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Effects of hyperbaric oxygen therapy on circulating interleukin-8, nitric oxide, and insulin-like growth factors in patients with type 2 diabetes mellitus

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Abstract

Background: The potential benefits of hyperbaric oxygen therapy (HBOT) have been reported in diabetic patients with foot ulcers. However, the roles of HBOT on wound healing-associated growth factors and inflammatory mediators are not completely understood in diabetes mellitus (DM).

Objectives: The aim of this study was to investigate the effects of HBOT on circulating cytokines, NO, and insulin-like growth factors (IGF) in patients with type 2 DM.

Design and methods: Serum samples were collected from patients with type 2 DM ($n=31$) and healthy subjects ($n=29$) before (baseline) and after the first and third exposure.

Results: Before HBOT, body mass index (BMI) and serum HbA1c were significantly greater, whereas serum IGF-I was significantly lower in diabetic patients compared to healthy subjects (one-way ANOVA, $p<0.05$). After adjusting for age, gender, and BMI, serum insulin, growth hormone (GH), IGF-II, IGF-binding protein (IGFBP)-1, IGFBP-3, leptin, interleukin (IL)-8, and NO were not significantly altered by HBOT in diabetic patients and healthy subjects (repeated-measures ANOVA). Change in serum insulin (baseline to the third exposure) was a positive predictor of changes in leptin and NO in healthy subjects and diabetic patients, respectively.

Conclusions: Our results suggest that short-term HBOT may not alter the circulating insulin, IGF, leptin, IL-8, and NO levels. In addition, healthy subjects and diabetic patients showed differential responses to HBOT in the relationships of leptin, insulin, and NO. Further studies are needed to clarify the mechanism of HBOT-improved wound healing in diabetic patients with foot ulcers.

Keywords: Hyperbaric oxygenation; Type 2 diabetes mellitus; IL-8; Nitric oxide; Leptin; Insulin-like growth factors

Introduction

Oxygen is an essential material for cell metabolism and is in especially increased demand during reparative processes, such as cell proliferation and collagen synthesis [1]. Evidence from animal and cell line studies has shown that hyperbaric oxygen therapy (HBOT), the administration of pure oxygen at pressures greater than 1 atmosphere absolute (ATA), results in increased growth factor production, such as platelet-related growth factor, transforming growth factor-β1 [2,3], vascular endothelial growth factor [3,4], and reactive oxygen species-related hypoxia-inducible factor-1 [1,5] in wound healing. These growth-promoting effects revealed that HBOT may be used as an adjuvant to promote collagen synthesis and to improve wound healing for diabetic venous, arterial, and pressure ulcers [6,7]. In addition, after 20 to 40 exposures, the beneficial effects of HBOT on the healing of foot ulcers and

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glucose metabolism have been reported in patients with diabetes mellitus (DM) [8–11].

Existing evidence indicates that insulin-like growth factor (IGF)-I, a proinsulin-like growth factor that modulates tissue growth and repair, and IGF-binding proteins (IGFBPs) play important roles in glucose homeostasis in diabetic patients [12,13]. In addition, circulating level of leptin, a polypeptide hormone with a tertiary structure similar to cytokines [14,15], is related to body weight, body fat mass, glycemic status, and disease duration in diabetic patients and may be related to peripheral insulin sensitivity [16,17]. To date, no study has addressed the relationship of IGFs, IGFBPs, and leptin to HBOT-induced wound healing and blood glucose control. However, several studies have focused on the effects of HBOT on serum levels of nitric oxide (NO) and cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and interferon-γ. Our previous results showed that serum IL-8, a chemotactic cytokine related to diabetic risk and treatment of diabetic complications [18], was significantly increased, and serum NO was significantly decreased in children with diabetes [19]. Recent evidence indicates that the low serum NO in diabetic patients with non-healing ulcers could be effectively corrected by HBOT [20]. There is little information regarding the effects of HBOT on IL-8 in diabetic patients and healthy subjects.

