

ORIGINAL

Long-term treatment with hyperbaric air improves hyperlipidemia of db/db mice

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Abstract : Hyperbaric air (HBA) is used to improve healing of wounds including diabetic ulcer. The aim of this study was to clarify the effects of HBA exposure on lipid and glucose metabolism in db/db mice. HBA did not influence the weight of db/db mice. Serum levels of free fatty acid and triglyceride, but not glucose and insulin, were significantly decreased after 6 weeks of treatment with HBA. The mRNA expressions of CPT-1, PPAR α and PGC-1 α genes, which are related to lipid metabolism, were significantly up-regulated in the muscle and liver. Increases in TNF α and MCP1 mRNA, which impaired lipid metabolism, were also attenuated by HBA treatment. These results suggest that exposure of HBA could have beneficial effects on lipid metabolism in patients with type 2 diabetes mellitus. *J. Med. Invest.* 57 : 224-231, August, 2010

Keywords : HBA, hyperlipidemia, TNF α , MCP1

INTRODUCTION

Type 2 diabetes mellitus is characterized by a chronic hyperglycemic state due to decreased insulin sensitivity in target tissues, including skeletal muscle, adipocytes and the liver, and/or due to the impairment of insulin secretion (1, 2). Obesity is a robustly pandemic and pathological disease and is responsible for type 2 diabetes mellitus, hyperlipidemia and hypertension (3). Increased serum levels of free fatty acid (FFA) or triglyceride (TG) deteriorate hyperglycemia through peripheral

insulin resistance, finally resulting in cerebral infarction and cardiovascular disease (4, 5). Thus, in obese type 2 diabetes patients, treatment of hyperlipidemia is clinically important to prevent these comorbidities.

Hyperbaric oxygen (HBO) therapy is a therapeutic procedure that provides tissues with hyperoxygenation by inhalation of high oxygen density at a pressure of more than one atmosphere in a hyperbaric chamber (6). HBO has been utilized for the treatment of various diseases, including gas poisoning (7, 8) and autism (9). In diabetic patients, HBO

Abbreviations used

GPO : glycerol-phosphate oxidase, DAOS : sodium n-ethyl-n-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, ACS : acyl-CoA synthetase, ACOD : acyl coenzyme A oxidase, CPT-1 : carnitine palmitoyltransferase-1, PPAR α : peroxisome proliferator-activated receptor α , PGC-1 α : peroxisome proliferator coactivator-1 α , TNF α : tumor necrosis factor-1 α , MCP-1 : monocyte chemoattractant protein-1, RT-PCR : reverse transcription polymerase chain reaction, C/EBP : CCAAT/enhancer-binding protein. UCPs : Uncoupling proteins. FBS : Fasting blood sugar.

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is also utilized for therapy of gangrene (10) and retinopathy (11). It has been reported that levels of blood glucose in patients with hypertension and type 2 diabetes were significantly lowered by exposure to HBO (12). In animal experiments, HBO treatment prevented an increase in blood glucose level during growth and changed the muscle type to slow twitch subtype (13-15). On the other hand, HBO treatment significantly increased blood glucose levels in type 1 diabetic rats compared with the levels in non-diabetic controls (16). However, there has been no investigation of the effects of HBO on lipid metabolism except for decreased oxidized low-density lipoprotein (17). In contrast to its various beneficial effects, HBO treatment has been shown to have serious adverse effects, including oxidative stress and oxygen poisoning, because of high oxygen concentrations (18-20).

Hyperbaric air (HBA) therapy is a therapeutic method for exposing patients to a pressure that exceeds one atmosphere while maintaining oxygen density at a normal level. It is thought that HBA treatment has less adverse effects than those of HBO treatment. Recently, an HBA chamber has been used commercial for athletes to recover from muscle fatigue. However, there have been no beneficial effects of HBA therapy for diseases such as diabetes or hyperlipidemia. In this study, using obese diabetic mice, db/db mice, we examined the effects of HBA on diabetes and hyperlipidemia.

