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Investigation of Vascular Endothelial Growth Factor and Endostatin Levels in Some Rat Tissues in Response to Cold Stress and Diet

Filiz Çoban[1], Muhittin Yürekli[2*]

1 Inönü University, Institute of Science and Technology, Malatya, TR
2 Inönü University, Faculty of Arts and Sciences, Malatya, TR

*** Corresponding Author**: Muhittin Yürekli **E-mail:** muhittin.yurekli@inonu.edu.tr

ABSTRACT

Objective: Obesity, the disease of our age, is a condition that occurs when there is an excess of fat tissue in the body. It is not merely a concern about weight gain, but rather a medical issue that elevates the risk of various diseases including heart disease, diabetes, high blood pressure, and certain cancers. This study aimed to explore the impact of a high-fat diet under normal conditions and cold stress, as well as the influence of propolis as a dietary supplement, on vascular endothelial growth factor (VEGF) and endostatin levels in rats fed with propolis.

Material and Methods: Thirty-six 3-month-old female Wistar rats (6 rats in each group) sourced from Inonu University Experimental Animal Production and Research Center were utilized for the study. Propolis was administered by gavage, dissolved in water, at a dosage of 2 mL per day for two weeks.

Results: The group exhibiting at least a 20% increase in weight due to high-fat diet consumption was categorized as the obese group. Tissues including heart, liver, lung, brown adipose, and white adipose tissues were procured from the obese, propolis-treated, and control groups. Endostatin and vascular endothelial growth factor levels were assessed in the tissues using the ELISA method. The study revealed an elevation in VEGF levels in brown adipose tissue in both cold stress and propolis treatment groups, accompanied by a reduction in white adipose tissue compared to the control group. Additionally, VEGF levels displayed a general increase in lung, liver, and heart tissues. Conversely, endostatin levels, an antiangiogenic factor, decreased in brown adipose tissue while increasing in white adipose tissue. In liver, lung, and heart tissues, endostatin levels exhibited a general decrease.

Conclusion: The findings suggest that both cold stress and propolis treatment influence VEGF and endostatin levels in various rat tissues, indicating potential implications for obesity-related conditions and angiogenesis regulation.

Keywords: VEGF, Obesity, Endostatin, Propolis

Research Article

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INTRODUCTION

Adipose tissue is an important metabolic organ and plays a key role in energy homeostasis. Depending on cell morphology and tissue function, mammals have two types of adipose tissue: white and brown. These adipose tissues have different physiological roles: White adipose tissue (WAT) is highly adapted to store excess energy in the form of triglycerides, while Brown adipose tissue (BAT) radiates energy to generate heat by converting glucose and fatty acids into the resulting proton-movement force. Brown adipose tissue thermogenesis depends on uncoupling protein 1 (UCP1), specifically expressed in brown fat mitochondria and responsible for the unique metabolic function of brown adipose tissue. UCP1 is known to distribute the proton gradient across the inner mitochondrial membrane, thereby uncoupling the electron transfer system from adenosine triphosphate synthesis, resulting in energy dissipation as heat. Brown adipose tissue plays a crucial role in regulating energy expenditure to adapt to cold environments (1).

