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ORIGINAL PAPER

Tryptophan reduces the degree of brown adipose tissue whitening in rats with visceral obesity

Roman Yanko , Mikhail Levashov , Sergey Safonov

Department of Clinical Physiology of Connective Tissue, Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine, Kiev, Ukraine

ABSTRACT

Introduction and aim. The relationship between brown adipose tissue (BAT) and visceral obesity (VO) is a topic of growing interest in scientific and medical research. The aim of this work was to investigate the effect of L-tryptophan on histomorphological abnormalities in BAT induced by a high calorie diet (HCD).

Material and methods. The study was performed on male Wistar rats 3 months of age. Control rats (group I) were fed a standard diet. VO in animals (groups II and III) was modelled by exposure to an HCD (45% fat and 31% carbohydrates) for 12 weeks. The rats in group III were also given L-tryptophan (80 mg/kg). Histological preparations were prepared from the interscapular bodies of the BAT. Indicators of lipid metabolism, oxygen consumption, subcutaneous oxygen tension and basal temperature were measured in the rats.

Results. It was found that the group of rats on HCD lead to the development of VO, and histomorphological changes occur in BAT indicating a decrease in its activity. Supplementation with L-tryptophan reduced the structural abnormalities in BAT, namely the accumulation of fat, the whitening of brown adipocytes, and prevented excessive loss of activity due to the deleterious effects of HCD.

Conclusion. Supplementation with L-tryptophan may have a potential benefit in preventing the development of excessive VO by preserving BAT activity.

Keywords. brown adipose tissue, obesity, tryptophan

Introduction

The global increase in obesity has become a serious problem with numerous consequences for the health of individuals and society as a whole.[1] Obesity often leads to the development of cardiovascular diseases, diabetes, fatty hepatosis, hormonal disorders, and even cancer.[2] At the same time, the effectiveness of existing methods of treatment and prevention of obesity is insufficient.

In recent years, researchers have turned their attention to brown adipose tissue (BAT) and its potential impact on obesity.[3] Unlike white adipose tissue, which stores energy in the form of triglycerides, BAT has a higher metabolic activity due to its thermogenic function, which allows it to burn calories and dissipate energy in the form of heat.[4] Therefore, increasing the activity of BAT may be of great importance in caloric expenditure and contribute to the control of visceral obesity (VO).

At this time, the search for BAT-activating factors is relevant. These include: the influence of low temperatures; physical activity; the effect of some food additives, e.g. capsaicin in chilli peppers; the effect of hormones, especially irisin and natriuretic peptides, etc.[5-8] BAT

Corresponding author: Roman Yanko, email: biolag@ukr.net

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transplantation has also been shown to be a promising potential therapeutic approach in the fight against obesity.[9] Although BAT activation has the potential to control obesity, the individual variability of its activity has certain complications. Not all people respond in the same way to stimuli that activate BAT, so individual approaches and the search for a universal factor that activates BAT are needed.

One such method is the use of the essential amino acid tryptophan. It is known that tryptophan is involved in the regulation of energy metabolism and food intake and has a direct effect on adipose tissue.[10] Most studies on the effects of tryptophan on obesity have been conducted in animals. It has been shown that tryptophan reduces the weight of visceral fat in rats fed a high calorie diet (HCD).[11] Tryptophan metabolites (mainly serotonin and melatonin) have been studied for the activity of BAT and the development of obesity. It has been shown that the central and peripheral pools of serotonin act in opposite directions. For example, centrally acting serotonin, synthesized in the brain stem, inhibits food intake and reduces body weight, while peripheral serotonin, mainly from the gut, stimulates the development of obesity.[12] Melatonin has been found in most studies to inhibit the development of obesity by increasing BAT activity and energy expenditure.[13,14] The effect of tryptophan on obesity in humans is poorly understood. For example, oral tryptophan (750 mg, twice daily for 3 months) was found to significantly increase weight loss in severely obese people.[15] Other authors showed that administration of L-tryptophan (in a dose of 1-3g) reduced appetite and accelerated weight loss in patients.[16]

