Int J Clin Exp Pathol 2010;3(2):177-188
www.ijcep.com /IJCEP911003

Original Article

Oxidative stress and gene expression of antioxidant enzymes in the streptozotocin-induced diabetic rats under hyperbaric oxygen exposure

Tokio Matsunami, Yukita Sato, Takuya Sato, Satomi Ariga, Toshiko Shimomura, Masayoshi Yukawa

Laboratory of Biomedical Science, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Fujisawa 252-8510, Japan.

Received November 17, 2009; accepted November 25, 2009; available online November 30, 2009

Abstract: Diabetes mellitus (DM) causes not only hyperglycemia but oxidative stress, resulting mainly enhanced production of mitochondrial reactive oxygen species (ROS). Hyperbaric oxygen (HBO) treatments are applied various diseases including diabetic patients with unhealing foot ulcers, however, and also increases the formation of ROS. Recently, it has been reported that oxidative stress worsens many pathological conditions including DM and obesity suggesting possible changes in regulation of genes associated with the oxidative stress, however, effects of HBO which could induce ROS on the gene expressions of oxidative stress parameters in DM animals are unknown. The purpose of this study is to investigate the effect of HBO exposure on the gene expression of three important antioxidant enzymes, cytosolic superoxide dismutase (Cu-Zn SOD), cytosolic glutathione peroxidase (GPx-1), and catalase (CAT) in DM rats, respectively. We used streptozotocin-induced DM model rats and examined both mRNA expressions and the activities of these antioxidant enzymes in the liver, skeletal muscle, and pancreas. The mRNA expressions of Cu-Zn SOD and CAT decreased significantly ($p < 0.001$), and GPx increased significantly ($p < 0.001$) in all the studied organs of DM rats under HBO exposure compared to those from DM-induced rats not exposed to HBO. Similarly, activities of these three enzymes changed in accordance with the mRNA levels. These results suggested that DM induction and HBO exposure might synergistically affect antioxidant enzymes, resulting increase of oxidative stress state. Thus, HBO exposure seems to be an excellent model system for investigating oxidative stress.

Key words: Diabetes mellitus, hyperbaric oxygen, oxidative stress, superoxide dismutase, glutathione peroxidase, catalase

Introduction

Hyperbaric oxygen (HBO) therapy, as defined by the Undersea and Hyperbaric Medical Society (UHMS), comprises the intermittent inhalation of 100% oxygen under a pressure greater than one atmosphere absolute (ATA). There are various well-established therapeutic uses of HBO which have been applied successfully for the treatment of 13 different illnesses, such as carbon monoxide poisoning, decompression sickness, osteomyelitis, and even diabetic foot [1]. However, it is known that exposure to oxygen at high ambient pressure can cause damage to mammalian cells. It has been suggested that the detrimental effects of exposure to high concentrations of oxygen can lead to an abundance of reactive oxygen species (ROS) [2]. In addition, it has been demonstrated that HBO also increases the formation of ROS, which are known to cause cellular damage through the oxidation of lipid, protein, and DNA [3]. In situations where a body's anti-oxidant defenses are inadequate, increased free radical formation is likely to increase the damage. This situation is generally termed "oxidative stress".

The oxidative effects of HBO have been investigated in animals and humans [4-6]. One study in rats revealed that, after 2 h of HBO exposure at 3 ATA, elevated levels of the oxidative stress markers, thiobarbituric acid

reactive substances (TBARS) and total superoxide dismutases (SOD), were found in the lung, brain and erythrocytes [5], and glutathione peroxidase (GPx) and nitrate/nitrite (NOx) activities were found to be elevated in the brain [6]. Thus, HBO exposure seems to be an excellent model system for investigating oxidative stress.

Oxidative stress occurs in some illnesses without exposure to HBO treatment. For example, persistent hyperglycemia caused through diabetes induces ROS production by glucose autoxidation [7, 8], activation of protein kinase C, and increase flux through the hexosamine pathway [9]. Oxidative stress has been also associated with diabetic status in animals and humans [10-14]. One of these studies using streptozotocin (STZ)-induced diabetic rats, a recognized model of type 1 diabetes mellitus (T1DM), showed that levels of lipid peroxidation had increased, as indicated by TBARS, an oxidative stress marker [10]. Oxidative stress-related changes occurred in the mRNA and protein expressions of Cu-Zn SOD and CAT in STZ-induced diabetic rat liver tissues [14]. Thus, pathological conditions of diabetes mellitus could be a model for studying oxidative stress.

HBO therapy is used in the treatment of diabetic patients with unhealing foot ulcers [1]. However, the side-effects of HBO treatment in diabetic patients and animals have been poorly investigated. It has been shown that the levels of TBARS and advanced oxidation protein products (AOPPs) increased in diabetic patients who received HBO therapy for diabetic foot ulcers [15]. In our previous study, a clinically-recommended HBO treatment significantly increased TBARS levels and decreased SOD activity, clearly demonstrating that HBO causes oxidative stress in the T1DM state [16]. However, to our knowledge, there is no study with regard to the effects of HBO on the gene expressions of oxidative stress parameters in diabetic animals. In this animal study, we compared the expression of the genes for the three important antioxidant enzymes, Cu-Zn SOD, GPx, and CAT of liver, skeletal muscle, and pancreas between non-diabetic and diabetic rats under HBO exposure.

Materials and methods

Animals and animal welfare policy

Twenty adult, 8-week-old, male Wistar rats (weight range, 240–250 g), bred in our laboratory, were used for the experiment. They were housed at 23 to 25 °C with light from 7:00 AM to 7:00 PM and free access to water at all times. All rats were fed a commercial diet during the experiment. All study procedures were implemented in accordance with the Institutional Guidelines for Animal Experiments at the College of Bioresource Sciences, Nihon University under the permission of the Committee of Experimental Animal in our college.