MATERIALS AND METHODS

Animals and treatments

Six-week-old male db/db diabetic mice (n=12) and db/+m non-diabetic mice (n=12) (Japan Charles River, Kanagawa, Japan) were randomly assigned to HBA groups (n=6) and control groups (n=6), respectively. Mice in the HBA groups were exposed to 1.3 atmospheric pressure by a commercially available hyperbaric chamber (Oasis O₂, Nihon Light Service, Inc., Tokyo, Japan) for 6 hours (10:00-16:00) per day, and mice in the control groups were kept in an environment similar to that for mice in the HBA groups but at normal atmospheric pressure. Food intake and body weight were measured, and blood samples were collected from the tip of the tail vein weekly in each group before HBA exposure at 10:00. Blood samples were immediately centrifuged to collect serum supernatant. Serum samples were stored at -80°C until use for

measurement of metabolic parameters. Mice were sacrificed 8 weeks later to obtain tissue samples of the liver, soleus muscle and epididymal fat. The tissues were immediately frozen in liquid nitrogen and stored at -80°C until used for RNA preparation. The mice were housed at a constant room temperature of 23±2°C with a 12-h light/dark cycle and were fed a normal chow diet (Oriental Yeast, Tokyo, Japan) with water *ad libitum*. This study was approved by the Ethics Committee of the University of Tokushima for Animal Studies.

Measurement of lipid parameters

Plasma TG and FFA concentrations were measured by the GPO-DAOS method and ACS-ACOD method (Wako Pure Chemical Industries, Osaka, Japan), respectively.

Quantitative real-time RT-PCR

Total RNA was extracted from the liver, soleus muscle and epididymal fat by using an RNeasy kit (Qiagen, Valencia, CA), and then total RNAs were reverse-transcribed using a Takara PrimeScript RT reagent kit (Takara, Kyoto, Japan). Quantitative real-time PCR was performed with the LightCycler system (Roche Diagnostics, Switzerland) using Takara SYBR Premix Ex Taq II (Takara, Kyoto, Japan). The following gene-specific primers were used: CPT-1a (sense: 5'-cttccatgactcggctcttc-3'; antisense: 5'-agcttgaacctctgctctgc-3'), CPT-1b (sense: 5'-cccatgtgctcctaccagat-3'; antisense: 5'-ccttgaagaagcgaccttg-3'), PPAR α (sense: 5'-agaccctcggggaacttaga-3'; antisense: 5'-cagagcgctaagctgtgatg-3'), PGC-1 α (sense: 5'-tcacaccaaaccacagaaa-3'; antisense: 5'-tctggggtcagaggaagaga-3'), TNF- α (sense: 5'-atggcctccctctcatcagtt-3'; antisense: 5'-acaggctgtcactcgaatttg-3'), MCP-1 (sense: 5'-cccaatgagtagctggaga-3'; antisense: 5'-tctggaccattcctcttg-3') and 18S ribosomal RNA (sense: 5'-aaacggctaccacatccaag-3'; antisense: 5'-ggcctcgaaagagtctgta-3'). After the PCR reaction, each PCR product was confirmed for its single amplification by analyzing a melting curve of the PCR products.

Statistical analysis

Data are expressed as means±SEM. Data were analyzed by ANOVA or unpaired Student's t-test. A *p*-value <0.05 was accepted as statistically significant.

RESULTS

Serum FFA and TG concentrations were decreased in db/db mice after HBA treatment but not in db/+m mice.

To determine the effects of HBA on lipid and glucose metabolism in obese diabetic mice, db/db mice were exposed to HBA for 6 hours, which is the same duration as that used in a previous study in which diabetic rats were exposed to HBO (14). The food intake in the db/db mice groups was much higher than that in the db/+m mice groups. Change in body weight during a period of 8 weeks was not altered by HBA exposure in either the db/db mice groups or db/+m groups (Figure 1A). The food intake, however, was significantly increased by HBA exposure in the db/db mice but not in the db/+m mice (Figure 1B).