However, the effect of the influence of tryptophan on the histomorphometric changes in BAT that develop with VO has not been fully investigated. The mechanism by which tryptophan, according to most literature, is able to reduce the manifestations of VO and its negative consequences on the body is not known.[17,18] Perhaps it does so by activating BAT. The question of the mechanisms of the relationship between the effect of tryptophan, the state of BAT and energy metabolism and their role in the pathogenesis of VO remains open and requires further study. Therefore, this study was conducted to investigate the efficacy of L-tryptophan supplementation in reducing the severity of obesity-related BAT structural disorders. And also to evaluate the possibility of using tryptophan in reducing the development of VO by preserving BAT activity.

Aim

The aim of this work was to investigate the effect of L-tryptophan on histomorphometric changes in brown adipose tissue induced by a high-calorie diet and to evaluate the possibility of its use in the prevention of visceral obesity.

Material and methods

Rats

Three month old Wistar rats (weight 360±20 g) obtained from the vivarium of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine were used in the experiments. The rats were maintained at a temperature of 20°C and humidity of 40–60% with a 12-hour light/dark cycle. All protocols were approved by the Committee on Biomedical Ethics of Animal Care and Use of the Bogomoletz Institute of Physiology (Protocol No. 5, dated 31.11.19). Rats were sacrificed by decapitation under isoflurane anesthesia in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

Modeling visceral obesity

The rats were randomly divided into 3 groups (12 animals in each group): I – control; II – animals that received HCD for 12 weeks; III – rats that received L-tryptophan (Ajinomoto Eurolysine S.A.S, France) at a dose of 80 mg/kg in addition to HCD.

Each rat of the control group received 20 g of standard feed daily with a fat content of 6%, protein content of 23%, and carbohydrate content of 55% (recipe K120-1 "Rezon-1", Ukraine) and had free access to water. The daily caloric content per rat was 66 kcal. Experimental rats of II and III groups received HCD with a total daily caloric intake of 116 kcal per animal. The composition of such a diet, in addition to standard mixed feed, included pork lard, white breadcrumbs and sunflower seeds (fats – 45%, proteins – 9% and carbohydrates – 31%). It is this selected composition of HCD that leads to the development of pronounced signs of VO (the reproducibility of this pathology is 100%). The diet used ensures a high rate of food intake by the animals (97–100%).[19] In addition, every second day the experimental rats received a 10% fructose solution instead of water, increasing the caloric content to 140 kcal per rat. This drinking regime does not reduce the animals' consumption of dry food and fluids and does not threaten to disrupt the body's water balance. The choice of fructose was based on the fact that its addition to the diet increases the negative effect of HCD on the development of obesity. As a high-fat, high-carbohydrate diet mimics the Western human diet, it is widely used to model obesity.

During the study period, the completeness of food intake was monitored daily in both control and experimental rats. Their general condition was assessed. Animals were weighed weekly. The presence of VO in the rats was diagnosed at the end of the experiment by determining the weight of visceral fat and the concentration of total lipids, triglycerides and cholesterol in the blood serum. These indicators can be used to determine the presence and degree of development of VO. Visceral fat was extracted mechanically from the peritoneal cav-

ity. BAT was removed from the interscapular area of the rat and its weight was determined.

Histomorphological analysis of the BAT

For histomorphometric studies, tissue samples were randomly selected from the interscapular bodies of the BAT, from which histological preparations were made according to the standard method.[20] Tissue samples were fixed in Bouin's fluid, dehydrated in alcohols of increasing concentration and embedded in paraffin. Paraffin sections of 6 μm thickness were prepared on a sliding microtome (MS-2, Reichert, Austria). The sections were stained with hematoxylin-eosin and by the Van Gieson method. Photomicrography was carried out using a light-optical microscope "Nikon Eslirse E100" (Japan) with a digital camera ("Levenhuk", USA). Morphometric analysis of photographs of serial sections was performed using the program "ImageJ 1.34".