Study design

The rats were allowed to acclimatize for 1 week prior to treatment, and then at the start of the experiment they were randomly divided into four groups, 5 rats per group. The groups were as follows: non-diabetic induction and non-HBO group (Control), non-diabetic induction and HBO group (HBO), diabetic induction and non-HBO group (DM), and diabetic induction and HBO group (DM + HBO). In this study, diabetes developed on the third day after diabetic induction, and then rats in the DM + HBO group were exposed to HBO once daily for 7 days. Rats in the HBO group were also exposed to HBO once daily for 7 days. The mean body weight of the animals in all groups was measured at the start and end of the study period.

Induction of diabetes

In two groups (DM and DM + HBO), diabetes was induced by a single intraperitoneal (i.p.) injection of Streptozotocin (STZ: 40 mg/kg body weight) dissolved in 0.05 M citrate buffer (pH 4.5), as described previously [17]. Control and HBO groups were treated with equal volume and concentration of STZ injection vehicle, citrate buffer. Diabetes was confirmed in the STZ-induced rats by measuring the fasting plasma glucose concentration 48 h post-injection. After an overnight fast, blood was obtained from the tail vein, centrifuged at 3,000 × g for 5 min, and then the plasma was collected. Fasting plasma glucose and insulin concentrations were measured every day by a commercial kit, the GLU-P Ⅲ (FUJIFILM, Tokyo, Japan) and Rat Insulin ELISA KIT; AKRIN-130 (Shibayagi, Gunma, Japan), respectively.

Diabetes was considered to have been induced when the blood glucose level reached at least 14 mmol/l [18].

HBO exposure

For all experiments, HBO was applied at a clinically-used pressure of 2.4 ATA for 90 min, once daily for 7 days, in a hyperbaric chamber for small animals (Nakamura Tekkosho, Tokyo, Japan). The ventilation rate was 4–5 L/min. Each exposure was started at the same hour in the morning (10 AM) to exclude any confounding issues associated with changes in biological rhythm.

Tissue preparation

After the final HBO exposure, all rats were weighed, anesthetized with sodium pentobarbital (40 mg/kg i.p.), and decapitated. Liver, skeletal muscle and pancreas were measured, and liver, skeletal muscle, and pancreas samples were collected in either liquid nitrogen for analysis of antioxidant defense enzymes and lipid peroxidation, or RNAlater solution (Qiagen, Hilden, Germany) for molecular, and stored at −80°C until analysis.

Liver, skeletal muscle, and pancreas homogenates were prepared as a 1:10 (w:v) dilution in pH 7.4, 10 mM potassium phosphate buffer using a Ultra-Turrax® (IKA® Japan, Nara, Japan). Samples were centrifuged at 3000 rpm for 10 min at 4°C, and the supernatants were collected and immediately assayed for enzyme activities. Blood samples were collected by cardiac puncture and divided into heparinised tubes, and separated into plasma and erythrocytes by centrifugation at 10,000 × g for 10 min at 4°C. The erythrocyte samples were washed three times with cold physiological saline (PS) and then hemolyzed by adding a fourfold volume of ice-cold HPLC-grade water. Hemolyzed erythrocyte samples were centrifuged at 10,000 × g for 10 min at 4°C, and the supernatants were sampled for determination of Cu-Zn SOD, GPx, and CAT activity, and TBARS levels.

Biochemical analysis

Plasma glucose and insulin concentrations were measured using a commercial kit as stated above. Lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the method of Ohkawa *et al*. [19]. The activities of antioxidant enzymes such as Cu-Zn SOD [20], GPx [21], and CAT [22] were assayed.

Total RNA isolation and RT-PCR

RNA isolation was performed by homogenization with a Micro Smash-100R™ (TOMY SEIKO, Tokyo, Japan) using Isogen (NIPPON GENE, Tokyo, Japan) for all organs. RNA purification was carried out using the RNeasy Mini kit (Qiagen, Hilden, Germany) in all organs. All samples were treated with DNase I (RNase Free DNase set, Qiagen). The concentrations of total RNA were measured by absorbance at 260 nm using a NanoDrop® ND-1000 (NanoDrop, USA). The purity was estimated by the 260/280 nm absorbance ratio. Reverse transcription of 1 μg total RNA was subjected to reverse transcription using an oligo(dT)$_{12\text{-}18}$ primer and M-MuLV reverse transcriptase (SuperScript™ Ⅲ First-Strand Synthesis, Invitrogen Life Science, USA) according to the manufacturer's instructions. Multiplex PCR was performed to quantify the expression of mRNA for antioxidant enzymes (Cu-Zn SOD, GPx and CAT) relative to *β*-actin mRNA as an internal control. The oligonucleotide primer pairs used for multiplex PCR are shown in **Table 1**. One microliter of cDNA mixture was amplified in a 25 μl of PCR reaction mixture containing 10× PCR reaction buffer, 25 mM $MgCl_2$, 2.5 mM dNTPmix, 10 μM of each primer, and 5.0 Unit Ex Taq® polymerase (TAKARA BIO INC, Shiga, Japan). cDNA mixture was amplified in a PCR reaction, which was carried out by using GeneAmp® PCR system 9700 (Applied Biosystems, CA, USA) and involved the following steps: initial denaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 58°C for Cu-Zn SOD and GPx, and 60°C for CAT for 30 s, extension at 72°C for 45 s (28 cycle), and final extension at 72°C for 5 min. After amplification, PCR products were run on 2% agarose gels, and the intensity of each band was measured with Image J software [23]. The levels of Cu-Zn SOD mRNA, GPx mRNA, and CAT mRNA were determined by calculating the intensity ratio of Cu-Zn SOD mRNA/ *β*-actin mRNA, GPx mRNA/ *β*-actin mRNA, and CAT mRNA/ *β*-actin mRNA.