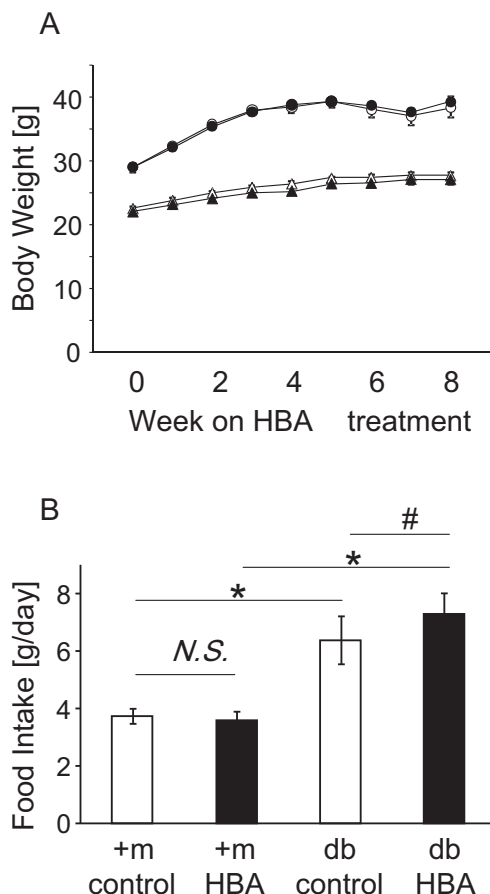


Figure 1. Body weights and food intakes of control groups or HBA groups. The body weight (A) of db/db mice was greater than that of db/+m mice, and HBA treatment did not alter the body weight during a period of 8 weeks. Food intake (B) of db/db mice was greater than db/+m mice, and it was increased after HBA treatment. ○ control group of db mice, ● HBA group of db mice, △ control group of +m mice, ▲ HBA group of +m mice. Data are means \pm SEM (n=6). * : $p < 0.05$, # : $P < 0.01$. N.S. : no significant difference.

The weights of the slow twitch muscle : soleus muscle, liver and fat tissues were not significantly altered by HBA exposure either in the db/db or db/+m mice (not shown). The concentration of fasting blood glucose and insulin sensitivity assessed by an oral glucose tolerance test and insulin tolerance test, respectively, were not altered significantly by HBA exposure either in the db/db or db/+m mice (Figure 2A, 2B and not shown). Interestingly, the concentrations of serum FFA and TG were significantly decreased by HBA exposure in the db/db mice but not in the db/+m mice (Figure 2C, 2D).

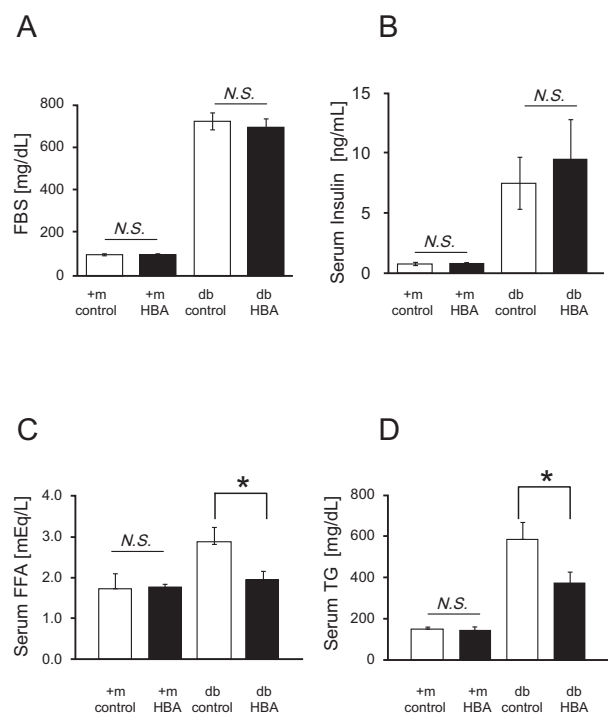


Figure 2. Serum levels FBS, Insulin, FFA and TG after HBA treatment. Serum concentrations of FBS (A), Insulin (B), FFA (C) and TG (D) of db/db mice were greater than that of db/+m mice and these values were decreased by HBA treatment for 8 weeks. Data are means \pm SEM (n=6). * : $p < 0.05$. N.S. : no significant difference.