For histomorphometric analysis, 5 sections of the BAT were taken from each rat. The relative areas of parenchyma, connective tissue and vessels were determined on the histological sections of the BAT. The stromal-parenchymal index (the ratio of the relative area of the connective tissue to the area of the parenchyma) and the trophic index (the ratio of the relative area of the vessels to the area of the parenchyma and connective tissue) were calculated.[21] The mean diameter and cross-sectional area of adipocytes, their nuclei and cytoplasm were measured in 100 cells. The number of adipocytes was counted in 100 areas of 1000 μm^2. The number of nucleoli was counted per 100 adipocyte nuclei. The number and area of lipid droplets in adipocytes were calculated. According to the number of lipid droplets, the adipocytes were divided into 3 types: A1 – contain 1 large lipid droplet; A2 – contain 1 large lipid droplet and several small ones; A3 – contain many small droplets. 100 adipocytes were counted on different sections. The number of adipocytes of each type was expressed as a percentage of the total number of cells counted. A morphometric grid was superimposed on the photomicrographs to facilitate cell counting and to determine the relative areas of the components of the BAT. Histomorphometric analysis of the BAT was performed on 10 photomicrographs from each rat at 800× magnification (23,000 μm^2).

Assessment of lipids in blood serum

The concentrations of total lipids, triglycerides, cholesterol, and high-density lipoproteins in rat blood serum were determined by a colorimetric-enzymatic method using standard reagent kits (Filisit-Diagnostics, Ukraine) and a biochemical analyzer (Sinnova, China). Standardized protocols were used to determine these indicators in blood serum.

Determination of oxygen consumption and basal body temperature

The determination of total oxygen consumption (VO_2) in rats was performed on an empty stomach in a closed gas exchange research system. The value of VO_2 was calculated in ml per 1 kg of body weight in 1 hour and brought to standard physical conditions (STPD): dry gas at a temperature of 0°C and a pressure of 760 mmHg. Rectal temperature was measured with an electronic medical thermometer "Beurer FT 09/1". Oxygen tension (PO_2) in the subcutaneous tissue of the rats' dorsal trunk was measured by *in situ* polarography using an open platinum electrode.

Statistical analysis

Statistical processing of the results was performed using the "SigmaPlot 14.5" software (Inpixon, CA). Data were tested for normality of distribution using the Shapiro-Wilk test. One-way analysis of variance was used to compare the samples. Homogeneity of variance was checked using Levene's test. Differences at $p<0.05$ were considered significant. Quantitative data are presented as mean ± standard deviation.

Results

In rats, signs of VO were found after 12 weeks on the HCD (group II). First of all, this indicated the increase in weight of visceral fat by 130.21% ($p<0.05$). In the blood serum an increase in the concentration of total lipids, triglycerides and cholesterol by 52.38%, 67.76% and 26.44% ($p<0.05$), respectively, compared with the control. In rats of group III receiving L-tryptophan in combination with HCD, indicators of lipid metabolism did not differ from control values (Table 1). The weight of visceral fat was 40.22% lower ($p<0.05$) than in the rats of group II. The weight of interscapular brown fat was 78.51 and 50.33% ($p<0.05$) higher in rats of groups II and III, respectively.

Table 1. Indicators of lipid metabolism in blood serum[a]

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Lipids, mmol/l	2.52±0.21	3.84±0.40*	2.60±0.24^
Triglycerides, mg/dl	94.9±3.8	159.2±9.3*	97.6±3.8^
Cholesterol, mmol/l	1.74±0.09	2.20±0.11*	1.74±0.09^
High density lipoproteins, mmol/l	1.72±0.07	0.80±0.03*	1.68±0.08^

[a] * – $p<0.05$ – significance of differences compared with the control, ^ – $p<0.05$ – significance of differences compared with rats that received a high-calorie diet

Microscopic examination of sections from interscapular BAT of rats showed that they were composed of specialized adipocytes, which are different from white fat cells. The adipocytes contained a nucleus with several nucleoli, usually located in the center. The nucleus is