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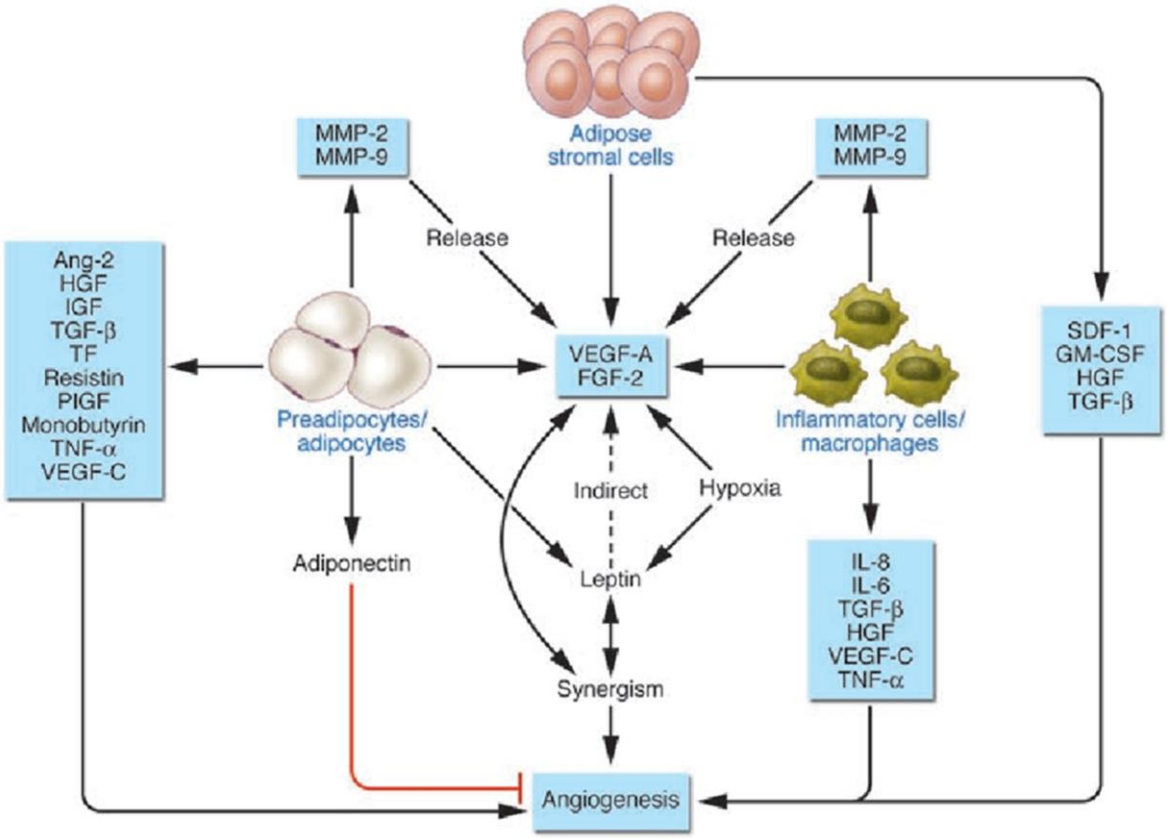

Figure 1. Regulation of adipose tissue angiogenesis by multiple factors (22).

Table 1. Angiogenic and antiangiogenic factors involved in angiogenesis

Angiogenesis inducers	Angiogenesis inhibitors
VEGF (Vascular endothelial growth factor)	Thrombospondin- 1
PGF (Placental growth factor)	Endostatin
FGF (Acidic, basic fibroblast growth factor)	Vasostatin
TGF-α (Transforming growth factor-α)	Vascular eldothelial growth factor inhibitor
TGF-β (Transforming growth factor-β)	Platelet factor-4 fragment
EGF (Epidermal growth factor)	Prolactin derivative
HGF (Hepatocyte growth factor)	Restin
TNF-α (Tumor necrosis factor-α)	Interferon-α-β
PDGF (Platelet-derived growth factor)	Angiopoetin-2
GCSF (Granulocyte colony stimulating factor)	Antithrombin-3 fragment
IL-8 (Interleukin-8)	Interferon-inducible protein- 10
Angiogenin	
Proliferin	

Adipose tissue is highly vascularized and each adipocyte is supplied by an extensive capillary network [2, 3, 4]. Adipose tissue growth is dependent on angiogenesis and can be inhibited by angiogenesis inhibitors. Treatment with angiogenesis inhibitors leads to weight loss and adipose tissue loss, adipose tissue mass is sensitive to angiogenesis inhibitors, and its vascularization can be regulated [5, 6, 7]. Angiogenesis, the formation of new capillaries from existing blood vessels, is a complex, multi-stage process involving a series of cellular events leading to new vascularization [8, 9]. Angiogenesis plays a central role in various physiological processes in the human body during fetal development and tissue repair after surgery or trauma. Angiogenesis is a hallmark of wound healing, the menstrual cycle, cancer and various ischemic and inflammatory diseases [10, 11].

