POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 38(1): 89–101, Y. 2023

DOI: 10.31648/pjns.8689

EFFECT OF *L*-TRYPTOPHAN ON THE MORPHO-FUNCTIONAL CHANGES OF WHITE ADIPOSE TISSUE AN INDUCED VISCERAL OBESITY RAT MODEL*

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Key words: *L*-tryptophan, white adipose tissue, induced visceral obesity rat model.

Abstract

Visceral obesity (VO) is a widespread issue that contributes to the development of various diseases. Consequently, there is a need to identify effective methods for preventing VO. One of the ways to prevent VO can be the use of the amino acid tryptophan. This study aimed to investigate the impact of *L*-tryptophan on morpho-functional changes in white adipose tissue (WAT) in rats with VO and assess its potential for disease prevention. Male Wistar rats were involved in the study. Control animals (Group I) followed a standard diet, while Group II rats were fed a high-fat and high-carbohydrate diet for 12 weeks. Group III animals received a high-calorie diet supplemented with *L*-tryptophan (80 mg/kg). Blood and tissue samples of WAT were collected for standardized biochemical, histological, and biophysical evaluations. The inclusion of *L*-tryptophan in the high-calorie diet led to inhibition of visceral fat accumulation and reduced levels of total lipids, cholesterol, and triglycerides in the blood serum. Rats in Group III exhibited smaller adipocyte sizes, larger cell nucleus areas, and a decreased relative area of connective tissue in the WAT compared to Group II animals. Tryptophan also mitigated disturbances in WAT bioimpedance among obese rats. These findings suggest that *L*-tryptophan can attenuate the manifestations of VO and reduce fat accumulation in WAT, thus holding promise for disease prevention.

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* The work was performed as part of the state assignment “Bogomoletz Institute of Physiology of NAS of Ukraine” (No. 0119U103965).

Introduction

Visceral obesity (VO) is a chronic, multifactorial disease characterized by the excessive accumulation of abdominal fat, primarily triglycerides (KONG et al. 2022). The primary cause of VO is overeating and the consumption of high-calorie foods. This condition contributes to various metabolic disorders, hormonal imbalances, increased insulin resistance, hypertension, type II diabetes, cardiovascular diseases, and cancer (APARECIDA et al. 2020, YANKO and LEVASHOV 2022). These complications significantly impact quality of life, leading to long-term disability and premature death (TCHERNOF and DESPRES 2013). The increasing prevalence of VO, along with its asymptomatic nature, limited understanding of its pathogenesis, and insufficient knowledge about prevention and treatment strategies, emphasizes the importance of addressing this medical and social concern (SHUSTER et al. 2012). The etiology of obesity involves complex physiological and biochemical mechanisms that are not yet fully understood. Consequently, there is a growing need to study the pathogenesis of VO and develop effective prevention and treatment methods. One potential approach for preventing VO is the use of the amino acid tryptophan.

Tryptophan is known to participate in the regulation of energy metabolism, food consumption, and has a direct impact on adipose tissue (LISCHKA et al. 2022). Administration of tryptophan to rats on a high-calorie diet has been shown to normalize body weight by reducing visceral fat (SHIPELIN et al. 2021). Furthermore, studies have indicated that tryptophan levels decrease in the blood with obesity (BRANDACHER et al. 2006). However, despite the available literature, there is a lack of sufficient research on the use of tryptophan for obesity prevention, particularly regarding its direct influence on biochemical indicators of lipid metabolism, histomorphological changes, and biophysical properties of visceral white adipose tissue (WAT) in VO.

Notably, the process of obesity involves not only an increase in the amount of WAT but also changes in its quality. These morpho-functional, biochemical, and biophysical alterations in WAT can significantly impact the efficacy of drugs used for obesity treatment and prevention. However, this aspect of the problem remains poorly investigated.

Moreover, the use of different doses of *L*-tryptophan, variations in the duration of research, differences in experimental models of obesity, and varying ages of animals have resulted in ambiguous results. Therefore, comprehensive studies that evaluate the role of tryptophan and its mechanisms of influence on WAT with existing signs of VO are necessary.

The aim of this study was to examine the effect of *L*-tryptophan on morpho-functional changes in WAT in rats with VO and determine its potential for disease prevention. By elucidating the impact of tryptophan on VO, we can contribute to a better understanding of its therapeutic potential and facilitate the development of effective preventive strategies.

Materials and Methods

Research object and experiment design

The study was conducted on 30 male Wistar rats (10 animals in each group), which were taken into the experiment at the age of 3 months. Rats were divided into 3 groups: I – control; II – rats that received a high-calorie diet (HCD) for 12 weeks; III – animals that were on the HCD and additionally received *L*-tryptophan ("Ajinomoto Eurolysine S.A.S", France) in a dose of 80 mg/kg of body weight. VO in rats was induced by feeding them a high-calorie diet containing fat (45% from the mass of the ration) and easily assimilable carbohydrates (31% from the mass of the ration) for 12 weeks. Each rat received daily: 6 g of specially prepared granulated feed (70% standard compound feed with the addition of 30% pork lard); 6.8 g of pork lard; 3.6 g of white breadcrumbs; 3.6 g of sunflower seeds. The total calorie content of the daily diet was 116 kcal. Experimental animals received feed *ad libitum* under daily monitoring of the completeness of its consumption. A day later, instead of water, experimental rats received a 10% fructose solution (YANKO et al. 2021, YANKO et al. 2022). A rat of the control group consumed 20 g of standard compound feed daily, the calorie content of which was 66 kcal. At the end of the experiment, visceral fat was obtained from the abdominal cavity by a mechanical separation method. The weight of visceral fat was determined by the gravimetric method. The degree of obesity was judged by the weight of isolated visceral fat.

