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Original Research

Effect of Hyperbaric Oxygen Therapy on Fatty Acid Composition and Insulin-like Growth Factor Binding Protein 1 in Adult Type 1 Diabetes Mellitus Patients: A Pilot Study

Ivana Resanović MSc [a,*]; Zoran Gluvić MD, PhD [b]; Božidarka Zarić PhD [a]; Emina Sudar-Milovanović PhD [a]; Vesna Vučić PhD [c]; Aleksandra Arsić PhD [c]; Olgica Nedić PhD [d]; Miloš Šunderić PhD [d]; Nikola Gligorijević MSc [d]; Davorka Milačić MD [e]; Esma R. Isenović PhD [a,f,*]

[a] Institute of Nuclear Sciences Vinča, Laboratory of Radiobiology and Molecular Genetics, University of Belgrade, Belgrade, Serbia
[b] Clinic for Internal Medicine, Zemun Clinical Hospital, School of Medicine, University of Belgrade, Belgrade, Serbia
[c] Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, University of Belgrade, Belgrade, Serbia
[d] Department for Metabolism, Institute for the Application of Nuclear Energy, University of Belgrade, Belgrade, Serbia
[e] Department of Hyperbaric Medicine, Zemun Clinical Hospital, Belgrade, Serbia
[f] Faculty of Stomatology, Pančevo, University Business Academy, Novi Sad, Serbia

Key Messages

- Metabolic changes occurring in type 1 diabetes mellitus (T1DM) patients may lead to tissue hypoxia, chronic inflammation, and atherosclerosis.
- Hyperbaric oxygen therapy (HBOT) exerts anti-inflammatory and antiatherogenic effects on T1DM patients.
- HBOT has beneficial effects in T1DM through changes in lipid profile and plasma fatty acid composition and expression of insulin growth factor binding protein 1.

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ABSTRACT

Objective: Metabolic changes in type 1 diabetes mellitus (T1DM) impair vasodilation, and this leads to tissue hypoxia and microvascular pathology. Hyperbaric oxygen therapy (HBOT) can significantly improve the outcome of ischemic conditions in T1DM patients and reduce vascular complications. The aim of our study was to assess the effects of HBOT on plasma fatty acid (FA) composition, and expression of insulin-like growth factor binding protein 1 (IGFBP-1) in T1DM patients.
Methods: Our study included 24 adult T1DM patients diagnosed with peripheral vascular complications. The patients were exposed to 10 sessions of 100% oxygen inhalation at 2.4 atmosphere absolute for 1 hour. Blood samples were collected at admission and after HBOT for measurement of metabolic parameters, FA composition and IGFBP-1. Measurement of plasma FA composition was determined by gas chromatography. Expression of IGFBP-1 in the serum was estimated by Western blot analysis.
Results: HBOT decreased blood levels of total cholesterol ($p<0.05$), triglycerides ($p<0.05$) and low-density lipoprotein ($p<0.05$). HBOT increased plasma levels of individual FAs: palmitic acid ($p<0.05$), palmitoleic acid ($p<0.05$), docosapentaenoic acid ($p<0.05$) and docosahexaenoic acid ($p<0.01$), and decreased levels of stearic acid ($p<0.05$), alpha linolenic acid ($p<0.05$) and linoleic acid ($p<0.01$). Expression of IGFBP-1 ($p<0.01$) was increased, whereas the level of insulin ($p<0.001$) was decreased in the serum after HBOT.
Conclusions: Our results indicate that HBOT exerts beneficial effects in T1DM patients by improving the lipid profile and altering FA composition.

* Address for correspondence: Ivana Resanović MSc and Esma R. Isenović PhD, Institute of Nuclear Sciences Vinča, Laboratory of Radiobiology and Molecular Genetics, University of Belgrade, PO Box 522, 11000 Belgrade, Serbia.
E-mail addresses: ivana_resanovic@yahoo.com; isenovic@yahoo.com

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RÉSUMÉ

Objectif : Les modifications du métabolisme lors du diabète de type 1 (DT1) nuisent à la vasodilation et, par conséquent, entraînent une hypoxie tissulaire et une pathologie microvasculaire. L'oxygénothérapie hyperbare (OHB) peut améliorer de manière significative l'évolution des maladies d'origine ischémique chez les patients atteints du DT1 et réduire les complications vasculaires. L'objectif de notre étude était d'évaluer les effets de l'OHB sur la composition plasmatique des acides gras (AG) et l'expression de la protéine de liaison au facteur de croissance insulinomimétique de type 1 (IGFBP-1, de l'anglais *insulin-like growth factor binding protein 1*) chez les patients atteints du DT1.

Méthodes : Notre étude regroupe 24 patients adultes atteints du DT1 qui ont reçu un diagnostic de complications vasculaires périphériques. Nous avons fait subir aux patients 10 séances d'inhalation d'oxygène à 100 % à une pression atmosphérique absolue de 2,4 durant 1 heure. Nous avons effectué la collecte d'échantillons de sang à l'admission et après l'OHB pour mesurer les paramètres métaboliques, la composition des AG et l'IGFBP-1. Nous avons déterminé la composition plasmatique des AG au moyen de la chromatographie en phase gazeuse. Nous avons estimé l'expression de l'IGFBP-1 dans le sérum à l'aide de l'immunobuvardage de western.