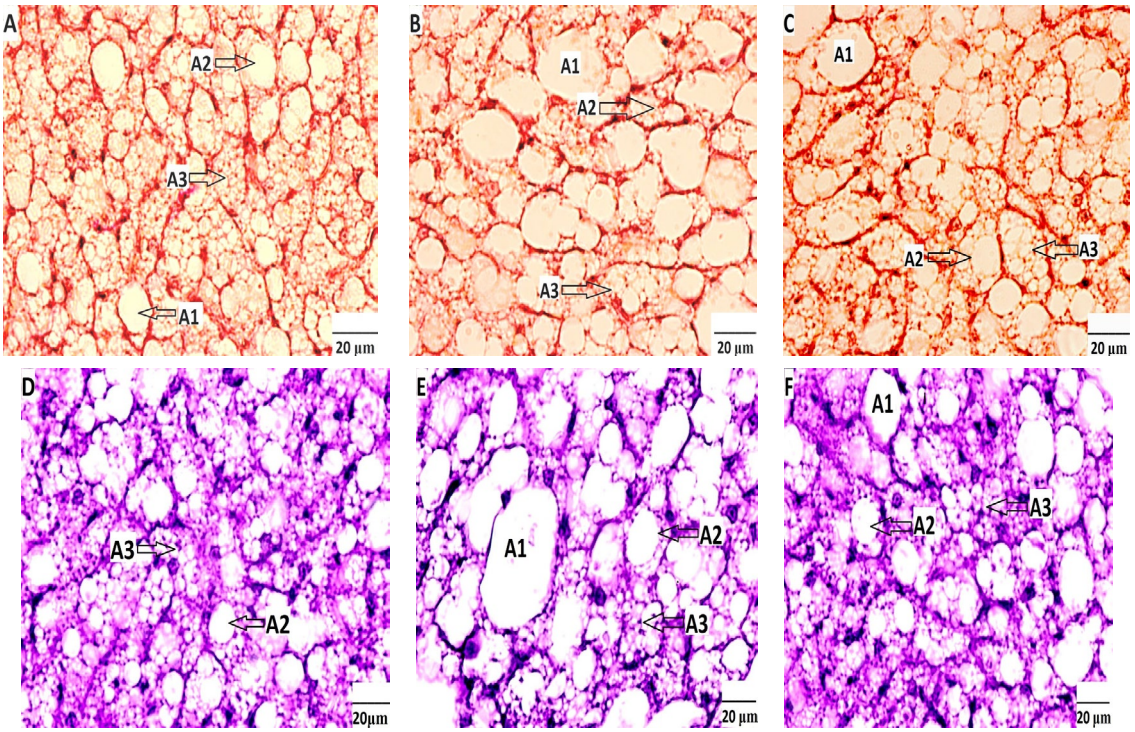

Fig. 1. Representative images of histological slides of brown adipose tissue (A, D – control rats, B, E – rats on high-calorie diet and C, F – rats treated with L-tryptophan in addition to high-calorie diet). A-C – Van Gieson stain, D-F – hematoxylin-eosin stain (800x), A1 – adipocyte with 1 large lipid droplet; A2 – adipocyte with one large and several small drops; A3 – adipocyte with many small drops

surrounded by many lipid droplets that are well separated from each other. This gave the cells a multi-chambered appearance. Such cells are actually functionally active brown adipocytes (type A3). In the HCD rats, the presence of adipocytes with a large lipid droplet and a nucleus displaced to the membrane was observed. We called this type of adipocyte A1. In terms of structure and function, these cells took on the characteristics of white adipocytes. The greater the degree of VO in the animal, the greater the number of white adipocytes in the BAT. In control animals, the white adipocytes in the BAT were small. Adipocytes were also found with a large lipid droplet surrounded by several small ones (type A2). These cells are a transitional type between white and brown adipocytes (Fig. 1).

