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Original Research

Investigation of endocrine and immunological response in fat tissue to hyperbaric oxygen administration in rats

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Abstract: Though HBO treatment is becoming more common, the mechanism of action is not fully known. The positive effects of HBO administration on the inflammatory response is thought to be a possible basic mechanism. As a result, we aimed to research whether endocrine and immunological response of fat tissue changes in rats given HBO treatment model. This research was carried out on Wistar albino rats, they were treated with hyperbaric oxygen therapy. Their fatty tissue were taken from the abdomen, gene expression of the cytokines and adipokines were analyzed with Real time PCR method. When the gene expression of hormones and cytokines by fat tissue was examined, the leptin, visfatin, TNF-α, IL-1β and IL-10 levels in the HBO treatment group were statistically significantly increased compared to the control group (p=0.0313, p=0.0156, p=0.0156, p=0.0156, p=0.0313). In conclusion, in our study we identified that HBO administration affected the endochrinological functions of fat tissue.

Key words: Hyperbaric oxygen treatment, fat tissue, adipokines, cytokines.

Introduction

Hyperbaric oxygen (HBO) treatment is administration of 100% oxygen at pressure higher than atmospheric pressure at sea level in a special environment (1). Since the 1900s it has been used as therapeutic, and is reported to be effective for diseases such as acute carbon monoxide intoxication, decompression disease, air embolism, necrotizing fasciitis, chronic refractory osteomyelitis, diabetic foot injuries and crush injuries (2). Though HBO treatment is becoming more common, the mechanism of action is not fully known. The positive effects of HBO administration on the inflammatory response is thought to be a possible basic mechanism (3).

Until recent years it was accepted that fat tissue is basically responsible for triglyceride storage. In 1994 with the discovery of the first adipokine, it was accepted that fat tissue is an independent endocrine organ. In white adipose tissue in adults, synthesis of a variety of cytokines such as IL1, IL6 and TNF-α, chemokines and hormone-like factors like adiponectin and leptin (adipokines) has been identified. Adipokines are important in regulation of a variety of processes including glucose and lipid metabolism, energy balance, nutritional behavior, insulin sensitivity, inflammation, immunity, adipogenesis, vascular function or coagulation (4).

There are many diseases treated with HBO administration, or where it leads to improvements in pathogenesis, that are known to be related to adipokines and cytokines released from fat tissue. As a result, we aimed to research whether endocrine and immunological response of fat tissue changes in rats given a HBO treatment model.

Materials and Methods

Animals

The research is performed in Canakkale Onsekiz Mart University Experimental Research Center (COMUDAM). Wistar Albino rats weighing 200-350 g (4-5 months old) are used in this research. All the rats are housed in controlled temperature (23–25 °C), 12-h light/dark cycles (light, 08:00–20:00 h; darkness, 20:00–08:00 h), and given free access to food and tap water. All the rats used in present study received human care in compliance with institutional animal care guidelines, and were approved by the Local Ethics Committee. All experimental procedures were performed in accordance with ethical animal care guidelines.

Study design

Group I (Control group, n=7): The rats in this group were given two sessions per day in a hyperbaric oxygen tank for seven days, respiring normal room air. There was 6 hours between sessions and each session lasted 60 minutes. At the end of the study rats were euthanized and at least 100 mg fat tissue was removed from the abdominal region and used in analysing the gene expression levels of adipokines.

Group II (HBO treatment group, n=7): The rats in this group were given two sessions per day of hyperbaric oxygen treatment for 7 days. Hyperbaric oxygen treatment was given in a hyperbaric oxygen

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Table 1. The primer series for the genes.