Table 1. Primer sequences and expected product size for antioxidant enzymes and internal standard *β*-actin

cDNA	Accession No.	Forward primer	Reverse primer	Product size
β-actin	V01217	5'-CCTGCTTGCTGATCCACA	5'-CTGACCGAGCGTGGCTAC	505 bp
Cu-Zn SOD	X05634	5'-GCAGAAGGCAAGCGGTGAAC	5'-TAGCAGGACAGCAGATGAGT	387 bp
GPx	NM_030826	5'-CTCTCCGCGGTGGCACAGT	5'-CCACCACCGGGTCGGACATAC	290 bp
CAT	AH004967	5'-GCGAATGGAGAGGCAGTGTAC	5'-GAGTGACGTTGTCTTCATTAGCACTG	670 bp

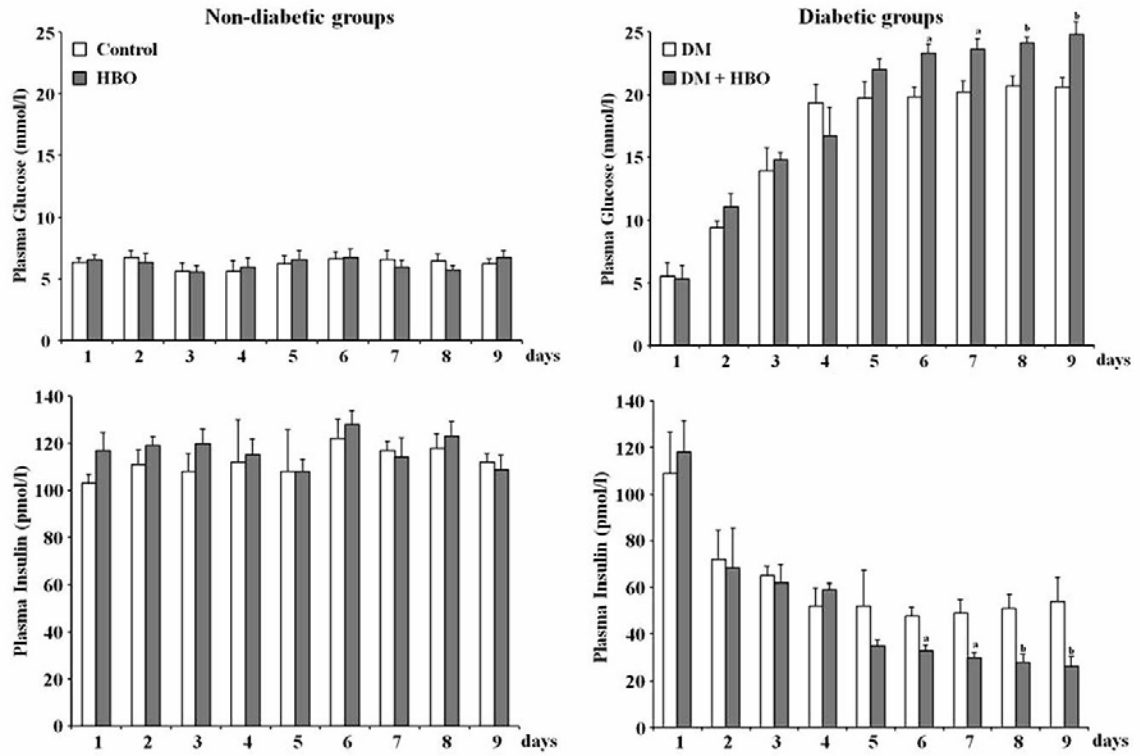

Figure 1: Plasma glucose and insulin concentrations in non-diabetic groups and diabetic groups. Values are expressed as mean ± SEM (n = 6). [a] $p < 0.05$; [b] $p < 0.01$ compared with control value.

Statistical analysis

The results are expressed as means ± standard error of mean (SEM). An unpaired *t*-test with a *P* value of < 0.05 was used to determine statistical significance.

Results

The baseline body weight of the rats at the beginning of the study was similar in all groups. At the end of the treatment, there was no difference in body weight between control and HBO rats (mean group weights ranged from 250 to 265 g). However diabetic rats presented with weight loss, and the body weight of DM + HBO rats (220 ± 12 g; mean ± SEM.), in particular, decreased significantly ($p < 0.05$), when compared with the rats in the other 3 groups (control, 260 ± 5 g; HBO, 257 ± 7 g; DM, 241 ± 8 g).

Hyperglycemia occurred within 3 days of STZ administration. Fasting plasma glucose concentrations of the DM + HBO group were significantly higher at 6, 7, 8, and 9 days after diabetes induction ($p < 0.01$ - 0.05) compared with the DM group (**Figure 1**). Moreover, fasting plasma insulin concentrations of the DM + HBO group were significantly decreased compared with DM group (**Figure 1**). No significant differences were observed between any other groups (control and HBO groups) or experimental periods.