Résultats : L'OHB diminuait les concentrations sanguines du cholestérol total ($p < 0,05$), des triglycérides ($p < 0,05$) et des lipoprotéines à faible densité ($p < 0,05$). L'OHB augmentait les concentrations plasmatiques individuelles des acides gras, soit l'acide palmitique ($p < 0,05$), l'acide palmitoléique ($p < 0,05$), l'acide docosapentaénoïque ($p < 0,05$) et l'acide docosahexaénoïque ($p < 0,01$), et diminuait les concentrations de l'acide stéarique ($p < 0,05$), de l'acide alpha-linolénique ($p < 0,05$) et de l'acide linoléique ($p < 0,01$). Nous avons noté une augmentation de l'expression de l'IGFBP-1 ($p < 0,01$), mais une diminution de la concentration de l'insuline ($p < 0,001$) dans le sérum après l'OHB.

Conclusions : Nos résultats indiquent que l'OHB exerce des effets bénéfiques chez les patients atteints de DT1 puisqu'elle permet d'améliorer le profil des lipides et de modifier la composition des AG.

Introduction

Type 1 diabetes mellitus (T1DM) is characterized by the destruction of insulin-secreting pancreatic beta cells under the influence of genetic and/or environmental factors (1). Factors involved in T1DM pathogenesis trigger lymphocyte infiltration in pancreatic beta cells, and various proinflammatory cytokines responsible for the destruction of pancreatic beta cells are produced (2). Metabolic changes occurring in T1DM patients may lead to chronic inflammation and atherosclerosis (3). Hyperglycemia is one of the major risk factors in the development of vascular complications due to consequent tissue hypoxia and insufficient tissue nutrition in T1DM patients (3).

Fatty acids (FAs) are structural components of triglycerides (TGs), phospholipids and cholesterol esters. Changes in FA composition influence cells by modifying membrane properties, altering intracellular signalling pathways and gene expression (4). A link between inflammation, altered FA composition and type 2 diabetes mellitus (T2DM) has been demonstrated (5). An elevated level of n-3 polyunsaturated FA (PUFA) may lower the risk of destruction of pancreatic beta cells and reduce inflammation by inhibiting secretion of proinflammatory cytokines and lymphocyte proliferation in T1DM patients (6,7). In addition, the elevated level of n-3 PUFA in plasma phospholipids has positive effects on glucose and lipid metabolism, which prevents the progression of arteriosclerosis in T1DM and T2DM (8). Evidence shows that changes of n-3 PUFA level in the membrane phospholipids may be a useful therapeutic approach for inflammation and the vascular complications that occur in T1DM (9).

Insulin-like growth factor 1 (IGF-1) plays a major role in glucose metabolism by lowering insulin level, increasing insulin sensitivity and improving the lipid profile, and thus affecting cardiovascular physiology (10,11). Altered IGF-1 activity is a major predisposing factor in T2DM-related vascular complications and contributes to the development of cardiovascular disorders (10,11), whereas insulin-like growth factor binding protein 1 (IGFBP-1) has been shown to inhibit action of IGF-1 by forming a IGF-1/IGFBP-1 complex (10,11). The IGFBP-1, as a 30-kD circulatory protein, is predominantly expressed in the liver, and IGFBP-1 protein has been involved in metabolic homeostasis (12). IGFBP-1 has been proposed as a player in insulin counter-regulation (13). In contrast, insulin inhibits the transcription of IGFBP-1 in the liver and decreases levels of IGFBP-1 in the blood (14). Results from a cross-sectional study showed that concentration of circulatory IGFBP-1 correlates with insulin sensitivity (15). Data from a longitudinal study showed that circulating IGFBP-1 concentration could predict the development of glucose intolerance and T2DM (16). In addition, some data also suggest that IGFBP-1 may contribute to the pathophysiology of the cardiovascular system (17,18). The concentration of IGFBP-1 has been shown to be inversely associated with microvascular and overt macrovascular disease (17). Thus, an increase in IGFBP-1 levels could play a role in the management of patients with insulin resistance, hypertension, T2DM and other conditions with accelerated atherosclerosis (19). When compared with T2DM, patients with T1DM demonstrated contrasting results with regard to IGFBP-1 levels, and it was shown that high serum IGFBP-1 levels were associated with T1DM (20).

Considering that insulin therapy does not always result in adequate daily glycemic control and this may contribute to the development of vascular complications, additional therapies are needed in T1DM patients to reduce vascular complications. An ideal treatment for the vascular complications that follow T1DM should combine strategies aimed to reduce hyperglycemia and inflammation, promote angiogenesis and restore functional pancreatic beta-cell mass. Hyperbaric oxygen therapy (HBOT) is used widely for the treatment of ischemic lesions and vascular complication caused by T1DM and T2DM (21). The relatively noninvasive nature and good safety profile of HBOT make it attractive for treatment of vascular complications related to T1DM and T2DM. HBOT includes therapeutic inhalation of 100% oxygen under elevated pressure and, therefore, increases the level of dissolved oxygen available to tissues (22).