Primers		
Hormones secreted from adipose tissue		
Adiponectin	F:TGTTGGAATGACAGGAGCTGAA R:*CACTGAACGCTGAGCGATACA*	(31)
Resistin	F:CCTTTTCTTCCTTGTCCCTGAA R:*ACAGGGAGTTGAAGTCTTGTTTGAT*	(31)
Visfatin	F:TTTTGAACACATAGTAACACAGTTCTCATC R:*GGTCTTCACCCCATATTTTCTCA*	(31)
Leptin	F:CAGCCTGCCTTCCCAAAA R:*CATCCAGGCTCTCTGGCTTCT*	(31)
Cytokines		
TNF-alpha	F:ACTGAACTTCGGGGTGATCGGT R:*TGGTTTGCTACGACGTGGGCTA*	(32)
IL-1Beta	F: AATGCCTCGTGCTGTCTGACCCAT R:*CCAAGGCCACAGGGATTTTGTCGTT*	(32)
IL-10	F: AAAAGCAAGGCAGTGGAGCAGGTG R:*TGGCCTTGTAGACACCTTTGTCTTG*	(32)
IL-6	F: ACCACTTCACAAGTCGGAGGCTT R:*CTGACAGTGCATCATCGCTGTTCA*	(32)

tank with 100% oxygen given at 2.4 atm pressure. There was 6 hours between sessions and each session lasted 60 minutes. At the end of the study rats were euthanized and at least 100 mg fat tissue was removed from the abdominal region and used in analysing the gene expression levels of adipokines.

HBO administration

HBO therapy was administered in a hyperbaric tank produced for use with small laboratory animals, for 1 hour at 2.4 atm pressure respiring 100% oxygen. The chamber was flushed with 100% oxygen at a rate of 5 L/min to avoid carbon dioxide accumulation. The pressure chamber temperature was maintained between 23 and 25 °C. To minimize the effects of diurnal variation, all HBO exposures were started at approximately 9:00 am and 05:00 pm.

qReal-Time PCR procedure

At the end of the experimental procedure rats in both groups had 100 mg visceral fat tissue removed and these were stored in DNAse/RNAse free tubes at -80 °C until the beginning of genetic analyses. Of the 100 mg fat tissue obtained from each rat, 25 mg was homogenized (RETSCH MM 400 Tissue Homogenizer) and total RNA sampling procedure was begun immediately with the aid of a kit (PureLink® RNA Mini Kit, 50 rxns). All isolated RNA samples underwent cDNA synthesis procedure manually (High-Capacity cDNA Reverse Transcription Kit, 200 rxn). Analysis of expression of the stated genes was completed using Power SYBR® Green PCR Master Mix, 1 x 5 mL (200). With the qRT-PCR method gene normalization of the gene expression levels examined was completed with the house-keeping gene of β-actin. All expressions were determined using a StepOne™ Real-Time PCR System (Applied Biosystems®) device. The primer series for the genes with expression levels evaluated in fat tissue are given in the table 1.

Statistical analysis

Initially beta-actin normalization was completed. To determine expression differences, the 2-ΔCt method (Ct Target gene- Ct Reference gene) was used for calculations. Statistical results were completed with the GraphPad Prism program. The Wilcoxon rank test was used to compare groups and p value <0.05 was accepted as statistically significant.

Results

In our study when the gene expression of hormones synthesized by fat tissue is examined, the leptin and visfatin levels in the HBO treatment group were statistically significantly increased compared to the control group (p=0.0313, p=0.0156) (Figure 1). In our experiment there was a increase observed in adiponectin levels, however this was not statistically significant. There was a reduction in resistin levels in the study group given HBO compared to the control group. However this change was not found to be statistically significant. Again in fat tissue when the gene expression of cytokines are investigated, there was a statistically significant increase in TNF-α, IL-1β and IL-10 levels compared to the control group (p=0.0156, p=0.0156, p=0.0313) (Figure 2).