The realization that tumor growth is associated with new blood vessels has led to the search for biochemical factors mediating angiogenesis, expanding knowledge of pathological processes and opening new possibilities for diagnosing and treating diseases [12]. The basic process of angiogenesis can be simply described as multiple stages. First, the stimuli of angiogenesis cause increased endothelial cell permeability and cell proliferation or cell division as the newly formed capillaries elongate (13). The second is the degradation of basement membrane components by proteolysis, which promotes the invasion of endothelial cells into the stroma of adjacent tissue, where the joint activity of the plasminogen activator (PA) system and matrix metalloproteinases (MMPs) is required (14). Third, since the newly formed capillaries form a multicellular structure,

migrating endothelial cells trigger lumen formation, forming a new capillary channel. Finally, the capillary membrane, adhesive junction and endothelial cells are formed.

Adipose Tissue-derived Angiogenesis Factors

Growing adipose tissue contains a variety of cell types, including adipocytes, adipose stromal cells, endothelial cells and inflammatory cells (15). The diversity of heterogeneous cell populations determines the expression of multiple growth factors and cytokines that individually or co-regulate vessel growth, but little is known about the functional interaction between these factors (16). Growing adipocytes contain angiogenic factors such as leptin, VEGF, FGF-2, HGF, IGF, TNF-α, TGF-β, placental growth factor (PlGF), VEGF-C, resistin, tissue factor (TF), neuropeptide Y (NPY), heparin-binding epidermal growth factor (17-19). Preadipocytes and adipocytes also produce small non-protein lipid molecules such as monobutyrin, stimulating angiogenesis in adipose tissue (20, 21). The regulation of adipose tissue angiogenesis by multiple factors is shown in Figure 1 (22).

VEGF plays a central role in the growth or development of most healthy and pathological tissues. The omentum expresses the highest level of VEGF among all adipose tissues studied in the body (23). Localization studies have shown that adipocytes are the primary source of VEGF, which can function as a factor of angiogenesis and vascular survival for the omental vasculature. In addition, adipose-infiltrating inflammatory cells and adipose stromal cells also contribute significantly to VEGF production (15, 24, 25).

Inactivation of PlGF (placental growth factor) function in mice results in impaired adipose tissue development due to defective angiogenesis, suggesting that other VEGF members also modulate adipogenesis through the vascular system (26). Gene expression profiling analysis in rat WAT shows a distinct pathway of adipokines including leptin, adiponectin and resistin growing against stagnant adipose tissues (17). Resistin, a specific adipokine, is a novel angiogenesis factor that directly promotes endothelial cell proliferation, migration and tube formation (22). IGF-1 and TNF-α are two other angiogenic factors increased in the expansion of adipose tissues (27). IGF-1 serves as a survival factor for various cell types and assumes a critical role in preserving vascular integrity within adipose tissue (28). In addition to its direct angiogenesis activity, TNF-α is a potent inflammatory cytokine that links inflammation, angiogenesis and adipogenesis (29). IL-8 becomes a survival factor for adipocytes in vivo, possibly through stimulation of angiogenesis (30). Thus, inflammatory cells in growing adipose tissues are coordinators for coupling the simultaneous events of adipogenesis and angiogenesis (Table 1) (22).

Endostatin, a naturally occurring 20-kDa C-terminal fragment, is derived from collagen XVIII through proteolytic cleavage at its carboxylic terminus. Endostatin administration has been reported to reduce adipose tissue expansion, inhibit the regulation of angiogenesis, and suppress tumor activity by interfering with the action of angiogenic factors such as vascular endothelial growth factor (VEGF) (218). Endostatin is an endogenous inhibitor of angiogenesis. It interferes with TNF a activation of C-Jun N-terminal Kinase, and blocks pro-angiogenic factors required for angiogenesis (5).