We hypothesized that exposure of T1DM patients to HBOT would improve the lipid profile and affect FA composition and

expression of IGFBP-1 and, consequently, contribute to the reduction of vascular complications. Thus, we evaluated the effect of HBOT on plasma lipid profile, plasma composition of FA and expression of serum IGFBP-1 in T1DM patients.

Methods

Chemicals and reagents

Protease inhibitor (Complete, Ultra Mini, without ethylenediamine tetraacetic acid) and phosphatase inhibitor (PhosStop) cocktails were obtained from Roche (Mannheim, Germany). A GLUC-PAP commercial kit (Randox, Crumlin, United Kingdom) was utilized. Kits for determination of total cholesterol, TG and high-density lipoprotein (HDL) were obtained from Cobas, Roche Diagnostics (Indianapolis, Indiana, United States), and a radioimmunoassay kit for determination of insulin was obtained from INEP (Belgrade, Serbia). FA methyl ester commercial standards (PUFA-2) used for calibration of the gas chromatography (GC) column were obtained from Supelco (Bellefonte, Pennsylvania, United States). Polyclonal goat anti-IGFBP-1 and antibeta-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, California, United States). Secondary biotinylated horse antigoat antibody was obtained from Vector Laboratories (Burlingame, California, United States). Enhanced chemiluminescent (ECL) reagent was obtained from Pierce, Thermo Scientific (Rockford, Illinois, United States).

Subjects

Our study included 24 adult T1DM patients (19 males and 5 females), 41 to 80 (62.6±1.7) years of age, with diagnosed peripheral vascular complications. The patients were recruited from the Department of Hyperbaric Medicine, Zemun Clinical Hospital, Belgrade, Serbia, from October 2016 to December 2017. This prospective, randomized pilot study was approved by the ethics committee of Zemun Clinical Hospital and informed written consent was obtained from all patients. A total of 10 sessions of HBOT were performed in a hyperbaric chamber (Dräger 1000/1200, Drägerwerk AG & Co, Lübeck, Germany). Therapy was applied as 1 session/day, over a 2-week period, with 100% oxygen inhalation at 2.4 atmosphere absolute (ATA) for a 1-h duration. The demographic properties and medical history of all patients were recorded at the first visit, including gender, body mass, height, body mass index (BMI), current T1DM management, hypertension, and other concomitant therapy (aspirin, statins, fibrates, antiplatelet therapy, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, calcium-channel and beta blockers). The presence of vascular complications was confirmed by a standard questionnaire. Serum and plasma were immediately extracted from blood samples collected on admission and after 10 sessions of HBOT, and then stored at −20°C until further analysis.

Measurement of serum glucose concentration

The concentration of the serum glucose was measured using the standard GLUC-PAP method (Randox) in an Rx Daytona Automatic Biochemical Analyzer (Randox) according to the manufacturer's manual. Serum blood glucose concentration was expressed in millimoles per litre (mmol/L).

Measurement of serum insulin concentration

Serum insulin concentration was measured using a standard insulin radioimmunoassay method (INEP) according to the manufacturer's manual. The insulin concentration was expressed in milli−international units per litre (mIU/L).

Measurement of lipid profile

Concentrations of total cholesterol, TG and HDL cholesterol (HDL-C) in the plasma were measured using an enzymatic colorimetric kit according to the manufacturer's guidelines using a Cobas Integra 400+ analyzer (Roche Diagnostics, Indianapolis, Indiana, United States). The concentration of HDL-C was determined by the precipitation method with sodium phosphowolframate/magnesium chloride. Non-HDL-C was calculated as: total cholesterol − HDL-C. The values of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald equation: LDL-C = total cholesterol − HDL − 0.45 × TG. Concentrations of total cholesterol, TG, HDL-C, non-HDL-C, and LDL-C were expressed in mmol/L and presented as fold change vs before HBOT. In addition, we used the measured parameters to calculate atherogenic and cardiovascular risks. Atherogenic risk represents a logarithmically transformed lipid ratio of molar concentrations of LDL-C to HDL-C. Cardiovascular risk is calculated as logarithmically transformed lipid ratio of molar concentrations of total cholesterol to HDL-C (23).