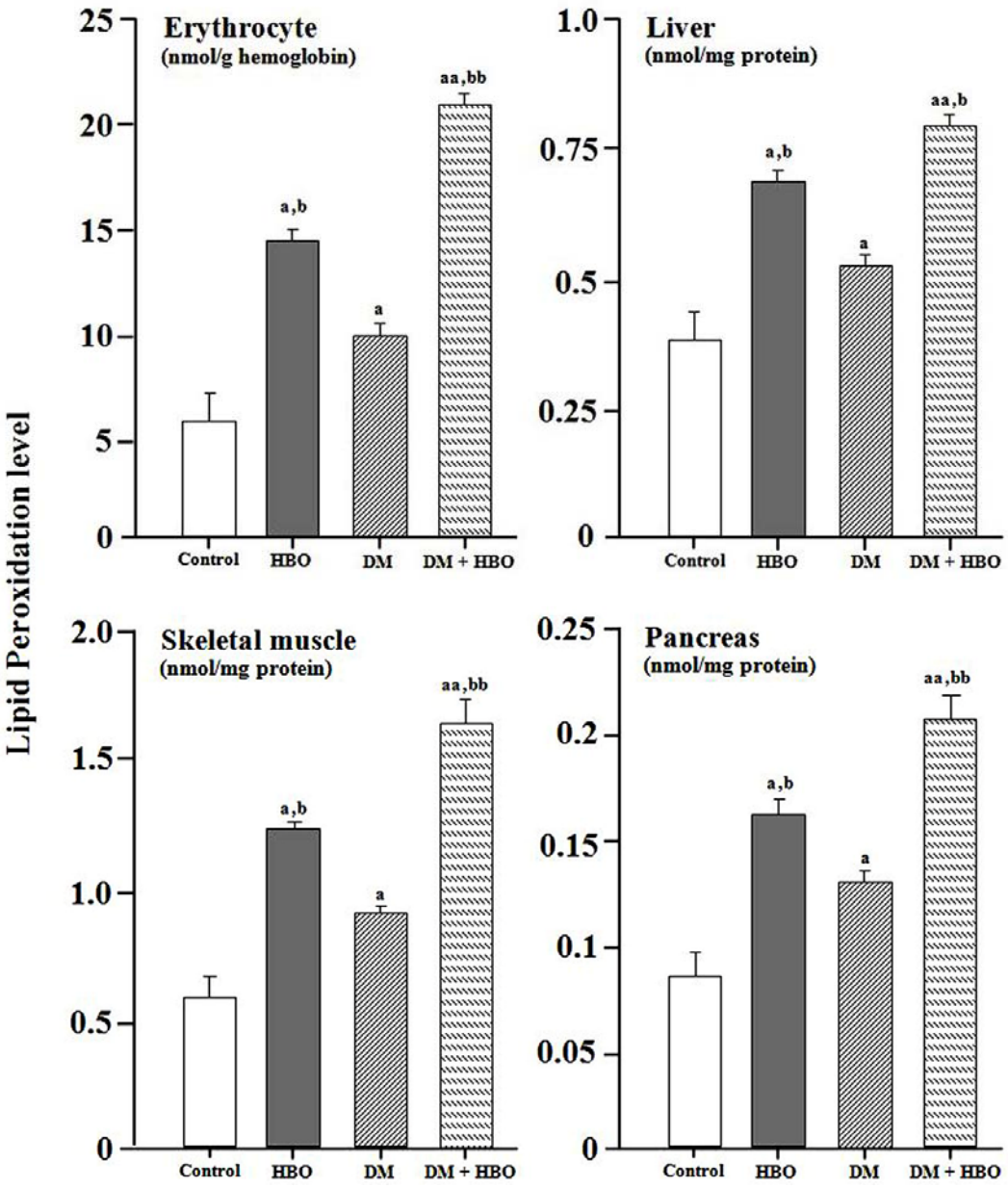

Figure 2: Comparison of lipid peroxidation levels (TBARS) in the erythrocyte, liver, skeletal muscle, and pancreas among non-diabetic rats in the non-HBO (control), non-diabetic rats in the HBO (HBO), diabetic rats in the non-HBO (DM), and diabetic rats in the HBO (DM + HBO) groups. TBARS values are indicated as nmol/g hemoglobin in erythrocytes and as nmol/mg protein in the various organs. Values are expressed as mean ± SEM ($n = 6$). [a] Represents significance at $p < 0.05$ and [aa] represents significance at $p < 0.005$ compared with the control groups. [b] Represents significance at $p < 0.05$ and [bb] represents significance at $p < 0.005$ compared with the DM groups.

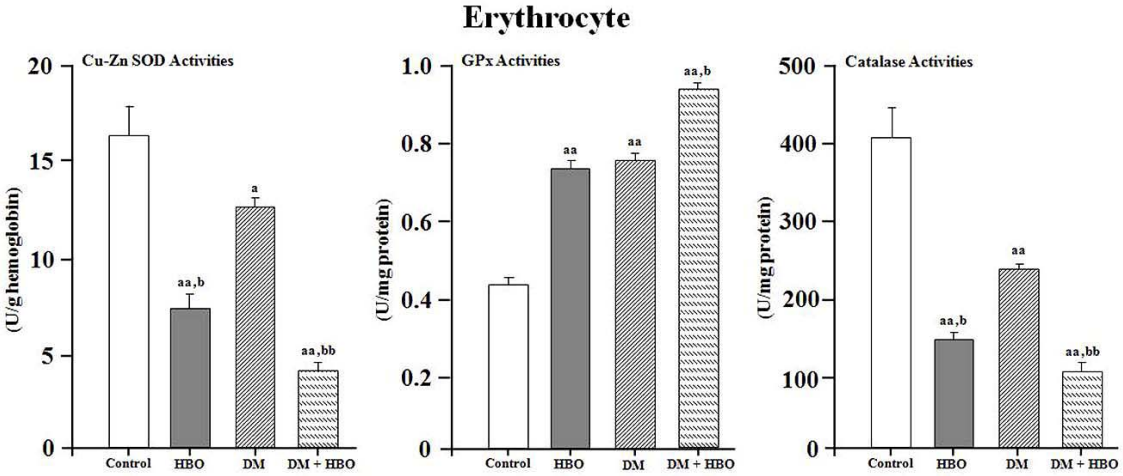

Figure 3: Comparison of the antioxidant enzyme activities (Cu-Zn SOD, GPx, and CAT) in the erythrocyte among non-diabetic rats in the non-HBO (control), non-diabetic rats in the HBO (HBO), diabetic rats in the non-HBO (DM), and diabetic rats in the HBO (DM + HBO) groups. These antioxidant enzymes values are given as U/g hemoglobin in erythrocytes. Values are expressed as mean ± SEM (n = 6). [a] Represents significance at $p < 0.05$ and [aa] represents significance at $p < 0.005$ compared with the control groups. [b] Represents significance at $p < 0.05$ and [bb] represents significance at $p < 0.005$ compared with the DM groups.