Propolis extracts are considered as nutritional products with potential for managing obesity and comorbidity. However, the composition of propolis extracts is highly variable and depends on the botanical origin of the plants used by bees to produce propolis. Propolis is a resinous substance collected by bees from buds, trees or shrubs in the plant ecosystem close to the hive. Propolis is used as a folk medicine in Europe, Asia and South America. The various biological and pharmacological effects reported for propolis activity are related to phenolic compounds, which can vary widely in quality and quantity depending on the plant source and place of collection. Three types of propolis, namely brown propolis from Populus sp., have been subjected to in-depth investigations in terms of identification, characterization, and biological activities. It is characterized by the presence of polyphenolic taxonomic markers such as pinocembrin, chrysin and galangin, and substituted cinnamic acids, especially phenylethyl caffeate (CAPE) esters. Green propolis from Baccharis dracunculifolia from the Minas Gerais region in Brazil is characterized by the presence of artepellin C, p-coumaric and drupanin as taxonomic markers and red propolis from Dalbergia ecastophyllum from the Algolas region. Brazilian is characterized by the specific presence of isoflavans, isoflavones or pterocarpan class represented by vestitol, formononetin and medicarpin (31).

MATERIAL and METHODs

- Experimental Animals Used in the Study

Three-month-old female Wistar rats, bred by the İnönü University Experimental Animal Production and Research Center, were utilized in this study. The rats were housed in specialized cages under controlled conditions at 24°C, with a 12-hour light/dark cycle, until the commencement of the experiment. Rats weighing between 220-250 g were included in the study. The animals were divided into six groups:

Group 1 (Normal weight- Normal feeding): Fed with pellet feed without additives.

Group 2 (Obese group - High-fat diet): Obesity was achieved by high-fat diet (HFD).

Group 3 (Normal weight- Cold stress): They were fed with pellet feed without additives and cold stress was applied.

Group 4 (Obese group - Cold stress): They were fed a high-fat diet (HFD), and cold stress was applied.

Group 5 (Normal weight - Propolis treatment): They were fed with pellet feed without additives and propolis was applied.

Group 6 (Obese group - Propolis application - Cold stress): Rats in this group were fed a high-fat diet (HFD) and subjected to both propolis administration and cold stress.

Rats were weighed at the beginning of the experiment, and their weights were recorded. Afterwards, the rats were weighed every week until the day of slaughter and their weights were recorded.

- Normal Nutrition

Rats in the caloric fed groups were fed standard chow and water was given ad libitum. The feeds were placed on the cage.

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-High Fat Diet

Rats in the high-fat diet groups were fed with DIO Rodent Purified Diet w/60% Energy From Fat- Blue special rat chow and given as much water as they could drink.

-Propolis Application

In our study, we utilized liquid, alcohol-free commercial propolis produced in Turkey, sourced from Asitane company. Propolis was administered via gavage every day for a duration of 2 weeks at a dosage of 100 mg/kg/day, diluted in drinking water.

-Cold Stress Practice

The cages for cold stress application were kept at 12±2o C for 48 hours. Rats were controlled by observation during cold stress application.

-Tissue Harvesting

Firstly, 1500 µL/kg ketamine and 500 µL/kg xylazine were administered intramuscularly for anesthesia. The unconsciousness of the rats was controlled by pinching their hind legs with pliers. An incision was made in the abdomen of anesthetized rats and cut up to the rib cage. The rib cages were also opened, and the vena cava was cut. Perfusion was completed by injecting 5 ml of saline into the right and left ventricles of the heart.

-Collection and Homogenization of Liver, Lung, Heart, Brown and White Fat Tissues

After perfusion, the rat was euthanized by removing the heart and the tissues were removed one by one. Lung tissue and then some of the liver tissue were removed. They were re-perfused in saline and wrapped in labelled aluminum foils. Intestines were removed, and retroperitoneal white adipose tissue was removed. The dorsal part of the rat was opened and interscapular brown adipose tissue was removed. Each tissue was placed in liquid nitrogen without waiting.