Determination of plasma FA composition

FA composition of total lipids in plasma was determined using the method described by Glaser et al, with slight modifications (24). Briefly, we added 3 mol/L hydrochloric acid mixed with methanol, in 100 μL of plasma, with heating to 85°C for 45 minutes. After cooling to room temperature, we added 1.5 mL of hexane and centrifuged at 1,800*g* for 10 minutes, then the hexane layer was dried under a stream of nitrogen. A sample was dissolved in 10 μL of hexane and 1 μL was injected into the Shimadzu 2014 GC device (Restek). The GC was equipped with a capillary column (Rtx 2330, Restek) with an inner diameter of 60 meters × 0.25 mm and film the thickness of 0.2 μm. The temperature program was 140°C to 210°C for 3 minutes. Individual FAs (palmitic acid [PA, 16:0], stearic acid [SA, 18:0], palmitoleic acid [16:1], vaccenic acid [VA, 18:1n-7], oleic acid [OA, 18:1n-9], alpha linolenic acid [ALA, 18:3n-3], eicosapentaenoic acid [EPA, 20:5n-3], docosapentaenoic acid [DPA, C22:5n-3], docosahexaenoic acid [DHA, C22:6n-3], linoleic acid [LA, 18:2n-6], gamma linolenic acid [GLA, 18:3n-6], dihomo gamma linolenic acid [DGLA, 20:3n-6], arachidonic acid [AA, 20:4n-6] and docosatetraenoic acid [adrenic acid, AdA, 22:4n-6]) were identified according to the retention time of FA methyl ester commercial standards (PUFA-2, Supelco). The level for each particular FA is expressed as a percent of total FA and presented as fold change vs before HBOT.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis and Western blotting

Serum samples were prepared by boiling for 5 minutes with the addition of 5× Laemmli sample buffer and separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (25). Each lane of the sodium dodecyl sulfate polyacrylamide electrophoresis gel was loaded with 40 μg of total proteins. Separated proteins from the serum were transferred to nitrocellulose membranes, blocked with 5% nonfat milk and then probed with the goat anti-IGFBP-1 antibody. The membranes were washed and incubated with biotinylated horse antigoat antibody. The anti-actin monoclonal antibody was used as a loading control. Signals originating from immunoreactive proteins were detected using electrochemiluminescent reagent. The densitometric analysis of protein bands was performed using ImageJ

Table 1
Anthropometric parameters and glucose level of T1DM patients at admission

Parameter	Mean ± SEM (n=24)
Age (years)	62.6±1.7
Body mass (kg)	83.5±3.1
Height (m)	1.75±0.02
BMI (kg/m^2)	27.3±1.1
T1DM duration (years)	16.1±1.4
Glucose level (mmol/L)	9.4±0.7

BMI, Body mass index; *HBOT*, hyperbaric oxygen therapy; *T1DM*; type 1 diabetes mellitus; *SEM*, standard error of the mean.

software (National Institutes of Health, Bethesda, Maryland, United States). Level of IGFBP-1 is presented as fold change vs before HBOT.

Statistical analysis

Numerical variables are reported using descriptive statistics (mean ± standard error); categorical variables are reported as a percent. SPSS 18.0 for Windows (SPSS, Chicago, Illinois, United States) was used for all statistical calculations. Pearson's correlation coefficient was calculated to test correlations between C-reactive protein (CRP) and individual FA. Differences between groups were analyzed by Student's *t*-test. Statistical significance was set at $p<0.05$.

Results

Our prospective pilot study included 24 T1DM patients with vascular complications. The anthropometric parameters and blood glucose levels of T1DM patients at admission are presented in Table 1.

Exposure to HBOT decreased the plasma levels of total cholesterol by 0.15-fold vs before HBOT, $p<0.05$ (before HBOT/after HBOT: 1 to 5.7/0.7 to 2.5 mmol/L) (Figure 1A); non-HDL-C by 0.22-fold vs before HBOT, $p<0.05$ (before HBOT/after HBOT: 0.5 to 4.2/0.4 to 2 mmol/L) (Figure 1B); TG by 0.18-fold vs before HBOT, $p<0.05$ (before HBOT/after HBOT: 0.3 to 1.9/0.2 to 1.2 mmol/L) (Figure 1C); and LDL-C by 0.22-fold vs before HBOT, $p<0.05$ (before HBOT/after HBOT: 0.4 to 3.6/0.2 to 1.5 mmol/L) (Figure 1D). HDL-C level was unchanged (before HBOT/after HBOT: 0.2 to 0.9/0.2 to 1 mmol/L) (Figure 1E). The calculated atherogenic and cardiovascular risks for T1DM patients show that exposure to HBOT decreased atherogenic risk ($p<0.05$), whereas no significant influence on cardiovascular risk was detected (Figure 1, inset).

Regarding plasma FA composition, exposure to HBOT did not change the level of total plasma saturated FA (SFA), although HBOT increased the level of PA ($p<0.05$) and decreased the level of SA ($p<0.05$). Exposure to HBOT did not changed the level of total monounsaturated FA (MUFA) and specific MUFA: VA and OA, whereas the level of palmitoleic acid increased significantly after HBOT ($p<0.05$). In addition, the level of total n-3 PUFA and specific n-3 PUFA EPA did not change after exposure to HBOT. Furthermore, HBOT increased levels of DPA ($p<0.05$) and DHA ($p<0.01$) and decreased plasma level of ALA ($p<0.05$). Exposure to HBOT did not change the level of total n-6 PUFA nor the level of specific n-6 PUFA: GLA, DGLA, AA, and AdA. However, HBOT decreased the level of LA ($p<0.01$) in the plasma of T1DM patients (Table 2). In previous work, we found that CRP decreased after exposure to HBOT in T1DM patients (26). To evaluate the relationship between CRP and FA composition, we performed linear relationship analysis (Table 2). The results show a significant correlation between CRP and DHA after exposure to HBOT ($r = +0.508$, $p<0.05$).