As mentioned above, the number of white adipocytes was significantly increased in the BAT of rats on HCD, exceeding the control rate by 1800.12%. These rats had by 51.34% ($p<0.05$) fewer brown adipocytes (A3) and by 110.52% ($p<0.05$) more cells with one large lipid droplet and several small ones (A2) than the control (Fig. 2). The size of brown adipocytes was larger, namely: diameter by 18.72% ($p<0.05$), area by 40.9% ($p<0.05$) and their cytoplasmic area by 43.52% ($p<0.05$). The nuclear-cytoplasmic ratio was less than by 22.89% ($p<0.05$) compared with the control. The increase in adipocyte size is associated with an increase in lipid droplet area by 93.04% ($p<0.05$). The number of lipid droplets in the cells was less than by 45.51% ($p<0.05$) compared with the control. The total number of adipocytes was less than by 31.91% ($p<0.05$). The number of nucleoli in the nucleus of adipocytes in group II rats was less by 15.63% ($p<0.05$), indicating a decrease in synthetic activity. There was also an increase in the relative area of connective tissue by 63.04% ($p<0.05$) and a decrease in the area of vessels by 54.55% ($p<0.05$) in the BAT of these rats (Table 2). This indicates the inhibition of oxygen transport to the parenchymal elements, the deterioration of conditions for the course of metabolism. In other words, the morphometric data obtained indicate a decrease in the functional activity of the BAT and its transformation into white fat.

Rats receiving HCD together with L-tryptophan showed less histomorphometric changes in BAT and significantly less evidence of brown to white adipocyte transformation. This was evidenced by a lower number of type A1 adipocytes (by 63.12%, $p<0.05$), type A2 adipocytes (by 23.93%, $p<0.05$) and a higher number of type A3 brown adipocytes (by 58.21%, $p<0.05$) compared with group II rats (Fig. 2). When L-tryptophan was administered, the area of lipid droplets was smaller by 18.92% ($p<0.05$) and their number was larger by 44.33% ($p<0.05$). This resulted in a decrease in adipocyte area and cytoplasm by 15.92% and 16.91% ($p<0.05$), respectively, and an increase in nuclear-cytoplasmic ratio by 18.75% ($p<0.05$) compared with rats on

HCD alone. Administration of L-tryptophan also resulted in by 26.67% ($p<0.05$) decrease in the relative area of connective tissue and by 40.00% ($p<0.05$) increase in the area of blood vessels (Table 2). This indicates better perfusion and greater functional activity of the adipocytes than in the group II rats.

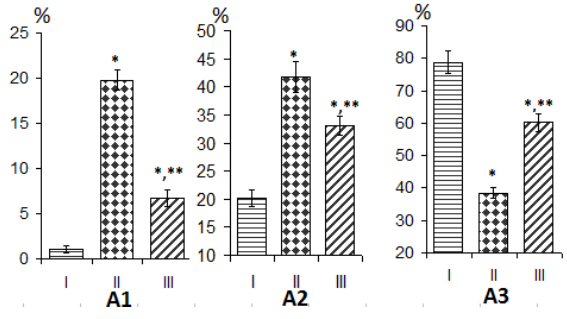

Fig. 2. The number of adipocytes of different types in BAT rats: A1 – contain 1 large lipid drop, A2 – contain 1 large lipid drop and several small, A3 – contain many small drops. The number of cells of each type is represented as a percentage of the total number of adipocytes. *p<0.05 – significance of differences compared with the group I, **p<0.05 – significance of differences compared with the group II. I – control rats, II – rats on HCD and III – rats treated with L-tryptophan in addition to HCD