After dissection, the tissues were removed from liquid nitrogen and stored at -40 °C until analysis. For homogenization, tissues were placed in pre-weighed microcentrifuge tubes, then weighed again. Subsequently, 20 µl/mg of PBS buffer (2 mM, pH 7.3) was added. Tissues were cut into smaller pieces with tissue scissors in the microcentrifuge tubes. Subsequently, tissues were sonicated using an ultrasonifier (PVC Kinematica Status) for 20 seconds on ice. The sonicated tissues were then stored at -40°C.

-Vascular Endothelial Growth Factor and Endostatin Measurement

The amount of VEGF and endostatin in liver, lung, heart, white and brown adipose tissue homogenate samples were determined with the Rat VEGF ELISA Kit (EK0540) and Rat Endostatin ELISA Kit (EK1377) manufactured by Boster Biological Technology Ltd. using a microplate reader (BioTek® Instruments, Inc., Eon) at 450 nm. As a result of the measurements, VEGF and Endostatin amounts were calculated using the standard graph according to the protocol given in the kit. GraphPad program was used for calculations.

-Statistical Method

Statistical evaluations were performed with SPSS for Windows Version 15.0 package program. Data related to measurable variables were given as mean ± standard error. The t-test method was used to determine the differences between the groups. The value found was evaluated at 5% significance level (95% confidence interval, $p<0.05$).

RESULTs

Weight (normal weight and obese), temperature (room temperature and cold stress treatment), nutrition (normal diet, high fat diet and propolis treatment) were taken into consideration in the evaluation of the findings. Among the groups formed according to these three factors, the "Normal weight and room temperature" treatment group was considered as the control group. **Table 2** shows the vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in brown adipose tissue increased in the obese- room temperature group compared to the normal weight- room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese-room temperature group (532.03±8.97ng/L). In the obese-room temperature group, there was a statistically significant increase compared to the normal weight propolis-fed room temperature group ($p<0.05$). Similarly, in the normal weight-cold stress group, a statistically significant increase was observed compared to the room temperature group ($p<0.05$). Furthermore, in the normal weight-propolis nutrition-room temperature group, there was a statistically significant increase compared to the room temperature group ($p<0.05$). In the obese-cold stress group, obese propolis nutrition showed an increase compared to the cold stress group and this increase was found to be statistically significant ($p<0.05$).

Table 2. Vascular endothelial growth factor (VEGF) levels in brown adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	244,40±23,43[a]
Obese - Room Temperature	532,03±8,97[b]
Normal Weight - Cold Stress	393,61±21,91[c]
Obese - Cold Stress	319,03±22,91[d]
Normal Weight - Propolis - Room Temperature	439,43±15,60[e]
Obese - Propolis - Cold Stress	257,46±14,47[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 3 shows the vascular endothelial growth factor (VEGF) levels in white adipose tissue. VEGF levels in white adipose tissue increased in the normal weight - room temperature group compared to the obese group - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the normal weight-room temperature group (394.36±21.53 ng/L). There was an increase in the obese group - room temperature group compared to the obese group - propolis nutrition - cold stress group, and this increase was found to be statistically significant ($p<0.05$). Obese group - cold stress group showed an increase compared to normal weight-cold stress group, which was found to be statistically significant ($p<0.05$).

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There was an increase in the obese group - propolis nutrition - cold stress group compared to the normal weight - cold stress group, and this increase was found to be statistically significant ($p<0.05$).

Table 3. Vascular endothelial growth factor (VEGF) levels in white adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	394,36±21,53[a]
Obese - Room Temperature	273,85±12,83[b]
Normal Weight - Cold Stress	63,99±4,88[c]
Obese - Cold Stress	149,80±11,52[g]
Normal Weight - Propolis - Room Temperature	82,36±4,35[e]
Obese - Propolis - Cold Stress	132,35±13,73[f]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 4 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in cardiac tissue increased in the obese-over-ambient temperature group compared to the normal weight-over-ambient temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was observed in the obese-room temperature group (228.62±9.11 ng/L). Additionally, there was a statistically significant increase in the obese-cold stress group compared to the obese-propolis nutrition-cold stress group ($p<0.05$). Moreover, there was a statistically significant increase in the obese-cold stress group compared to the normal weight-cold stress group ($p<0.05$). There was an increase in the obese-propolis nutrition-cold stress group compared to the normal weight-cold stress group and this increase was found to be statistically significant ($p<0.05$).