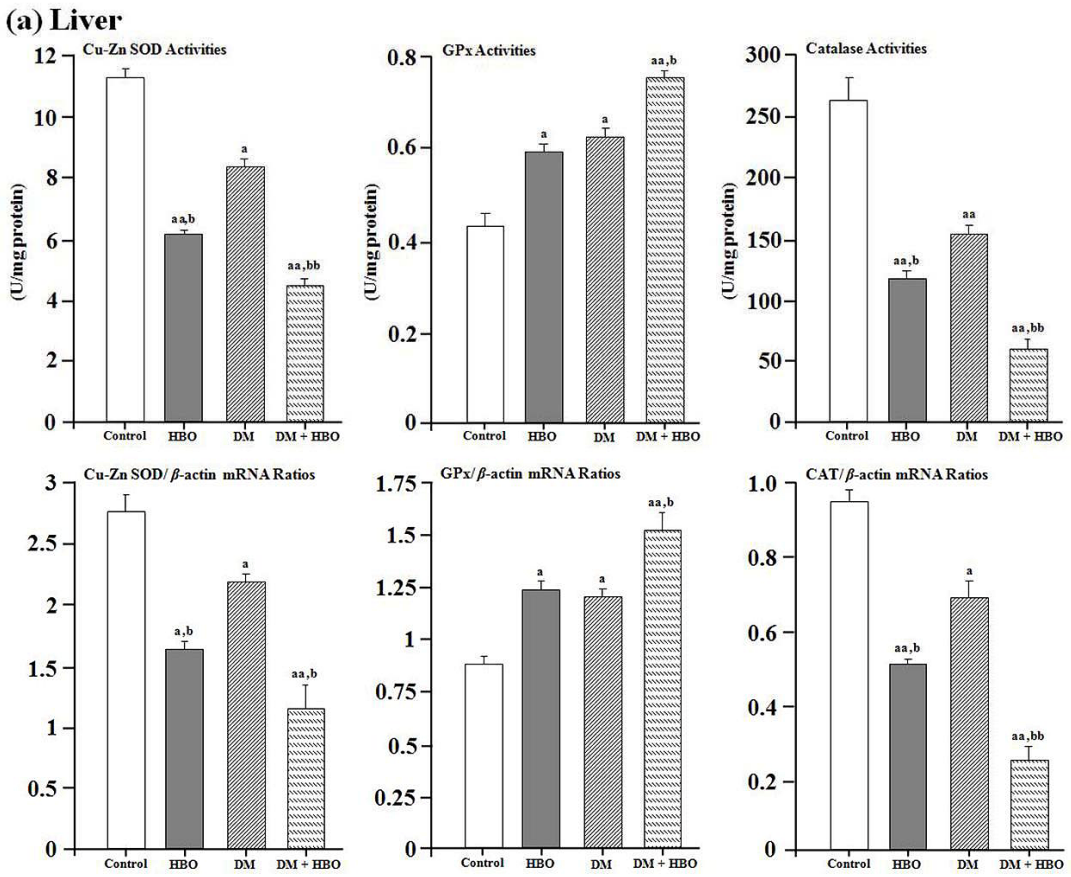

Figure 4: Comparison of the antioxidant enzyme activities and mRNA expressions (Cu-Zn SOD, GPx, and CAT) in the liver (**a**); (Refer to Figure 4b and 4c in next page).

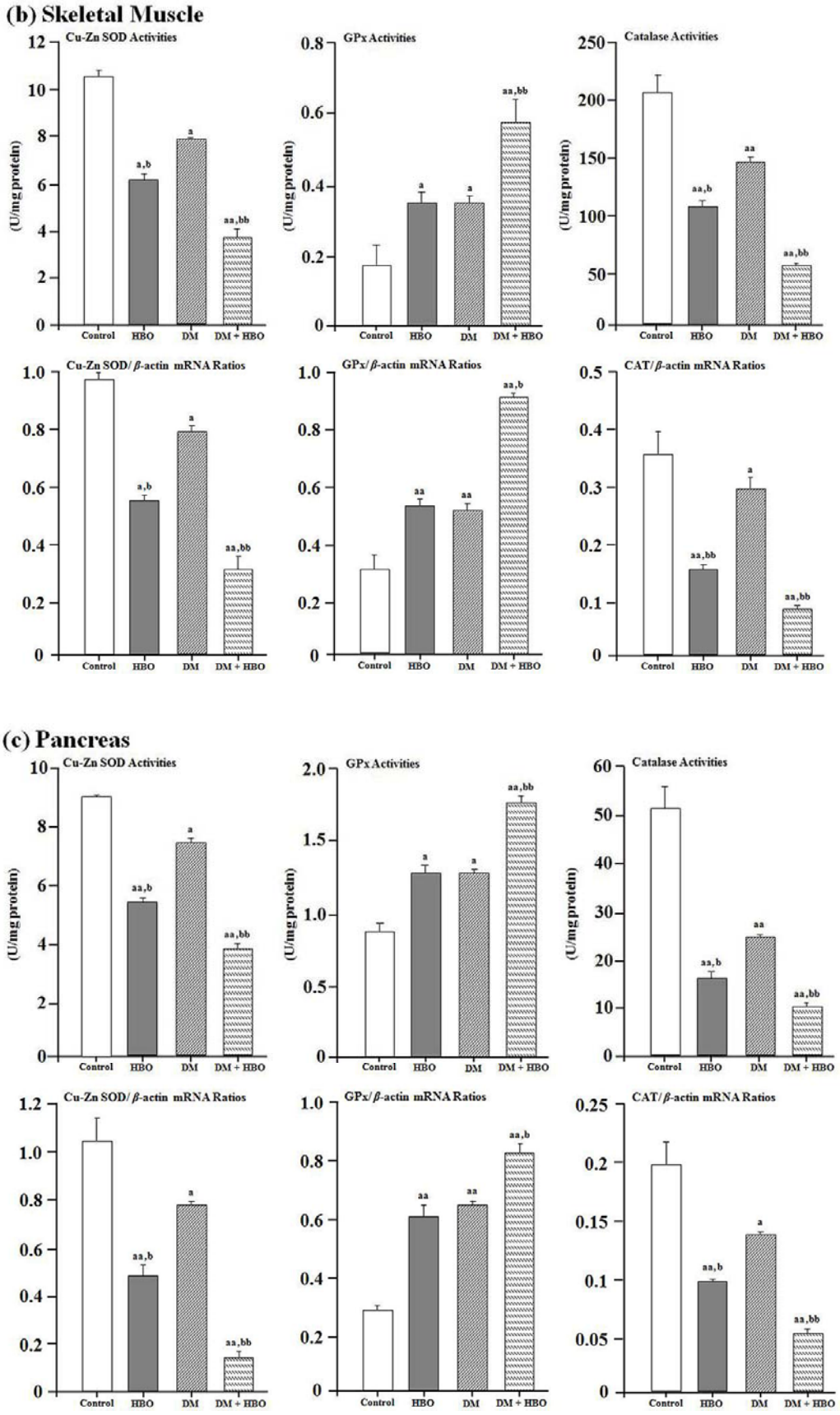
(b) Skeletal Muscle
Cu-Zn SOD Activities
(U/mg protein)
GPx Activities
(U/mg protein)
Catalase Activities
(U/mg protein)
Cu-Zn SOD/β-actin mRNA Ratios
GPx/β-actin mRNA Ratios
CAT/β-actin mRNA Ratios
Control
HBO
DM
DM + HBO
(c) Pancreas
Cu-Zn SOD Activities
(U/mg protein)
GPx Activities
(U/mg protein)
Catalase Activities
(U/mg protein)
Cu-Zn SOD/β-actin mRNA Ratios
GPx/β-actin mRNA Ratios
CAT/β-actin mRNA Ratios