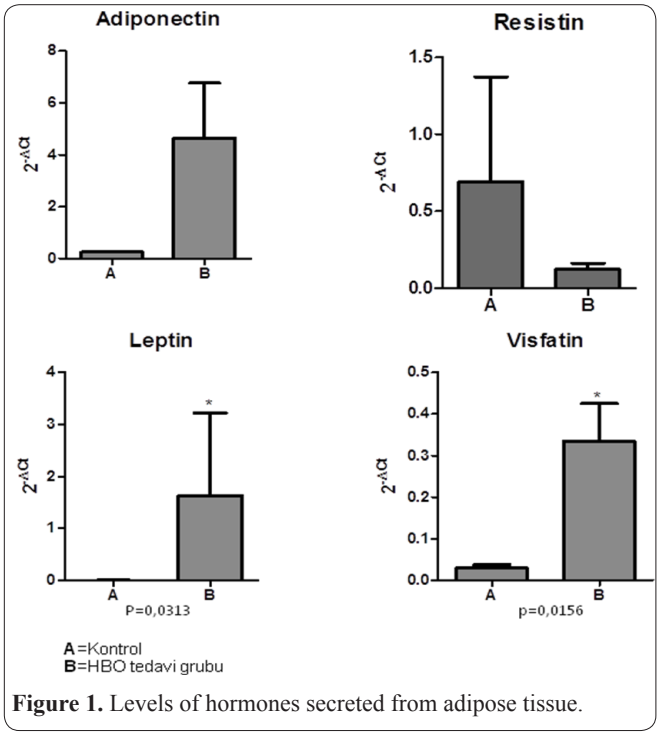

Figure 1. Levels of hormones secreted from adipose tissue.

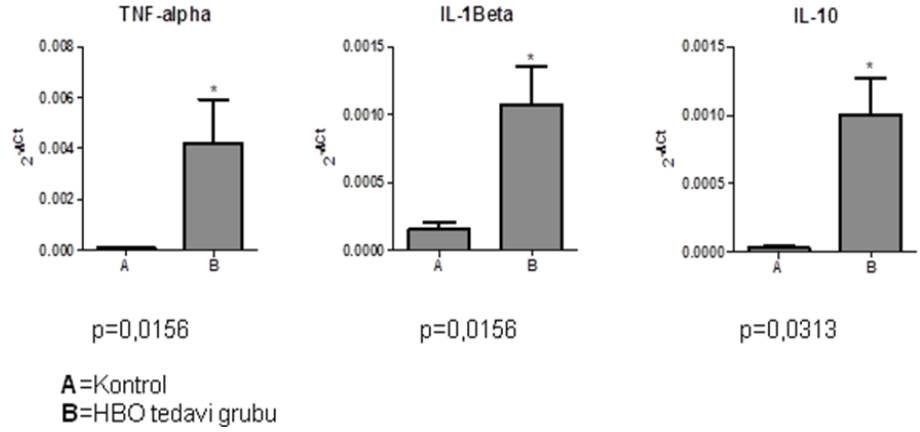

Figure 2. Levels of cytokines.

Discussion

In our animal study HBO administration was shown to be effective on hormone and cytokine synthesis functions of fat tissue. We identified that HBO therapy is increasing leptin and visfatin levels significantly. Additionally we observed that HBO administration significantly increased the levels of cytokines released from fat tissue (TNF α, IL-1β and IL-10). We found that HBO treatment was effective on the synthesis of other adipokines (adiponectin and resistin).

Leptin was the first hormone synthesized by adiopcyte to be discovered. Leptin levels are pulsatile and are related to fat tissue/BMI (5). Leptin is synthesized by adipocytes and affects through receptors on target cell surfaces in the central nervous system and peripheral tissues. It plays an important role in regulating satiation, appetite, nutritional intake, reproduction functions, activity and energy expenditure (6,7,8,9). In the hypothalamus it causes effects of reducing appetite (10). It is known that in obese individuals levels are low or there is resistance to its effect. Currently it is used for congenital leptin deficiency and treatment of lipodystrophy cases (11). Studies have emphasized that it may be used in treatment of diseases such as dyslipidemia, infertility and eating disorders like anorexia nervosa (12). In our animal study HBO administration caused a statistically significant increase in leptin synthesis. This result of our study leads to the consideration that HBO administration may be used for treatment of many diseases such as obesity and eating disorders in the future.

Visfatin, another adipokine synthesized by fat tissue, was described in 2005 by Fukura et al. Previously it was thought to have insulinomimetic effects (13). Many studies have shown that visfatin is related to obesity, insulin resistance, DM and chronic inflammation. Animal studies have identified that in DM groups given visfatin, insulin secretion in the pancreas increased and blood glucose levels fell. Visfatin is accepted as important for the regulation of pancreas beta cell functions. Especially in DM cases, increases in visfatin levels are reported as a possible physiological response to protect beta cell functions (14). In our study there was a statistically significant increase in visfatin levels identified with applied HBO therapy.