Table 4. Vascular endothelial growth factor (VEGF) levels in heart tissue in relation to obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	147,70±15,08[a]
Obese - Room Temperature	228,62±9,11[b]
Normal Weight - Cold Stress	74,52±4,37[c]
Obese - Cold Stress	203,72±13,50[d]
Normal Weight - Propolis - Room Temperature	156,56±11,86[a]
Obese - Propolis - Cold Stress	163,20±9,55[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 5 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in lung tissue increased in the obese-chamber temperature group compared to the normal weight-chamber temperature group and this increase was found to be statistically significant ($p<0.05$).

A statistically significant increase was observed in the obese-cold stress group compared to the obese-propolis nutrition-cold stress group ($p<0.05$).

The highest increase was recorded in the obese-cold stress group (236.50±12.29 ng/L). Furthermore, there was a statistically significant increase in the obese-cold stress group compared to the normal weight-cold stress group ($p<0.05$).

Table 5. Vascular endothelial growth factor (VEGF) levels in lung tissue concerning obesity, cold stress and propolis treatment.

Group	VEGF (ng/L)
Normal Weight - Room Temperature	122,66±8,39[a]
Obese - Room Temperature	166,98±8,43[b]
Normal Weight - Cold Stress	139,16±10,97[a]
Obese - Cold Stress	236,50±12,29[c]
Normal Weight - Propolis - Room Temperature	36,42±2,86[d]
Obese - Propolis - Cold Stress	193,62±8,77[e]

Statistically significant differences between groups, denoted by different letters, have been observed.

Table 6 shows vascular endothelial growth factor (VEGF) levels according to the groups. VEGF levels in the liver tissue increased in the obese-over-ambient temperature group compared to the normal weight-over-ambient temperature group, and this increase was found to be statistically significant ($p<0.05$). The obese-room temperature group had the highest increase (189.52±7.04 ng/L). There was an increase in the normal weight-propolis nutrition- room temperature group compared to the normal weight- room temperature group, and this increase was found to be statistically significant ($p<0.05$). A statistically significant increase was observed in the normal weight-cold stress group compared to the normal weight-room temperature group ($p<0.05$).

Table 6. Vascular endothelial growth factor (VEGF) levels in liver tissue concerning obesity, cold stress and propolis treatment.

Group	**VEGF (ng/L)**
Normal Weight - Room Temperature	88,32±5,43[a]
Obese - Room Temperature	189,52±7,04[b]
Normal Weight - Cold Stress	137,12±10,85[c]
Obese - Cold Stress	143,34±12,84[d]
Normal Weight - Propolis - Room Temperature	144,70±13,39[d]
Obese - Propolis - Cold Stress	138,12±14,70[d]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 7 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in brown adipose tissue decreased in the normal-cold stress group compared to the obese-cold stress group and this decrease was found to be statistically significant in the obese cold stress group ($p<0.05$). The most significant decrease was found in the obese-cold stress group (1.77±0.006 ng/L). A statistically significant increase was observed in the obese-propolis nutrition-cold stress group compared to the obese-cold stress group ($p<0.05$). There was an insignificant increase observed in the normal weight propolis nutrition room temperature group compared to the normal weight room temperature group ($p>0.05$).

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Table 7. Endostatin (ES) levels in brown adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	2,36±0,11[a]
Obese - Room Temperature	2,02±0,07[a]
Normal Weight - Cold Stress	2,28±0,18[a]
Obese - Cold Stress	1,77±0,06[b]
Normal Weight - Propolis - Room Temperature	2,12±0,08[a]
Obese - Propolis - Cold Stress	2,26±0,16[a]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 8 shows the treatment groups' vascular endostatin (ES) levels. ES levels in white adipose tissue increased in the obese - room temperature group compared to the normal - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese - room temperature group (3.00±0.14 ng/L). There was an increase in the obese - cold stress group compared to the normal - cold stress group, which was found to be statistically significant ($p<0.05$). A statistically significant increase was observed in the obese propolis nutrition group compared to the normal weight propolis nutrition room temperature group ($p<0.05$).