Expression of IGFBP-1 in the serum samples was analyzed by Western blotting. Results show that exposure to HBOT increased IGFBP-1 expression by 0.5-fold vs before HBOT, $p<0.01$ (Figure 2A). We also assessed insulin in the serum of T1DM patients and found that insulin level decreased after HBOT by 0.3-fold vs before HBOT, $p<0.001$ (before HBOT: 37.1±5.6 mIU/L; after HBOT: 21.9±3.8 mIU/L) (Figure 2B).

Discussion

This is the first report of the effects of HBOT on FA composition and expression of IGFBP-1 in T1DM patients. Our findings suggest that: 1) HBOT induced an increase in the level of PA, palmitoleic acid, DPA and DHA, and a significant decrease in SA, ALA and LA in the plasma of T1DM patients; 2) HBOT induced an increase in IGFBP-1 level and 3) HBOT affected the lipid profile. The T1DM patients with vascular complications were exposed to HBOT, which has previously been associated with a significant reduction in T1DM by inhibiting apoptosis of pancreatic beta cells in a T1DM mouse model (27). However, some of the beneficial effects of HBOT remain controversial and the exact mechanisms of action are still unclear. A deeper understanding of the molecular mechanisms involved in HBOT may improve and fine-tune treatment of vascular complications related to T1DM and lead to recognition of new molecular targets for T1DM treatment.

T1DM patients are prone to abnormalities in the lipid profile, such as high levels of plasma TG and LDL-C, and low levels of HDL-C (28). These abnormalities may lead to enhanced lipoprotein susceptibility to oxidation, altered FA composition and lipoprotein density and increased lipid glycosylation (28). The impaired lipid profile observed in T1DM patients could be explained by hyperglycemia, but also by peripheral hyperinsulinemia caused by the subcutaneous insulin administration (29). Changes in glucose and lipid metabolism may further suppress autophagy, implicated in the progression of T2DM and development of microvasculature abnormalities (30). Consistent with data from other studies (28,31), we found that HBOT decreased plasma levels of TG and LDL-C. The high level of non-HDL-C has been associated with increased risk of vascular complications in T1DM patients (32). We observed a decrease of non-HDL-C and total cholesterol level in the plasma after HBOT. Sun et al reported that an increased level of lipoproteins and decreased level of HDL-C was associated with a higher incidence of cardiovascular disease (33). However, we did not find any significant effect of HBOT on the level of HDL-C, probably due to other parameters, such as age and diet (34,35). Another reason for not detecting a significant effect on HDL-C could lie in the small sample size and small effect size. Eleftheriou et al observed that the lipid profile and overall atherogenic and cardiovascular risk factors were pronounced in T1DM patients (36). We found that HBOT led to a significant decrease in atherogenic risk and a nonsignificant decrease in cardiovascular risk, consistent with previous observations (37). As it is well known that elevated levels of TG and LDL-C, and atherogenic risk are directly related to vascular complications (31), our results suggest that exposure to HBOT improves the lipid profile in T1DM patients.

Because the abnormalities in the plasma FA composition are related to poor glycemic control (38), and interactions between FA and glucose may cause oxidative stress and lead to inflammation in T1DM and T2DM (39), we aimed to evaluate whether exposure of T1DM patients to HBOT has an effect on plasma FA composition. In addition, considering that FA may act as either a pro- or anti-inflammatory factor, depending on their chemical structure (4), we assessed levels of SFA, MUFA, and PUFA in plasma using the GC method. Grimble et al reported a link between altered FA composition and inflammation in lymphocytes (40). Results from our previous study (26) suggest that the anti-inflammatory effects of HBOT may be linked to the reduction in nuclear factor kappaB (NF-κB) and, consequently, to the reduction of activity and expression of inducible nitric oxide synthase in T1DM patients, so we aimed to evaluate whether HBOT exerts an additional anti-inflammatory effect by changing the FA composition.