Figure 4: Comparison of the antioxidant enzyme activities and mRNA expressions (Cu-Zn SOD, GPx, and CAT) in the liver (**a**), skeletal muscle (**b**), and pancreas (**c**) among non-diabetic rats in the non-HBO (control), non-diabetic rats in the HBO (HBO), diabetic rats in the non-HBO (DM), and diabetic rats in the HBO (DM + HBO) groups. These antioxidant enzymes values are given as U/mg protein in the various organs, mRNA expressions values are presented as the ratios of the densities of these antioxidants mRNAs and *β*-actin genes of all organs in all groups measured by Image J software. Values are expressed as mean ± SEM (n = 6). [a] Represents significance at $p < 0.05$ and [aa] represents significance at $p < 0.005$ compared with the control groups. [b] Represents significance at $p < 0.05$ and [bb] represents significance at $p < 0.005$ compared with the DM groups.

TBARS levels in the erythrocytes and all organs (liver, skeletal muscle, and pancreas) examined are presented in **Figure 2**. TBARS levels in the HBO, DM, and DM + HBO groups were significantly increased ($p < 0.005$ - 0.05) compared with the control group. In addition, TBARS levels in the erythrocytes and all organs were higher in the HBO and DM + HBO groups than in the DM group ($p < 0.005$ - 0.05). The Cu-Zn SOD and CAT activities in the erythrocytes and all organs (liver, skeletal muscle, and pancreas) examined in the HBO, DM, and DM + HBO groups were significantly decreased ($p < 0.005$ - 0.05) compared with the control group; the same finding was not seen with the GPx activity (**Figure 3**, **4a**, **4b**, and **4c**). Moreover, the Cu-Zn SOD and CAT activities in the erythrocytes and all organs were significantly lower in the HBO and DM + HBO groups than in the DM group ($p < 0.005$ - 0.05).

Figure 4a, **4b**, and **4c** present the ratios of the densities of Cu-Zn SOD, CAT, and *β*-actin genes of all organs (liver, skeletal muscle, and pancreas) in all groups measured by Image J software. As shown in all figures, the HBO, DM, and DM + HBO groups showed a decrease in mRNA levels of Cu-Zn SOD and CAT in all organs ($p < 0.05$), compared with the control group. The mRNA expressions of Cu-Zn SOD and CAT in all organs were significantly lower in the DM + HBO group compared to the control group ($p < 0.005$). In addition, mRNA levels of Cu-Zn SOD and CAT in all organs were significantly lower in the HBO and DM + HBO groups than in the DM group ($p < 0.05$).

The same series of figures present the ratios of densities of GPx and *β*-actin genes after densitometric analysis of the respective bands with Image J software (**Figure**s **4a**, **4b**, and **4c**). As seen from all figures, the HBO, DM, and DM + HBO groups showed an increase in mRNA levels of GPx in all organs ($p < 0.05$) compared with the control group. The mRNA expressions of GPx in all organs were higher in the DM + HBO group compared to the control group and these reductions were statistically significant ($p < 0.005$). In addition, mRNA levels of GPx in all organs were higher in the DM + HBO group than in the DM group ($p < 0.05$). For the HBO group, in all organs, the increases in the relative expression of GPx mRNA compared to the DM group were about 5 – 8%, but this difference was not statistically significant.

Discussion

Our study examines whether the levels of activity and mRNA expression of the antioxidant enzymes, such as Cu-Zn SOD, GPx, and CAT are differentially-affected in the liver, skeletal muscle, and pancreas of the diabetic rats receiving HBO treatment. We have shown for the first time that diabetic rats receiving HBO treatment had significantly increased ROS production. Hyperglycemia subsequent to diabetes causes oxidative stress**,** mainly leading to the enhanced production of mitochondrial ROS [9]. Oxidative stress induced by hyperglycemia leads to the activation of stress-sensitive signaling pathways, which worsen both insulin secretion and action, and promote the development of type 2 diabetes mellitus (T2DM) [24]. Oxidative stress and damage to the tissues and the blood in STZ-induced diabetic rats enhance glucose autoxidation, and may be a contributory factor to the complications associated with diabetes [10]. Similarly, HBO, which was used in our experiment, also increases the formation of ROS that can cause cellular damage through the oxidation of lipid, protein, and DNA [3]. Thus, our results of changes in antioxidant enzymes at the mRNA

level might correlate with the oxidative stress induced and enhanced by both diabetes induction and HBO exposure.

HBO exposure increases the amount of dissolved oxygen in the blood, resulting in the improvement of a variety of clinical conditions such as hypoxia, acute carbon monoxide intoxication, air embolism and diabetic lower limb wounds [1]. The most commonly-used doses of HBO for standard therapeutic purposes are 1.8–2.8 ATA for 60–90 min [25]. Although the oxidative effects of HBO have been investigated in animals and humans [5, 6], the HBO conditions applied in previous studies seemed to be higher than the usual therapeutic levels. One study in rats revealed that, after a single HBO exposure at 3 ATA, oxidative stress markers, including TBARS levels and GPx, and nitrate/nitrite (NOx) activity, were elevated in the brain [6]. In this present study, by using an HBO exposure protocol based on clinically-used pressures and duration (2.4 ATA for 90 min), we showed that plasma glucose concentrations were not improved and plasma insulin concentrations were decreased in HBO-treated diabetic rats, suggesting that even clinically-used HBO administration might enhance the functional disorder of pancreatic cells in diabetic conditions as demonstrated in our previous study [26].