Although the beneficial effects of HBOT have been demonstrated in animals, the clinical evidence regarding the effectiveness of HBOT on healing chronic wounds is sparse and difficult to interpret [7,21]. In contrast, serious adverse events during HBOT, such as barotraumatic lesions, oxygen toxicity, confinement anxiety, and ocular effects, have been reported [21–23]. These adverse effects might be related to HBOT-induced alterations in cytokine [24–27] and NO [28–30] productions. The relatively high medical cost of HBOT and the time and direct costs associated with daily travel for patients are other issues to be considered [6]. Therefore, routine application of HBOT remains controversial. The aim of this study was to investigate the effects of short-term HBOT on cytokines, NO, and IGFs in patients with type 2 DM. We also examined the safety of short-term HBOT in healthy subjects.

Methods

Subjects and study design

Ethical approval from the Institutional Review Board of Changhua Christian Hospital was obtained before conducting this study. Thirty-one patients with type 2 DM (the DM group) and twenty-nine healthy subjects (the CON group) were included in this study. All individuals completed the informed consent process before enrolling in the study. Subjects with any diabetic complications, such as nephropathy, neuropathy or retinopathy, acute or chronic diseases, or oral medication, were excluded. The twenty-nine healthy volunteers recruited by the advertisement of the hospital served as controls. The age ranged from 23 to 80 years in the CON group and 43 to 76 years in the DM group.

All subjects were exposed to 2.5 ATA for 90 min per day for three consecutive days. The 90 min of pure oxygen exposure was divided into three 25-minute periods and one final 15-minute period with 5-minute air breaks between each period as routine clinical operating procedures for preventing oxygen toxicity, such as seizure, visual disturbances, shortness of breath, and chest pain. Individuals who could not complete this process were excluded from this study. HBOT treatments were carried out in two hyperbaric chambers (Sigma II and Sigma PLUS, Perry Baromedical Co, FL) at the hyperbaric oxygen management center of Changhua Christian Hospital.

Demographic information for all subjects, such as age and gender were recorded. Using electrical scales and a wall-mounted stadiometer, body weight and body height of each subject were obtained by nurses. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Five milliliters of peripheral venous blood was drawn from each subject before the exposure (as baseline), immediately after the first exposure, and immediately after the third exposure of HBOT. Samples for laboratory uses were centrifuged, divided, and stored at −80°C for future assays.

Analytical measurements

Serum concentration of HbA1c was measured using a commercially available kit of high performance liquid chromatography (A1c 2.2, Tosoh Diagnostics, Japan). Serum concentrations of insulin, growth hormone (GH), IGF-I, IGF-II, IGFBP-1, and IGFBP-3 were determined by commercially available enzyme-linked immunosorbent assay kits (ELISA, Diagnostic Systems Laboratories, Webster, TX). Serum concentrations of leptin and IL-8 were analyzed using ELISA kits (DuoSet, R&D System, Minneapolis, MN and Pharmingen Inc., San Diego, CA). In addition, serum concentration of NO was indirectly measured as the total amount of nitrite and nitrate by the Griess reaction [31]. All of the serum samples were analyzed in one assay with a duplicate. The intra-assay coefficients of variance were 5–10%.

Statistical analysis

Values are expressed as mean±standard error of means (SEM). Statistical analysis was performed using SPSS for Windows (SPSS Advanced Statistics 7.5, SPSS, Chicago, IL, 1997). At baseline, age, body weight, body height, and BMI were analyzed by one-way analysis of variance (ANOVA) between the CON and DM groups. In addition, the biochemical parameters, such as HbA1c, insulin, GH, IGFs, IGFBPs, leptin, IL-8, and NO, were compared between the CON and DM groups at baseline, after the first exposure, and after the third exposure of HBOT, respectively (one-way ANOVA). After adjusting for age and gender in the general linear regression model, repeated-measures analysis of variance was used to determine the main effects of group, time, and BMI on the changes in biochemical parameters from the baseline to the third exposure of HBOT. Where there was a significant difference during the experimental period ($p<0.05$), Bonferroni post hoc