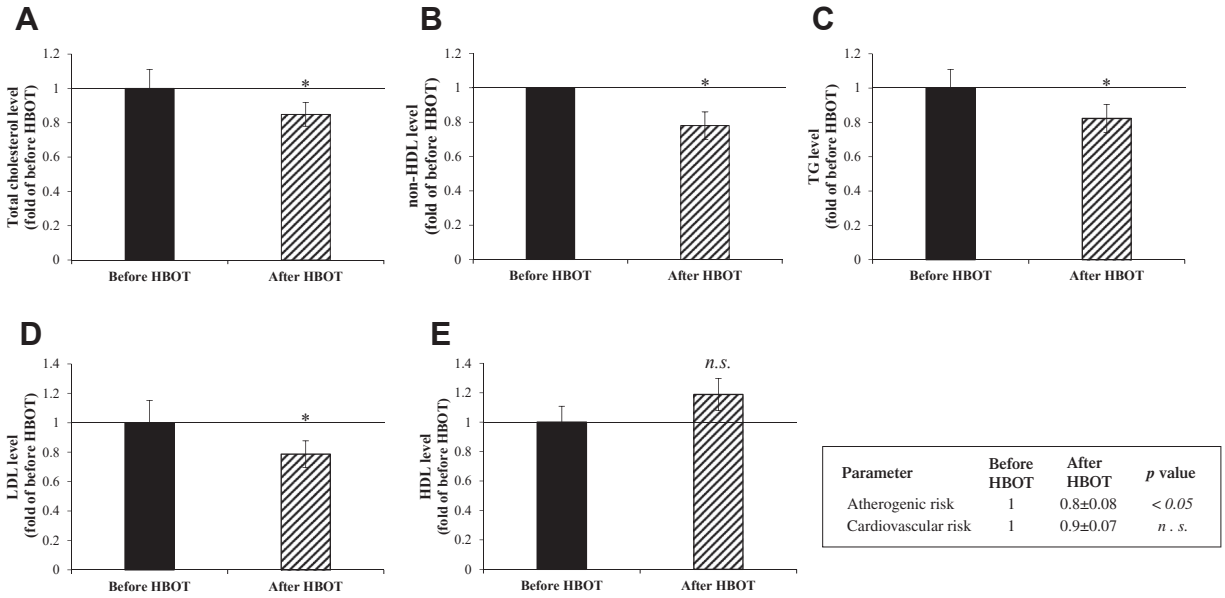

Figure 1. Effect of HBOT on lipid profile in the plasma of T1DM patients. (A) Level of total cholesterol in plasma, expressed as fold change vs before HBOT (arbitrary control set at 1). (B) Level of non-HDL in plasma, expressed as fold change vs before HBOT (arbitrary before HBOT set at 1). (C) Level of TG in plasma, expressed as fold change vs before HBOT (arbitrary before HBOT set at 1). (D) Level of LDL in plasma, expressed as fold change vs before HBOT (arbitrary before HBOT set at 1). (E) Level of HDL in the plasma, expressed as fold change vs before HBOT (arbitrary before HBOT set at 1). Inset: Atherogenic and cardiovascular risk assessment after HBOT (n=21–23); *p<0.05. *HBOT*, Hyperbaric oxygen therapy; *HDL*, high-density lipoprotein; *T1DM*, type 1 diabetes mellitus; *LDL*, low-density lipoprotein; *n.s.*, not statistically significant; *TG*, triglyceride.

The literature data show that T1DM patients have increased SFA levels in the plasma (38). We have found that HBOT did not change the total amount of SFA in plasma, although it significantly changed the level of specific SFAs—increasing the level of PA and decreasing the level of SA. Increased levels of PA and SA can trigger an inflammatory response by increasing secretion of proinflammatory cytokines and expression of transcription factors involved in inflammation, such as NF-κB (41). This profile, with increased levels of PA and SA, is often found in metabolic disorders and is considered unfavourable because PA has higher atherogenic potential than SA (42). In contrast, our results show that HBOT had no effect on the total amount of MUFA, whereas the level of palmitoleic acid was significantly increased. Talbot et al reported that, even the presence of an individual MUFA, palmitoleic acid is sufficient to prevent inflammatory activity induced by PA (43). These results suggest that exposure of T1DM patients to HBOT could be in part responsible for a reduction in inflammatory response triggered by PA in plasma.

Table 2
Changes in the plasma FA composition after HBOT in T1DM patients

FA	Before HBOT (%)	After HBOT (%)	p value
SFA	40.6±0.3	40.3±0.4	n.s.
Palmitic acid (16:0)	27.1±0.3	28.1±0.3	<0.05
Stearic acid (18:0)	13.3±0.2	12.4±0.2	<0.05
MUFA	16.7±0.4	17.0±0.5	n.s.
Palmitoleic acid (16:1)	1.3±0.1	1.7±0.1	<0.05
Vaccenic acid (18:1n-7)	1.6±0.1	1.6±0.1	n.s.
Oleic acid (18:1n-9)	13.4±0.4	14.2±0.4	n.s.
n-3 PUFA	3.5±0.2	3.8±0.1	n.s.
Alpha linolenic acid (18:3n-3)	0.3±0.04	0.2±0.02	<0.05
Eicosapentaenoic acid (20:5n-3)	0.5±0.05	0.5±0.04	n.s.
Docosapentaenoic acid (22:5n-3)	0.5±0.02	0.6±0.03	<0.05
Docosahexaenoic acid (22:6n-3)	2.1±0.1	2.5±0.1	<0.01
n-6 PUFA	39±2.1	38.7±2.1	n.s.
Linoleic acid (18:2n-6)	25.3±0.04	23.2±0.04	<0.01
Gamma linolenic acid (18:3n-6)	0.8±0.04	0.8±0.01	n.s.
Dihomo gamma linolenic acid (20:3n-6)	2.9±0.3	3.3±0.2	n.s.
Arachidonic acid (20:4n-6)	10.5±0.4	10.5±0.3	n.s.
Docosatrienoic acid (22:4n-6)	0.4±0.03	0.4±0.02	n.s.
Correlation analysis of CRP (mg/L) and docosahexaenoic acid (% of total FA) after HBOT (n=23)		r=+0.508	<0.05

CRP, C-reactive protein; *FA*, fatty acid; *HBOT*, hyperbaric oxygen therapy; *MUFA*, monounsaturated fatty acid; *n.s.*, not statistically significant; *PUFA*, polyunsaturated fatty acid; *SEM*, standard error of the mean; *SFA*, saturated fatty acid; *T1DM*, type 1 diabetes mellitus.
Note. Data are expressed as percent of plasma total FAs (mean ± SEM).