The primary ROS generated in the several organs is superoxide anion. SOD can catalyze dismutation of superoxide anion into hydrogen peroxide, which is then detoxified to water and molecular oxygen by CAT or GPx. In that respect, evidence obtained from clinical and animal studies suggested that diabetic patients and animals have significant decreased in antioxidant enzymes, and increased in lipid peroxidation [10, 13]. In the present study, we investigated the levels of TBARS of diabetic rats with HBO treatment. We have showed that diabetic rats receiving HBO treatment had significantly increased TBARS levels compared with both the control and DM groups. Moreover, we showed that the levels of TBARS were significantly higher in non-diabetic rats treated with HBO than in diabetic rats without HBO treatment, thus there is no doubt that HBO causes lipid peroxidation [27]. Furthermore, we showed that diabetic rats receiving HBO treatment had significantly decreased activities and mRNA expressions of Cu-Zn SOD and CAT, and significantly increased GPx activities and mRNA expressions compared with the diabetic control group (i.e., no HBO treatment). The diabetic state caused the activity of the antioxidant enzymes, Cu-Zn SOD and CAT to decrease and GPx to increase in liver tissues of rats [28, 29]. As with the TBARS levels, the activities of Cu-Zn SOD and CAT were significantly lower in non-diabetic rats with HBO treatment than in diabetic rats without HBO treatment. In addition, non-diabetic rats with HBO treatment had increased activity of GPx compared to diabetic rats without HBO treatment, but this difference was not statistically significant. Moreover, the mRNA expressions of Cu-Zn SOD and CAT in all organs were significantly lower in non-diabetic rats with HBO treatment than in diabetic rats without HBO treatment. These results indicated the activities of antioxidant enzymes such as Cu-Zn SOD, CAT, and GPx were associated with the expressions of each respective gene. Recent studies reported that the activities and mRNA expressions of antioxidant enzymes, Cu-Zn SOD and CAT, were decreased [30] and those of GPx were increased [14] in the liver tissue of rats, 2-3 weeks after induction of diabetes. On the other hand, rat liver tissue 5-6 weeks after diabetes induction showed an increase in Cu-Zn SOD mRNA expression together with an increase in the total SOD activity, and an increase in CAT gene expression in contrast to a decrease in enzyme activity in the renal cortex [10]. These results indicate that oxidative stress affects the antioxidant defense systems in the different organs in an organ-specific way. The mRNA expressions of antioxidant enzymes in animals and humans under HBO exposure have been poorly investigated, however, we have shown the dynamics of mRNA expressions of these antioxidant enzymes in the present study.

In this present study, we showed that Cu-Zn SOD and CAT activities were decreased in HBO-treated diabetic rats, suggesting that HBO treatment might enhance the superoxide radicals under diabetic conditions. Similarly, HBO treatment also increased the GPx activity. SOD and CAT are the major scavenging enzymes that remove radicals in vivo. A decrease in the activity of these antioxidant enzymes can lead to an excess availability of superoxide radicals, such as superoxide anion

and hydrogen peroxide [22]. Superoxide radicals are known to inhibit CAT activity, and then SOD activity is directly inhibited by increased superoxide radicals [31]. Additionally, in this study, we have shown that diabetic rats receiving HBO treatment had significantly increased GPx activities and mRNA expressions compared with the diabetic control group (i.e., no HBO treatment). A significant increase in GPx activities and mRNA expressions could suggest that CAT activities and mRNA expressions were inactivated by ROS, which are increased in diabetic rats and HBO exposure. Thus, the decreasing CAT activity and mRNA may be due to be counterbalanced by increasing GPx activities and mRNA expressions in diabetic rats [14] and HBO exposure. Elevated levels of cellular oxidative stress caused by diabetes and HBO exposure contribute to the inactivation or sensitive to the antioxidant enzymes, such as Cu-Zn SOD, GPx, and CAT. In addition to causing damage to cellular constituents, recent evidence suggests that ROS can affect signal transduction processes or the translocation of redox-sensitive transcription factors [32]. Therefore, the observed changes in the mRNA expressions of antioxidant enzymes in diabetes under HBO exposure could be due to oxidation of transcriptional factors responsible for the initiation machinery of antioxidant enzymes transcription process.

In conclusion, a decrease of Cu-Zn SOD and CAT mRNA expression, and an increase of GPx mRNA expression were associated with higher plasma glucose concentrations in diabetes following exposure to HBO. Thus, the synergistic elevation of plasma glucose concentrations between STZ-induced diabetes and HBO treatment lead to glucose autoxidation, which would increase the induction of ROS. It has been shown that oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol 3-kinase (PI3K) in adipocytes [33]. Oxidative stress produced via glucose autoxidation worsens many pathological conditions and induces diabetic complications [9]. Recent studies have reported that STZ-induced β-cell death was associated with oxidative stress caused by the production of excess intracellular ROS [34, 35], and that STZ could damage the pancreatic tissue through imposing oxidative as well as nitrosative stresses, which in turn could induce apoptosis in the pancreatic cells [36]. Furthermore, the oxidative stress caused by HBO exposure has been shown to induce apoptosis via the mitochondrion [37]. Future work should investigate the gene expression of the insulin receptor (IR), IRS, PI3K, and glucose transporter 1 or 4 (GLUT-1 or 4), which all have a wider spectrum of activity and are also responsible for the regulation mechanism of blood glucose levels. This would help us to arrive at a better understanding of the molecular mechanisms of the antioxidant defense system of diabetes under HBO exposure. Further studies also need to address the detailed mechanism of how oxidative stress caused by HBO activities is linked to the mitochondrial pathway of apoptosis. These observations suggest that the detection of a protection mechanism against oxidative stress caused by HBO exposure may be beneficial in humas with essential diabetes mellitus.