The mRNA expression levels of factors involved in lipid homeostasis were increased after HBA treatment.

To clarify the mechanism underlying the effect of HBA on lipid metabolism, mRNA expression of CPT-1, a rate-limiting enzyme for β -oxidation mainly in the soleus muscle and liver, was quantified by real-time RT-PCR. As shown in Figures 3A and 3D, the mRNA expression of CPT-1 (a of liver type and b of skeletal muscle type), but not that of CPT-2

(not shown), was increased significantly by HBA exposure both in the soleus muscle and liver of db/db mice. CPT-1 mRNA expression in the soleus muscle and liver was not altered by HBA exposure in db/+m mice.

The mRNA expressions of the transcription factors PPAR α and PGC-1 α were examined since the former was reported to control lipid metabolism (21, 22) and the latter was reported to increase β -oxidation in brown adipocytes (23) or in skeletal muscle with enhanced mitochondria function coordinated with exercise (24), even though PGC-1 α usually has roles in glucose metabolism to attribute a gluconeogenesis and mitochondria biosynthesis (25). Moreover, it has been shown that PGC-1 α can cooperate with PPAR α to express the genes of mitochondrial fatty acid oxidation enzymes such as CPT-1 in a hepatoma cell line (26). In the soleus muscle, mRNA expression of PPAR α and PGC-1 α in db/db mice was decreased significantly compared to that in db/+m mice. The mRNA expression of

PPAR α was increased after HBA treatment in the skeletal muscle of both db/db and db/+m mice (Figure 3B). In the liver, however, the mRNA expression of PPAR α was increased after HBA treatment only in db/db mice (Figure 3E). HBA treatment enhanced the mRNA expression of PGC-1 α in db/+m and db/db mice (Figure 3C). On the other hand, the mRNA expression of PGC-1 α was significantly greater in the liver of db/db mice than in the liver of db/+m mice. Exposure to HBA significantly enhanced the mRNA expression of PGC-1 α only in db/db mice (Figure 3F).

mRNA expression levels of TNF α and MCP-1 were decreased after HBA treatment.

In adipocytes, lipolysis from fat droplets rather than β -oxidation contributes to the development of hyperlipidemia. On the other hand, adipocytes become larger by accumulating TG and become smaller by lipolysis *via* output of FFA. In this study, however, the weight of adipose tissue with HBA

