletal muscle arterioles of hyperphagic Otsuka Long-Evans Tokushima Fatty rats. J Appl Physiol 109, 1203–1210.

19. Mikus CR, Roseguini BT, Uptergrove GM, Morris EM, Rector S, Libla JL, Oberlin DJ, Borengasser SJ, Taylor M, Ibdah JA, Laughlin MH and Thyfault JP (2013) Voluntary wheel running augments insulin-stimulated vasodilation in arterioles from white skeletal muscle of insulin resistant rats. Microcirculation 19, 729–738.

20. Nagatomo F, Takemura A, Roy RR, Fujino H, Kondo H and Ishihara A (2018) Mild hyperbaric oxygen inhibits the growth-related decline in skeletal muscle oxidative capacity and prevents hyperglycemia in rats with type 2 diabetes mellitus. J Diabetes 10, 753–763.

21. Quirinia A and Viidik A (1996) The impact of ischemia on wound healing is increased in old age but can be countered by hyperbaric oxygen therapy. Mech Ageing Dev 91, 131–144.

22. Tibbles PM and Edelsberg JS (1996) Hyperbaric-oxygen therapy. N Engl J Med 5, 1642–1648.

23. Yasuda K, Adachi T, Gu N, Matsumoto A, Matsunaga T, Tsujimoto G, Tsuda K and Ishihara A (2007) Effects of hyperbaric exposure with high oxygen concentration on glucose and insulin levels and skeletal muscle-fiber properties in diabetic rats. Muscle Nerve 35, 337–343.

24. Zhong MF, Shen WL, Tabuchi M, Nakamura K, Chen YC, Qiao CZ, He J, Yang J, Zhang C, Kamenov Z, Higashino H and Chen H (2012) Differential changes of aorta and carotid vasodilation in type 2 diabetic GK and OLETF rats: Paradoxical roles of hyperglycemia and insulin. Exp Diabetes Res 2012, 429020.