Acknowledgements

This study was partially supported by the Academic Frontier Project “Surveillance and control for zoonoses” and Strategic Research Base Development Program "International research on epidemiology of zoonoses and training for young researchers" from Ministry of Education, Culture, Sports, Science and Technology, Japan.

Please address all correspondences to: Yukita Sato, PhD, Laboratory of Biomedical Science, Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Fujisawa 252-8510, Japan. Phone and Fax: +81-466-84-3445; E-mail address: sato.yukita@nihon-u.ac.jp

References

[1] Feldmeier JJ and Undersea and Hyperbaric Medical Society: Hyperbaric oxygen 2003: indications and results: the Hyperbaric Oxygen Therapy Committee report. Edited by Feldmeier JJ. North Carolina, Durham, 2003, pp. 141

[2] Jamieson D, Chance B, Cadenas E and Boveris A. The relation of free radical production to hyperoxia. Annu Rev Physiol 1986;48:703-719.

[3] Speit G, Dennog C, Radermacher P and Rothfuss A. Genotoxicity of hyperbaric oxygen. Mutat Res 2002;512:111-119.

[4] Eken A, Aydin A, Sayal A, Ustündağ A, Duydu Y and Dündar K. The effects of hyperbaric oxygen treatment on oxidative stress and SCE

frequencies in humans. Clin Biochem 2005;38:1133-1137.
[5] Oter S, Korkmaz A, Topal T, Ozcan O, Sadir S, Ozler M, Ogur R and Bilgic H. Correlation between hyperbaric oxygen exposure pressures and oxidative parameters in rat lung, brain, and erythrocytes. Clin Biochem 2005;38:706-711.
[6] Korkmaz A, Oter S, Sadir S, Topal T, Uysal B, Ozler M, Ay H and Akin A. Exposure time related oxidative action of hyperbaric oxygen in rat brain. Neurochem Res 2008;33:160-166.
[7] Hunt JV, Smith CC and Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 1990;39:1420-1424.
[8] Wolff SP, Jiang ZY and Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med 1991;10:339-352.
[9] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-820.
[10] Kakkar R, Kalra J, Mantha SV and Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem 1995;151:113-119.
[11] Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y and Yamada Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. Diabetes 1999;48:927-932.
[12] Opara EC. Oxidative stress, micronutrients, diabetes mellitus and its complications. J R Soc Health 2002;122:28-34.
[13] Davi G, Falco A and Patrono C. Lipid peroxidation in diabetes mellitus. Antioxid Redox Signal 2005;7:256-268.
[14] Sadi G and Guray T. Gene expressions of Mn-SOD and GPx-1 in streptozotocin-induced diabetes: effect of antioxidants. Mol Cell Biochem 2009;327:127-134.
[15] Gürdöl F, Cimşit M and Oner-Iyidoğan Y. Early and late effects of hyperbaric oxygen treatment on oxidative stress parameters in diabetic patients. Physiol Res 2008;57:41-47.
[16] Matsunami T, Sato Y, Sato T and Yukawa M. Antioxidant status and lipid peroxidation in diabetic rats under hyperbaric oxygen exposure. Physiol Res 2009.
[17] Like AA and Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science 1976;193:415-417.
[18] Matkovics B, Kotorman M, Varga IS, Hai DQ and Varga C. Oxidative stress in experimental diabetes induced by streptozotocin. Acta Physiol Hung 1997;85:29-38.
[19] Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358.
[20] Sun Y, Oberley LW and Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
[21] Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-169.
[22] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-126.
[23] Rasband WS. ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/ 2008.
[24] Evans JL, Goldfine ID, Maddux BA and Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 2002;23:599-622.
[25] Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F and Canestrari F. Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. Clin Biochem 2004;37:312-317.
[26] Matsunami T, Sato Y, Morishima T and Yukawa M. Enhancement of glucose toxicity by hyperbaric oxygen exposure in diabetic rats. Tohoku J Exp Med 2008;216:127-132.
[27] Oury TD, Schaefer LM, Fattman CL, Choi A, Weck KE and Watkins SC. Depletion of pulmonary EC-SOD after exposure to hyperoxia. Am J Physiol Lung Cell Mol Physiol 2002;283:777-784.
[28] Maritim AC, Sanders RA and Watkins JB. Effects of alpha-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. J Nutr Biochem 2003;14:288-294.
[29] Anwar MM and Meki AR Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comp Biochem Physiol A Mol Integr Physiol 2003;135:539-547.
[30] Sadi G, Yilmaz O and Güray T. Effect of vitamin C and lipoic acid on streptozotocin-induced diabetes gene expression: mRNA and protein expressions of Cu–Zn SOD and catalase. Mol Cell Biochem 2008;309:109-116.
[31] Kono Y and Fridovich I. Superoxide radical inhibits catalase. J Biol Chem 1982;257:5751-5754.
[32] Sen CK and Packer L. Antioxidant and redox regulation of gene transcription. FASEB J 1996;10:709-720.
[33] Tirosh A, Potashnik R, Bashan N and Rudich A. Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. J Biol Chem 1999;274:10595-10602.
[34] Peschke E, Ebelt H and Brömme HJ. 'Classical' and 'new' diabetogens--comparison of their

effects on isolated rat pancreatic islets in vitro. Cell Mol Life Sci 2000:57:158-164.
[35] González E, Jawerbaum A, Sinner D, Pustovrh C, White V, Capobianco E, Xaus C, Peralta C and Roselló-Catafau J. Streptozotocin-pancreatic damage in the rat: modulatory effect of 15-deoxy delta12,14-prostaglandin j(2) on nitridergic and prostanoid pathway. Nitric Oxide 2002;6:214-220.
[36] Manna P, Sinha M and Sil PC. Protective role of arjunolic acid in response to streptozotocin-induced type-I diabetes via the mitochondrial dependent and independent pathways. Toxicology 2009;257:53-63.
[37] Weber SU, Koch A and Kankeleit J. Hyperbaric oxygen induces apoptosis via a mitochondrial mechanism. Apoptosis 2009;14:97-107.