Table 2. Histomorphometric indicators of brown adipose tissue

Indicators	Control	High-calorie diet	High-calorie diet + L-tryptophan
Adipocyte diameter, μm	18.7±0.7	22.2±0.6*	21.1±0.4*
Area, μm²			
adipocyte	300.0±15	422.7±18.6*	355.4±14*^
nucleus	23.1±0.9	25.3±1.1	25.2±0.9
cytoplasm	276.9±15.6	397.4±12.6*	330.2±15.1*^
nuclear-cytoplasmic ratio	0.083±0.008	0.064±0.004*	0.076±0.001^
Number of adipocytes, pcs/1000 μm²	2.57±0.11	1.75±0.09*	2.04±0.09*^
Number of nucleoli in the nucleus, pcs	1.60±0.07	1.35±0.05*	1.49±0.04
Number of lipid droplets in the adipocyte, pcs	17.8±0.6	9.7±0.4*	14.0±0.7*^
Area of lipid droplets, μm²	11.5±0.6	22.2±1.2*	18.0±0.9*^
Relative area, %			
parenchyma	92.1±1.1	91±1.4	92.4±1.3
connective tissue	4.6±0.8	7.5±0.5*	5.5±0.2^
vessels	3.3±0.4	1.5±0.2*	2.1±0.1*^
Stromal-parenchymal index	0.086±0.004	0.099±0.003*	0.082±0.005^
Trophic index	0.036±0.005	0.016±0.003*	0.023±0.002*^

[a] * – $p<0.05$ – significance of differences compared with the control, ^ – $p<0.05$ – significance of differences compared with rats that received a high-calorie diet

A significant change in oxygen metabolism and thermogenesis was observed in experimental rats receiving HCD. Rectal temperature increased the most in group II rats by 0.96°C ($p<0.05$). In these rats there was a clear tendency to increase total oxygen consumption (+11.30%) and subcutaneous oxygen tension (+2.70 mmHg). Rectal temperature was 0.82°C ($p<0.05$) higher in rats treated with HCD in combination with L-tryptophan than in control animals. Total oxygen consumption in this rats increased by 21.60% ($p<0.05$) and subcutaneous oxygen tension by 9.31 mmHg ($p<0.05$) compared with the control (Fig. 3). Thus, tryptophan activates oxygen metabolism, contributes to the utilization of excess dietary fats and increases heat production.

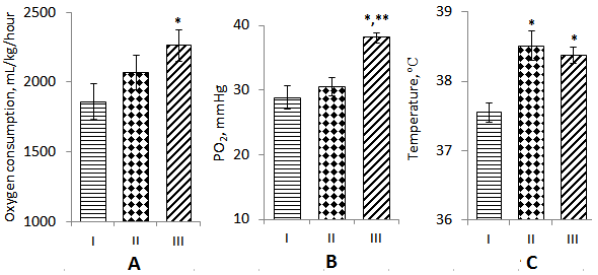

Fig. 3. Oxygen consumption (A), subcutaneous oxygen tension (B) and rectal temperature (C) of control rats (I), rats receiving high calorie diet (II) and rats receiving high calorie diet and L-tryptophan (III). *p<0.05 – significance of differences compared with the group I, **p<0.05 – significance of differences compared with the group II

Discussion

The relationship between BAT and VO is a topic of growing interest in scientific and medical research. The unique ability of BAT to burn calories through thermogenesis has led to its investigation as a potential target for obesity reduction strategies.[22] Although various methods of activating brown fat show promise, further research is needed to fully understand its role and how it can be used effectively and safely to prevent the development of excessive VO. Therefore, the search for new approaches to BAT activation may offer new opportunities in the fight against obesity and contribute to the improvement of global health care. The protective mechanisms against the excessive development of VO caused by tryptophan are complex and not yet fully understood. One possible mechanism is the activation of BAT by tryptophan or its derivatives.[13]

We found that a 12-week stay of rats in the HCD leads to the development of VO, as evidenced by a significant increase in the weight of visceral fat and indicators of lipid metabolism in blood serum. Histomorphological changes occur in BAT, indicating a decrease in its activity and intensification of brown adipocyte whitening processes. An increase in the size of lipid droplets and a change in the color of brown adipocytes made them more similar to cells of white adipose tissue, which are mainly involved in the accumulation of energy and not in its consumption, i.e. they do not contribute to an increase in body temperature. However, we did find an increase in body temperature in experimental rats. A high