Table 8. Endostatin (ES) levels in white adipose tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,55±0,18[a]
Obese - Room Temperature	3,00±0,14[b]
Normal Weight - Cold Stress	1,33±0,14[a]
Obese - Cold Stress	2,59±0,14[c]
Normal Weight - Propolis - Room Temperature	1,96±0,14d
Obese - Propolis - Cold Stress	2,59±,021[c]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 9 shows vascular endostatin (ES) levels according to the groups. ES levels in cardiac tissue increased in the obese - room temperature group compared to the normal - room temperature group and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the obese - room temperature group (2.34±0.18 ng/L). In the normal weight-cold stress group, there was a statistically significant increase compared to the obese-cold stress group ($p<0.05$). Additionally, in the obese room temperature group, there was a statistically significant increase compared to the obese-cold stress group ($p<0.05$). A statistically significant increase was observed in the normal weight propolis nutrition room temperature group compared to the obese propolis nutrition cold stress group ($p<0.05$).

Table 9. Vascular endostatin (ES) levels in heart tissue in response to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,68±,12[a]
Obese - Room Temperature	2,34±0,18[b]
Normal Weight - Cold Stress	1,69±0,08[a]
Obese - Cold Stress	1,22±0,14[c]
Normal Weight - Propolis - Room Temperature	1,79±0,04[d]
Obese - Propolis - Cold Stress	1,41±0,08[e]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 10 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in lung tissue increased in the normal - room temperature group compared to the normal - propolis feeding - room temperature group, and this increase was found to be statistically significant ($p<0.05$). The highest increase was found in the normal - room temperature group (1.77±0.06 ng/L).

Table 10. Vascular endostatin (ES) levels in lung tissue in response to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	1,77±0,06[a]
Obese - Room Temperature	1,56±0,12[b]
Normal Weight - Cold Stress	1,14±0,05[c]
Obese - Cold Stress	1,34±0,13[d]
Normal Weight - Propolis - Room Temperature	1,40±0,11[b]
Obese - Propolis - Cold Stress	1,32±0,11[d]

The difference between the groups indicated by different letters has been found to be statistically significant.

Table 11 shows vascular endostatin (ES) levels according to the treatment groups. ES levels in liver tissue increased in the normal - cold stress group compared to the obese - cold stress group and this increase was found to be statistically significant ($P<0.05$). The highest increase was found in the normal - cold stress group (2.47±0.15 ng/L). In the normal room temperature group, there was a statistically significant increase compared to the obese room temperature group ($p<0.05$). Additionally, there was a statistically significant increase observed in the normal room temperature group compared to the normal propolis nutrition room temperature group ($p<0.05$).

Table 11. Endostatin (ES) levels in liver tissue in relation to obesity, cold stress and propolis treatment.

Group	ES (ng/L)
Normal Weight - Room Temperature	2,22±0,20[a]
Obese - Room Temperature	1,39±0,12[b]
Normal Weight - Cold Stress	2,47±0,15[a]
Obese - Cold Stress	0,89±0,07[c]
Normal Weight - Propolis - Room Temperature	0,99±0,06[c]
Obese - Propolis - Cold Stress	0,71±0,03[c]

The difference between the groups indicated by different letters has been found to be statistically significant.

DISCUSSION

This study aimed to investigate the levels of VEGF, an angiogenic factor, and endostatin, an antiangiogenic factor, in liver, lung, heart tissue, white adipose tissue, and brown adipose tissue in cases of obesity induced by cold stress and propolis feeding. Upon evaluation of the findings, it was observed that VEGF levels increased in brown adipose tissue in both cold stress and propolis treatment groups, whereas they decreased in white adipose tissue. Similarly, VEGF levels showed a general increase in lung, liver, and heart tissues. Conversely, endostatin levels, as an antiangiogenic factor, decreased in brown adipose tissue but exhibited a general increase in white adipose tissue.