Adiponectin is an apidokine specifically synthesized by adipocytes and levels of 3-30 μg/ml have been identified in human plasma (15). Adiponectin levels in plasma are inversely related to fat deposition in the body, especially visceral fat, and plasma adiponectin levels in obese individuals are low (16). Clinical studies have shown that low adiponectin levels in plasma are related to many diseases linked to obesity (17,18). Additionally hypoadiponectinemia is related to insulin resistance, type 2 DM, HT, metabolic syndrome and atherosclerosis (17,19, 20, 21). Many studies have shown adiponectin has anti-inflammatory effect, and that reduced plasma levels may affect chronic inflammation forming the basis of the majority of obesity-sourced diseases (15).

In obese individuals increased visceral fat tissue, together with hypertrophy of adipocytes, increases hypoxia and inflammation in fat tissue. Several studies report that hypoxia may occur within adipose tissue due to the obesity-associated expansion of adipocytes and a concomitant reduction in capillary density and blood flow (22,23). Hypoxia formed by reduced blood flow causes disruption of fat tissue functions and reduces adiponectin synthesis. Cell culture studies have shown that oxygen intensity in different tissues is linked to different adiponectin levels, and as oxygen amount increases, there is a significant increase in adiponectin levels (24). In our study, HBO treatment increased oxygen presentation in fat tissue and in parallel an increase in adiponectin production was observed.

Currently it is known that adiponectin has anti-diabetic, anti-atherogenic and anti-inflammatory effects. In recent years studies on its usage in diabetes treatment have been emerged. In the near future increasings in adiponectin synthesis or its effect on pathways will comprise treatment aims. We believe the increase in adiponectin synthesis obtained with the HBO administration in our study may be a road marker in this sense.

Resistin is an adipokine synthesized in fat tissue in rodents. Synthesis is suppressed by inflammatory cytokines (for example, TNF-alpha) (25). In humans this is synthesized mainly by macrophages in fat tissue with synthesis of increased inflammatory cytokines (26). Studies have shown a close relationship between resistin and insulin resistance, atherosclerosis and diseases related to inflammation (27). In our study, there was a reduction in resistin gene expression in the HBO treatment administered group, however this was not statistically significant. In our study TNF-α increased with HBO administration, which we believe this may be linked to the reduction in resistin levels.

TNF-α is a proinflammatory adipokine with a central role in inflammatory and autoimmune diseases. It disrupts insulin secretion and insulin efficacy and is closely related to obesity, inflammation and DM. Other proinflammatory cytokines synthesized by fat tissue are immune cells of IL-6 and IL-1β. Similarly these are related to inflammatory diseases and DM development (28). IL-10 is an anti-inflammatory cytokine synthesized in fat tissue and though expression from fat tissue is known to increase in obese individuals, there is no known metabolic effect (29).

In our study we observed HBO administration increased the expression of TNF-α, IL-10 and IL-1β cytokines in fat tissue. We found that both inflammatory and anti-inflammatory cytokines increased together. Studies have shown that with increased oxygen amount in fat tissue, there is a migration of inflammatory cells to the region and synthesis of cytokines is induced (30). When we look at the literature, in rats with induced brain damage HBO therapy was administered and serum IL-6, IL-1β and IL-10 levels were studied. While there was a clear reduction in IL-6 and IL-1β levels, there was an increase observed in IL-10 levels (30). The brain damage induced in this study increased inflammatory cytokine levels and we believe that after HBO application there was a reduction in inflammatory cytokines linked to healing of injury. In our study healthy rats were used, and we believe that the increasing in IL-10 with anti-inflammatory effects are significantly high, similar to the previous study.

Successful results have been obtained through the using of HBO administration for many diseases; however the mechanism of action is not fully understood. The results of our study lead to the consideration that success in HBO treatment for diseases such as diabetic foot, necrotizing fasciitis and chronic refractory osteomyelitis may be linked to increases in IL-10 levels.

In conclusion, in our study we identified that HBO administration affected the endochrinological functions of fat tissue. Observation of significant increases in leptin, visfatin and IL-10 levels, leads to the consideration that in near future HBO administration may be applied as treatment for obesity, DM, eating disorders and obesity related diseases. More comprehensive studies designed for obesity, are required.

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