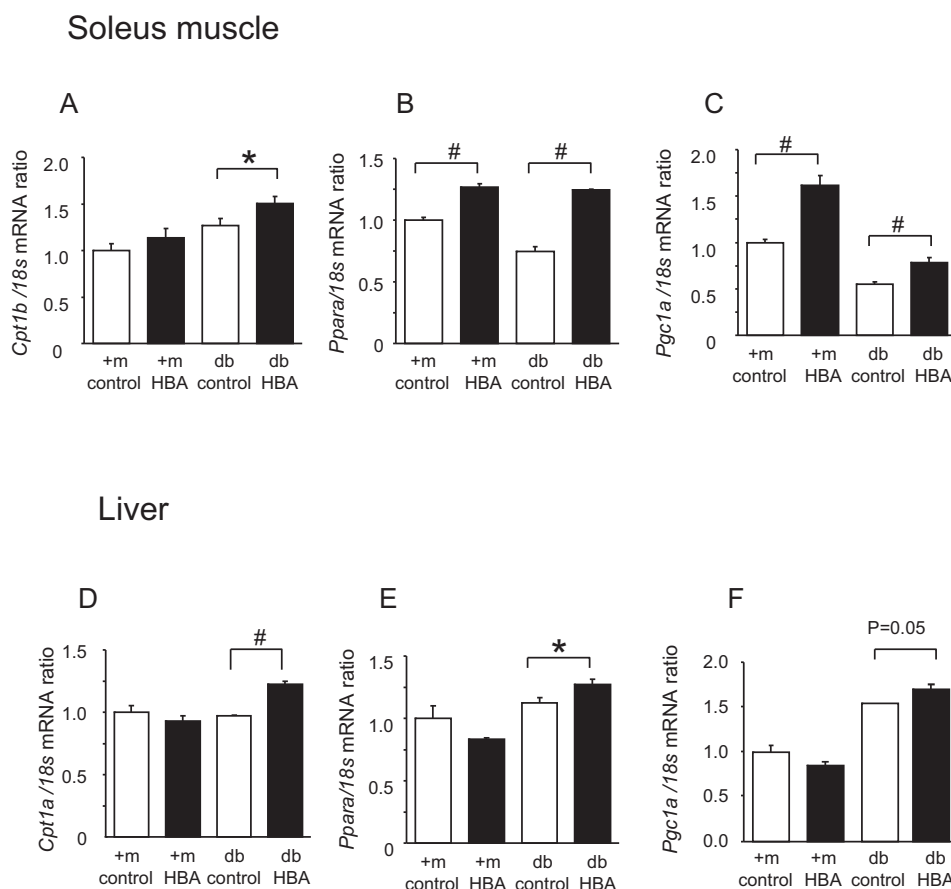


Figure 3. mRNA expression of factors involved in lipid homeostasis.

The soleus muscle and liver were obtained from db/db and db/+m mice with or without HBA exposure for 8 weeks. Total RNA isolated from these tissues was subjected to quantitative real-time RT-PCR with primers specific for CPT-1a/b (A, D) PPAR γ (B, E) and PGC-1 α (C, F) as described in the *Materials and Methods* section. Data were normalized by 18S ribosomal RNA (* : $P < 0.05$ and # : $P < 0.01$). Data are means \pm SEM (n=6).

exposure, as mentioned previously, did not differ from that without HBA exposure in db/db mice (not shown). Recently, it has been reported that adipocyte inflammation in obesity causes insulin resistance and subsequently type 2 diabetes or hyperlipidemia (27, 28). HBO treatment decreases lipopolysaccharide-induced production of proinflammatory adipokines production such as TNF α and IL6 (29) without changing body weight. Therefore, we studied the mRNA expression of adipokines. As shown in Figure 4, the mRNA expression levels of TNF α and MCP-1 were significantly decreased after HBA exposure in db/db mice. The mRNA expression level of adiponectin tended to decrease after HBA exposure in db/db mice, although it did not reach a level of statistical significance (not shown).

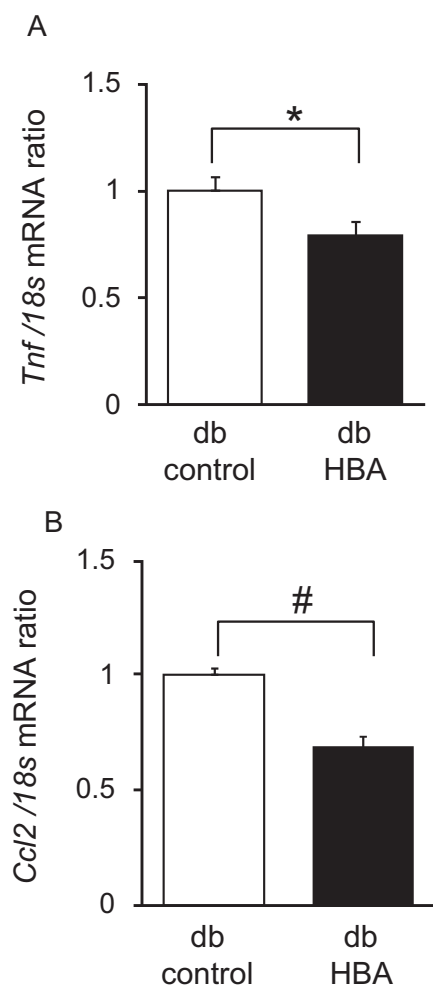


Figure 4. mRNA expression of TNF α and MCP-1 after HBA treatment.