Our findings show that exposure of T1DM patients to HBOT did not change the total amount of n-3 PUFA, but it significantly increased the level of DPA and DHA and decreased the level of ALA. This decrease in ALA level could be explained by the effect of HBOT on the induction of higher conversion of ALA to longer chain n-3 PUFA, considering also that ALA is a well-known precursor for synthesis of n-3 series PUFAs, such as DPA and DHA (42). Because we observed an increased conversion of ALA to DPA and DHA in plasma after HBOT, it would be of interest for our future work to explore the synergistic effect of treatment with n-3 PUFA supplementation and HBOT. Furthermore, in our previous study (26), we observed that the level of CRP was reduced after HBOT in T1DM patients. In the current study, we observed a significant correlation between CRP and DHA level in T1DM patients after HBOT. These results suggest that exposure of T1DM patients to HBOT leads to a reduction of inflammation and slowed progression of atherosclerosis. Recent studies indicated that an elevated level of DHA exerted strong anti-inflammatory and cardioprotective effects in the plasma of T2DM patients (44,45), and reduced inflammation by decreasing the generation of reactive oxygen species, production of proinflammatory cytokines and transcription of genes encoding proteins involved in the inflammatory response (e.g. NF-κB) (6,9). Also, an increased level of DHA is associated with a less atherogenic profile of LDL, by increasing the LDL size and reducing levels of TG, thus improving vascular function (46). Our results show that HBOT

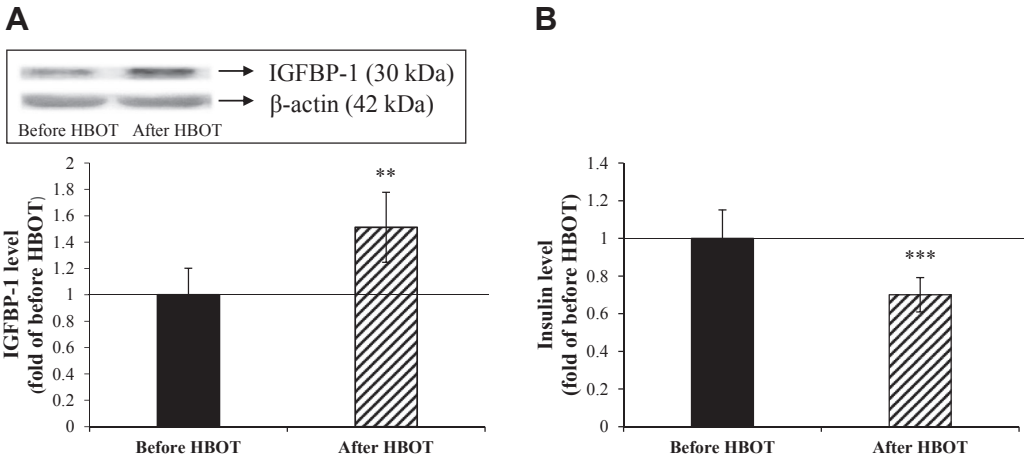

Figure 2. Effects of HBOT on the serum levels of IGFBP-1 and insulin in T1DM patients. (A) Level of IGFBP-1 in the serum, expressed as fold change of before HBOT (arbitrary control set at 1). (B) Level of insulin in the serum, expressed as fold change of before HBOT (arbitrary control set at 1). Inset: Representative Western blot (n=20). $^{***}p<0.001$; $^{**}p<0.01$. *HBOT*, Hyperbaric oxygen therapy; *IGFBP-1*, insulin-like growth factor binding protein 1; *T1DM*, type 1 diabetes mellitus.

exerts similar effects as dietary intake of long-chain n-3 PUFAs, which is of great interest in the context of their anti-inflammatory and antioxidative properties.

Because most of the n-6 PUFAs are well-known promoters of inflammation, and a strong association between level of n-6 PUFA and T2DM was observed (47), we also evaluated the level of total n-6 PUFA in T1DM patients after exposure to HBOT. We found that HBOT significantly decreased the plasma level of a specific n-6 PUFA, LA. Considering that increased LA can alter the function of proteins, increase the production of reactive oxidative spaces (48) and stimulate the transcription of NF-κB (49), and also considering our current and previous results (26), we suggest that HBOT modifies signalling pathways and transcription factors involved in the inflammatory response by changing the FA composition in T1DM patients. Changes in FA composition caused by HBOT may be partially responsible for the beneficial effects of HBOT on the vascular function of T1DM patients. The mechanism by which HBOT exerts its effects on FA composition is still unclear. However, Brown et al showed that HBOT at 2.2 ATA stimulated synthesis and elongation of FAs, and also changed the relative amounts of individual FAs in membranes by affecting enzymes involved in FA syntheses, such as elongase and desaturase (50). In the current study, we also found that after HBOT relative activity of elongase enzyme was increased (data not shown).