level of total oxygen consumption and an increase in basal body temperature in the presence of VO indicates the possible presence of a certain degree of disruption of oxidation and phosphorylation processes, but does not exclude other mechanisms of temperature increase. For example, the number of beige adipocytes with thermogenic capacity may increase in white adipose tissue. The remaining brown adipocytes may increase their thermogenic activity to compensate for the decrease in their number. Improved mitochondrial function and increased expression of UCP1 in existing brown adipocytes can lead to increased heat production.[23] Activation of the sympathetic nervous system can significantly increase the thermogenic activity of brown and beige adipocytes.[24] Obesity is often associated with a chronic inflammatory process of low severity. It is characterized by an increase in levels of pro-inflammatory cytokines, which can affect the center of temperature regulation.[25] Activation of the immune system in obesity may also contribute to the development of hyperthermia.[26] Further studies are therefore needed to fully understand the complex interactions between obesity, inflammation and thermoregulation in laboratory rats.

Supplementation with L-tryptophan reduces structural abnormalities in the BAT, thereby reducing the accumulation of fat in her and preventing excessive loss of activity due to the deleterious effects of HCD. At the same time, there is a decrease in the number of white adipocytes and an increase in the number of active brown cells, which intensively produce heat.[27] Adipocytes not involved in thermogenesis acquire a structure more similar to white cells.[28] As a result, after exposure to L-tryptophan, indicators of lipid metabolism returned to control values and visceral fat weight decreased.

It is known that in obesity the activity of BAT decreases and the process of „whitening" occurs – the transformation of brown adipocytes into white ones.[29] Possible mechanisms include inhibition of vascular endothelial growth factor, redoxisome proliferator-activated receptor gamma coactivator 1 alpha and bone morphogenetic protein 7, which disrupts vascularization, mitochondrial biogenesis and differentiation of brown adipocytes. This can lead to the infiltration of T cells that secrete interferon-gamma, increase autophagy and disrupt metabolism. Whitening of BAT is thought to be under the control of genes such as b-AR, BMP and mitochondrial transcription factor A, as well as microRNAs, which regulate many processes including brown adipocyte differentiation.[30-32]

Thus, new data were obtained regarding the role of tryptophan in the mechanisms of obesity development and related metabolic disorders. Namely, it has been shown that tryptophan reduces the severity of structural damage of the BAT caused by HCD. This helps to increase the activity of the BAT. Important links in the positive effect of tryptophan in VO are the activation of oxygen metabolism and the increase in the activity of thermogenesis, which contributes to the utilization of the excess amount of fats that come with food.

Study limitations

Although L-tryptophan at a dose of 80 mg/kg reduced the severity of BAT structural damage and the degree of adipocyte whitening induced by a HCD, the lack of a dose-dependent effect for the obesity model used limits the final conclusion.

Conclusion

Tryptophan supplementation may have a potential benefit for brown adipose tissue by reducing the severity of its structural damage and the extent of adipocyte whitening induced by a high calorie diet. Tryptophan also promotes increased basal metabolic rate and increased energy expenditure associated with the activation of thermogenesis. This may be of practical interest in the use of tryptophan and its derivatives in the clinic to prevent a decrease in brown adipose tissue activity and thus reduce the development of obesity. Enhancing the beneficial effects of tryptophan by combining dietary supplementation with other methods of brown adipose tissue activation will be the focus of our future research and may benefit obese patients.

Declarations

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Author contributions

Conceptualization, M.L. and R.Y.; Methodology, R.Y and S.S.; Software, R.Y.; Validation, M.L., R.Y. and S.S.; Formal Analysis, M.L.; Investigation, R.Y.; Resources, R.Y.; Data Curation, M.L.; Writing – Original Draft Preparation, R.Y.; Writing – Review & Editing, M.L.; Visualization, R.Y.; Supervision, M.L.; Project Administration, M.L.; Funding Acquisition, S.S.

Conflicts of interest

The authors declare no conflict of interests.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee on Biomedical Ethics of Animal

Care and Use of the Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine (protocol No. 5 dated 11/31/19).

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