Epididymal fat was obtained from db/db mice with or without HBA exposure for 8 weeks. Total RNA isolated from these tissues was subjected to quantitative real-time RT-PCR with primers specific for TNF α (A) and MCP-1 (B) as described in the *Materials and Methods* section. Data were normalized by 18S ribosomal RNA (* : $P < 0.05$ and # : $P < 0.01$). Data are means \pm SEM (n=6).

DISCUSSION

In previous studies, HBO treatment could decrease blood glucose levels in humans (12) and rat (13, 14), but investigations with HBO were not done for hyperlipidemia. In addition, the effects of HBA on diabetes and hyperlipidemia have not been studied, either. Therefore, in the present study, obese diabetic mice, db/db mice, were used to investigate the effects of HBA on diabetes and hyperlipidemia. The results showed that HBA treatment decreased serum FFA (Figure 2C) and TG (Figure 2D) concentrations and increased mRNA expression levels of CPT-1 enzyme (Figure 3A, 3D), PPAR α (Figure 3B, 3E) and PGC1- α (Figure 3C, 3F) in the liver and muscle of db/db mice. We also found that HBA treatment decreased mRNA expression levels of the proinflammatory adipokines, TNF α and MCP-1 in db/db mice (Figure 4).

The food intake was significantly increased by HBA exposure in the db/db mice (Figure 1A), but HBA had no effect of body weight in db/db mice (Figure 1B). The weight of liver, soleus muscle or epididymal fat was not changed in db/db mice with or without HBA though it was not examined the body composition of total fat or fat free mass. The mRNA of UCPs, which are important for energy expenditure, was not changed in these mice (not shown). Until now, it has been still not clear that the discrepancy of body weight and food intake.

FFA is metabolized by β -oxidation, the rate-limiting enzyme of which is CPT-1, mainly in the skeletal muscle and liver. PGC-1 α with PPAR α or either of them alone transcribes CPT-1 in the muscle and liver as mentioned in the results section. PPAR α as a molecular target of fibrates also improves hypertriglyceridemia. Chronic adipocyte inflammation is modulated by TNF α , which increases lypolysis, finally resulting in increased level of serum FFA (28). Therefore, HBA treatment not only up-regulated mRNA of CPT-1, PGC-1 α and PPAR α but also decreased TNF α expression, which might consequently decrease the serum levels of FFA and TG.

HBA increases oxygen contents of the blood by about 2.5%, much less than the increase induced by HBO (30). A previous study using microarray analysis of neurons showed that HBA increases the expression levels of more genes than does normobaric oxygen (31). The genes include genes for transporters, signal transduction, growth and metabolism. Interestingly, HBA also increases the expression levels of more genes than does HBO. The

expression levels of some genes, such as C/EBP family genes, which are increased by hyperbaric air are decreased by exposure to HBO. The effects of HBA on cells are complicated and might not be the same as the effects of HBO. It is speculated that high pressure of HBA may influence the lipid metabolism. On the other hand, HBO increased parasympathetic activities in healthy volunteers (32-34) and significantly decreased cortisol levels (35). Dominance of sympathetic activities causes high FFA, because β receptor signal stimulates lipolysis. Moreover, stimulation of parasympathetic activities attenuates the increase in TNF α responded in response to inflammation (36, 37). These findings suggest that HBA increases parasympathetic activities, leading to lipid homeostasis.

Different from the results of previous studies showing that HBO had an effect on glucose metabolism (12-15), HBA treatment did not influence glucose metabolism in our experiments (Figure 2A, 2B and not shown). Tissue hypoxia (38, 39) and TNF α (40) or MCP-1 (41) induce insulin resistance, and high pressure up-regulates glycolytic genes (31). The db/db mice have a profile of severe insulin resistance with obesity unlike the GK rats used in previous studies. We speculate that HBA treatment in our experiments could not overcome the phenotype of db/db mice even though HBA might decrease insulin resistance. To clarify this possibility, effects of HBA on glucose metabolism should be tested using mice having mild phenotypes of diabetes or using a combination of anti-diabetic drugs or exercise.

Taken together, the results indicate that HBA treatment might have beneficial effects on lipid metabolism in type 2 diabetes mellitus patients.