It was shown that insulin and IGF-1 with its binding proteins play an important role in the pathophysiology of vascular complications in T1DM and T2DM (51,52). Therefore, we aimed to explore the effect of HBOT on levels of insulin and IGFBP-1 in the blood. Our findings demonstrate that the insulin level was significantly decreased after exposure to HBOT. This effect of HBOT in T1DM patients could be explained by better T1DM control in the hospital environment, including prepared meals with specified portions and caloric intake and the absence of everyday stress. In addition, numerous studies in T1DM and T2DM patients showed that HBOT may improve insulin sensitivity, followed by decreased blood glucose levels (53–56). Beneficial effects of HBOT in foot ulcer healing or early beneficial effects on peripheral blood vessels' endothelial layer function can significantly decrease hyperglycemia, as these can be the source of poor T1DM and T2DM control (55,56). A positive influence of HBOT on control of T1DM may have led to the reduced insulin requirements, but the underlying mechanisms of HBOT effects in T1DM require further investigation.

IGFBP-1 has a major role in lipid and glucose metabolism, namely IGFBP-1, decreases lipid accumulation in different cells and enhances glucose uptake in peripheral tissues (57). Increased levels of IGFBP-1 are associated with vascular complications in T1DM patients (58). Because HBOT is known to reduce vascular complications in T1DM and T2DM patients (21), one of the aims of our study was to investigate whether HBOT affects the level of IGFBP-1 in the serum of T1DM patients. Surprisingly, we found that IGFBP-1 level was increased after HBOT. Regulation of IGFBP-1 is dependent on several factors, but the most important is insulin, and hepatic production of IGFBP-1 is inversely regulated by insulin in the circulation (58). The reduced level of insulin after HBOT observed in our study could be a reason for the increased level of IGFBP-1. Also, the aforementioned HBOT effects occur early, so at this time we cannot speculate on the further direction of serum IGFBP-1 change. However, conflicting results from other studies (58) exploring the effects of IGFBPs in T1DM and the lack of data on HBOT effects on IGFBPs levels call for further investigation, especially with regard to delayed effects. According to genetic studies, some additional epigenetic analyses of the IGFBP-1 gene may elucidate the link between the change in IGFBP-1 level and micro/macrovascular complications in T1DM patients (20).

This study has several limitations. As this was a pilot study, we recruited T1DM patients with different comorbidities and concomitant diseases and various drugs used for their management, and we observed wide individual variability in the parameters measured. Also, we included T1DM patients of both genders, with the exception of menstruating women (to exclude the beneficial effects of estrogen on vascular endothelium). Also, the number of patients was small, and we did not have any specific information about dietary data on the patients studied. We also recognize and appreciate that those interindividual variations in diet, medications, lifestyle, smoking and other environmental exposures can influence the FA composition of plasma phospholipids. Because of the small number of patients included in our study, we were unable to confirm definitively the effect of HBOT. In future work, we plan to recruit a larger number of T1DM patients to follow up all parameters assessed in this study after 10 HBOT treatments to estimate the long-term effects of the therapy. We also plan to follow up fluctuations of the studied parameters after each treatment to establish the minimum number of treatments needed to obtain beneficial effects in T1DM patients with vascular complications. Further study is necessary to elucidate the effects of confounding factors and more clearly outline the molecular mechanisms of HBOT's effects on the vascular endothelium.

In conclusion, in this work, we have identified a significant HBOT effect on FA composition and IGFBP-1 expression in T1DM patients. Based on our results, we suggest that HBOT exerts anti-inflammatory and antiatherogenic effects in T1DM patients, probably by a mechanism that involves changes in the lipid profile and plasma FA composition. The improvement seen in these patients after HBOT can contribute to a reduction in vascular complications. Our study is the first to examine the effects of HBOT on FA composition and IGFBP-1, and these observations deserve more attention and additional research. Future studies on a larger population and including dietary information are needed to better understand the molecular mechanism by which HBOT regulates FA composition and reduces vascular complications in T1DM patients. Both frequency and dose of HBOT still need to be determined to identify the maximum efficiency and minimum side effects in the treatment of T1DM patients.

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Author Disclosures

Conflicts of interest: None.

Author Contributions

I. Resanović, E. Sudar-Milovanović and B. Zarić performed the research, collected and analyzed the data and wrote the paper; D. Milačić recruited the patients and collected the data; A. Arsić, M. Šunderić and N. Gligorijević performed the research and analyzed the data; V. Vučić and O. Nedić critically reviewed the paper and provided funding; Z. Gluvić and E. R. Isenović designed the research, provided the funding and wrote and critically reviewed the article.

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