In liver, lung, and heart tissues, endostatin levels generally decreased.

When white adipose tissue and brown adipose tissue are evaluated in terms of energy and heat extraction, it is thought that there may be increases, while differences in other tissues may vary depending on the function of the tissues.

In a study on cold stress, it was reported that cold stress caused an increase in VEGF levels in brown adipose tissue of rats (32). The study we conducted is similar to the increase in VEGF level in brown adipose tissue due to cold stress application. In addition, a general increase in VEGF levels was found in white adipose tissue, lung, liver and heart tissue due to cold stress application. There is no study on endostatin, a proteolytic fragment of collagen XVIII, a potent angiogenesis inhibitor, in relation to exposure to cold stress. Our study detected a decrease in endostatin levels in rats exposed to cold stress for the first time. However, a study on diet-dependent endostatin was conducted by Wang et al. (2015) (33). Wang et al. (2015) reported that there was no change in the weight of the heart, liver, lungs and kidneys, while endostatin administration to mice fed a high-fat diet reduced the weight of white tissue. It was also reported that endostatin was effective in the inhibition of obesity development in mice fed a high-fat diet, with a detailed histological analysis of white adipose tissue revealing that the size of adipocytes in mice fed a high-fat diet given endostatin was smaller than in animals fed an endostatin high-fat diet.

Propolis is a natural product obtained by mixing bee secretions with plant exudates. Since propolis is rich in flavonoids and cinnamic acid derivatives, the application of propolis extracts in treatments against cancer, inflammation and metabolic diseases has been investigated. Accumulating evidence utilizing animal and cellular models suggests that propolis extracts have therapeutic effects on obesity by controlling adipogenesis, adipokine secretion, food intake and energy expenditure. Research in animal and cellular models has shown that propolis modulates oxidative stress, accumulation of advanced glycation end products and adipose tissue inflammation, all of which contribute to insulin resistance or impairments in insulin secretion (34). In the study performed by Culum and Yurekli (2020), the effects of resveratrol, which has anti-inflammatory, anti-oxidant and anticancer effects, on angiogenesis, white adipose tissue and brown adipose tissue, and its effects on vascular endothelial growth factor were investigated. It has been stated that resveratrol increases the level of VEGF in brown adipose tissue and that resveratrol may possibly have proangiogenic activity. Our study determined that rats given propolis, which has antibacterial, antitumor and antioxidant properties, generally caused an increase in VEGF levels (35). Propolis, produced both in our country and around the world and known to have various effects on human health, has antibacterial, cytostatic, free radical protective activity, while bactericidal, antifungal and antiparasitic activity, antioxidant, anticarcinogenic, wound healing, cell regenerative and aniogenic properties have also been researched (36). Studies have shown that propolis suppresses angiogenesis. In the study conducted by us, in addition to the features mentioned above, a study was conducted on the levels of angiogenic VEGF and antianiogenic endostatin in brown adipose tissue and white adipose tissue due to the application of cold stress to obesity. Especially considering obesity and cold stress, it is thought that propolis will generally increase VEGF levels in brown adipose tissue compared to control groups and that propolis will have beneficial effects in terms of energy efficiency/usage. When we look at endostatin data, it is important to investigate more aniogenic and antioaniogenic factors, since very clear data are not obtained as in VEGF. According to the literature, no study investigates the levels of VEGF and endostatin, which are angiogenic and antiangiogenic factors, due to propolis diet and cold stress application.

CONCLUSION

In our study, it was found that endostatin levels were generally increased in brown and white adipose tissue due to cold stress and obesity, while endostatin levels decreased in heart, lung and liver tissue. Our study studied the effects of cold stress application and propolis nutrition on angiogenic and antiangiogenic factors. According to literature data, it is known that propolis has angiogenic properties. Although the results obtained in our study are similar to the literature data, it is obvious that more studies are needed in the future, considering that there are many angiogenic-antiangiogenic factors. When propolis and cold stress were evaluated together, it was determined in our study that they may play a role in regulating energy efficiency in brown adipose tissue.

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