FOOTNOTE

First three authors contributed equally to this work.

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REFERENCES

1. Stumvoll M, Goldstein BJ, van Haeften TW : Pathogenesis of type 2 diabetes. *Endocr Res* 32 : 19-37, 2007
2. Guillausseau PJ, Laloi-Michelin M : [Pathogenesis of type 2 diabetes mellitus]. *Rev Med Interne* 24 : 730-737, 2003
3. Bluher M : Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes* 117 : 241-250, 2009
4. Mooradian AD : Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract Endocrinol Metab* 5 : 150-159, 2009
5. Wilding JP : The importance of free fatty acids in the development of Type 2 diabetes. *Diabet Med* 24 : 934-945, 2007
6. Robins M : Chamber advances for delivery of hyperbaric oxygen therapy. *Aviat Space Environ Med* 79 : 731, 2008
7. Fukaya E, Hopf HW : HBO and gas embolism. *Neurol Res* 29 : 142-145, 2007
8. Stoller KP : Hyperbaric oxygen and carbon monoxide poisoning : a critical review. *Neurol Res* 29 : 146-155, 2007
9. Rossignol DA, Rossignol LW : Hyperbaric oxygen therapy may improve symptoms in autistic children. *Med Hypotheses* 67 : 216-228, 2006
10. Levin ME : Prevention and treatment of diabetic foot wounds. *J Wound Ostomy Continence Nurs* 25 : 129-146, 1998
11. Chang YH, Chen PL, Tai MC, Chen CH, Lu DW, Chen JT : Hyperbaric oxygen therapy ameliorates the blood-retinal barrier breakdown in diabetic retinopathy. *Clin Experiment Ophthalmol* 34 : 584-589, 2006
12. Al-Waili, NS, Butler GJ, Beale J, Abdullah MS, Finkelstein M, Merrow M, Rivera R, Petrillo R, Carrey Z, Lee B, Allen M : Influences of hyperbaric oxygen on blood pressure, heart rate and blood glucose levels in patients with diabetes mellitus and hypertension. *Arch Med Res* 37 : 991-997, 2006
13. Yasuda K, Adachi T, Gu N, Matsumoto A, Matsunaga T, Tsujimoto G, Tsuda K, Ishihara A : 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, 2007
14. Yasuda K, Aoki N, Adachi T, Tsujimoto G, Gu N, Matsunaga T, Kikuchi N, Tsuda K, Ishihara A : Hyperbaric exposure with high oxygen concentration inhibits growth-associated increase in the glucose level of diabetic Goto-Kakizaki rats. *Diabetes Obes Metab* 8 : 714-715, 2006
 15. Matsumoto A, Nagatomo F, Yasuda K, Tsuda K, Ishihara A : 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, 2007
 16. Matsunami T, Sato Y, Morishima T, Mano Y, Yukawa M : Enhancement of glucose toxicity by hyperbaric oxygen exposure in diabetic rats. *Tohoku J Exp Med* 216 : 127-132, 2008
 17. Kudchodkar BJ, Pierce A, Dory L : Chronic hyperbaric oxygen treatment elicits an anti-oxidant response and attenuates atherosclerosis in apoE knockout mice. *Atherosclerosis* 193 : 28-35, 2007
 18. Speit G, Dennog C, Radermacher P, Rothfuss A : Genotoxicity of hyperbaric oxygen. *Mutat Res* 512 : 111-119, 2002
 19. Chavko M, Harabin AL : Regional lipid peroxidation and protein oxidation in rat brain after hyperbaric oxygen exposure. *Free Radic Biol Med* 20 : 973-978, 1996
 20. Harabin AL, Braisted JC, Flynn ET : Response of antioxidant enzymes to intermittent and continuous hyperbaric oxygen. *J Appl Physiol* 69 : 328-335, 1990
 21. Li AC, Glass CK : PPAR- and LXR-dependent pathways controlling lipid metabolism and the development of atherosclerosis. *J Lipid Res* 45 : 2161-2173, 2004
 22. Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O : Fatty acid regulation of hepatic gene transcription. *J Nutr* 135 : 2503-2506, 2005
 23. Feige JN, Auwerx J : Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol* 17 : 292-301, 2007
 24. Muoio DM, Koves TR : Skeletal muscle adaptation to fatty acid depends on coordinated actions of the PPARs and PGC1 alpha : implications for metabolic disease. *Appl Physiol Nutr Metab* 32 : 874-883, 2007
 25. Puigserver P, Spiegelman BM : Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha) : transcriptional coactivator and metabolic regulator. *Endocr Rev* 24 : 78-90, 2003
 26. Vega RB, Huss JM, Kelly DP : The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 20 : 1868-1876, 2000
 27. Rasouli N, Kern PA : Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 93 : S64-73, 2008
 28. Guilherme A, Virbasius JV, Puri V, Czech MP : Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 9 : 367-377, 2008
 29. Kudchodkar B, Jones H, Simecka J, Dory L : Hyperbaric oxygen treatment attenuates the pro-inflammatory and immune responses in apolipoprotein E knockout mice. *Clin Immunol* 128 : 435-441, 2008
 30. Weaver LK, Howe S, Snow GL, Deru K : Arterial and pulmonary arterial hemodynamics and oxygen delivery/extraction in normal humans exposed to hyperbaric air and oxygen. *J Appl Physiol* 107 : 336-345, 2009
 31. Chen Y, Nadi NS, Chavko M, Auker CR, McCarron RM : Microarray analysis of gene expression in rat cortical neurons exposed to hyperbaric air and oxygen. *Neurochem Res* 34 : 1047-1056, 2009
 32. Schipke JD, Pelzer M : Effect of immersion, submersion, and scuba diving on heart rate variability. *Br J Sports Med* 35 : 174-180, 2001
 33. Ceamitru N, Badiu G, Teren O, Petru A, Soare G : Study of heart rate of professional divers in hyperbarism during simulated diving in saturation with different respiratory mixture. *Rom J Physiol* 30 : 179-182, 1993
 34. Lund VE, Kentala E, Scheinin H, Klossner J, Helenius H, Sariola-Heinonen K, Jalonen J : Heart rate variability in healthy volunteers during normobaric and hyperbaric hyperoxia. *Acta Physiol Scand* 167 : 29-35, 1999
 35. Lund V, Kentala E, Scheinin H, Klossner J, Koskinen P, Jalonen J : Effect of hyperbaric conditions on plasma stress hormone levels and endothelin-1. *Undersea Hyperb Med* 26 : 87-92, 1999
 36. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ : Vagus nerve stimulation attenuates the systemic inflammatory

- response to endotoxin. *Nature* 405 : 458-462, 2000
37. Niederbichler AD, Papst S, Claassen L, Jokuszies A, Steinstraesser L, Hirsch T, Altintas MA, Ipaktchi KR, Reimers K, Kraft T, Vogt PM : Burn-induced organ dysfunction : vagus nerve stimulation attenuates organ and serum cytokine levels. *Burns* 35 : 783-789, 2009
38. Trayhurn P, Wang B, Wood IS : Hypoxia in adipose tissue : a basis for the dysregulation of tissue function in obesity? *Br J Nutr* 100 : 227-235, 2008
39. Regazzetti C, Peraldi P, Gremeaux T, Najem-Lendom R, Ben-Sahra I, Cormont M, Bost F, Le Marchand-Brustel Y, Tanti JF, Giorgetti-Peraldi S : Hypoxia decreases insulin signaling pathways in adipocytes. *Diabetes* 58 : 95-103, 2009
40. Tilg H, Moschen AR : Inflammatory mechanisms in the regulation of insulin resistance. *Mol Med* 14 : 222-231, 2008
41. Tamura Y, Sugimoto M, Murayama T, Ueda Y, Kanamori H, Ono K, Ariyasu H, Akamizu T, Kita T, Yokode M, Arai H : Inhibition of CCR2 ameliorates insulin resistance and hepatic steatosis in db/db mice. *Arterioscler Thromb Vasc Biol* 28 : 2195-2201, 2008