Rats were euthanized the day after the last dose of *L*-tryptophan. The work with rats was carried out in compliance with the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was approved by the biomedical ethics committee of the Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine (protocol No. 5 dated 11/31/19).

Histological studies

For histo-morphological studies WAT samples were randomly selected. The histological preparations were made according to the standard method: fixed in Buen's fluid, dehydrated in alcohols of increasing concentration and dioxane. The obtained samples were embedded in paraffin. Paraffin sections (6 μm thick) were made on a sled microtome. Staining of the obtained sections was carried out with Bemer's hematoxylin and eosin and according to Van Gieson (REHFELD et al. 2017, YANKO et al. 2021). Hematoxylin and eosin staining allows for the visualization of the general morphological structure of WAT, while Van Gieson staining facilitates the identification of connective tissue elements. The micropreparations were photographed on a microscope "Nikon Eclipse E100" (Japan) with the use of a digital camera. Morphometry was performed using the computer program "ImageJ 1.34p".

Several parameters were measured on the WAT micrographs, including the relative area of the parenchyma, connective tissue, and blood vessels. The average diameter and cross-sectional area of adipocytes, as well as the area of the adipocyte nucleus, were determined. The number and density of adipocyte placement per unit area were also assessed. Distribution of adipocytes by size (<50 μm, 50–100 μm, >100 μm) was carried out. The stromal-parenchymal index (the ratio of the relative area of vessels and 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) were determined (COSTA et al. 2011, MILJKOVIC et al. 2022).

Biochemical studies

The concentration of lipids, cholesterol, triglycerides, and high-density lipoproteins in the blood serum of rats was determined by the photometric method using standard sets of reagents ("Filisit-Diagnostika", Ukraine) on a biochemical analyzer ("Sinnowa", China). Standardized protocols were used to determine these indicators in blood serum. Normal values were established as follows: total lipids – 2.50 ±0.15 mmol/L, triglycerides – 94.9 ±3.9 mg/dL, cholesterol – 1.70 ±0.08 mmol/L, and high-density lipoproteins – 1.70 ±0.14 mmol/L.

Biophysical studies

The method of multifrequency bioelectrical impedance analysis (BIA) was used to assess the biophysical properties of WAT (SHCHELYKALINA et al. 2021). The BIA method is increasingly used in experimental and

clinical research as one of the highly informative methods for assessing the viability of biological tissues, their functional and metabolic activity, as well as for tissue histological verification (KHALIL et al. 2014). BIA testing of preparations of freshly removed WAT was carried out *ex tempore* on "LCR – meter Quad Tech 1920" (USA) in the mode of operation of the device with a parallel equivalent circuit. Absolute values of electrical parameters were determined at frequencies of 1000 Hz – 1 MHz. Measurements were made using 2 flat silver electrodes with an area of 25 mm^2. The impedance values obtained at the maximum (10^4 Hz) and minimum (10^6 Hz) polarization frequencies of the object were used for the analysis. The impedance dispersion coefficient was calculated from the obtained results as the ratio of its values measured at low and high frequencies ($D_Z = Z_{10}{}^4/Z_{10}{}^6$).

Data analysis

The obtained data were processed by the methods of variational statistics using the software "Statistica 6.0 for Windows" (StatSoft, USA) and "Excel 2010" (Microsoft, USA). Data are reported as the mean ± SD when normally distributed. Groups were analyzed by one-way analysis of variance followed by Bonferroni *t*-tests with a significance level of 0.05.

Results

Rats that received HCD for 12 weeks (Group II) showed signs of VO development. The absolute and relative weights of visceral fat were higher than those of control animals by 145% and 122%, respectively ($p < 0.05$). In rats from the Group III, which received HCD along with *L*-tryptophan, the absolute and relative weights of visceral fat were 38% and 23% lower ($p < 0.05$), respectively, compared to rats in the Group II (Table 1).

Table 1

Visceral fat weight (Mean ± SD)

Indicators	Absolute weight of visceral fat [g]	Relative weight of visceral fat [%]
Control (Group I)	19.0 ±1.4	0.046 ±0.005
High-calorie diet (Group II)	46.6 ±2.6*	0.102 ±0.010*
High-calorie diet + *L*-tryptophan (Group III)	28.7 ±1.3*#	0.079 ±0.006*#

* Different from Group I at $P < 0.05$, #Different from Group II at $P < 0.05$ (One way ANOVA followed by Bonferroni post hoc test, $N = 10$ rats/group)

Histologically, the WAT in control and experimental rats consisted of parenchymal and stromal components. Adipocytes, representing the parenchymal component, appeared optically empty with a narrow cytoplasmic rim and a flattened nucleus displaced to the cell's edge due to fat accumulation. Adipocytes were closely packed, but significant differences in size and shape were observed between control and obese rats. Control animals had smaller, predominantly round-oval-shaped cells, while obese rats showed hypertrophied adipocytes with irregular shapes (Figure 1). Thus, in the WAT of the animals that received HCD, the adipocytes were larger in size: diameter – by 36% ($p < 0.05$), area – by 50% ($p < 0.05$) compared to the control. Additionally, the number of adipocytes and their density per unit area were 19% ($p < 0.05$) lower in the Group II (Table 2).

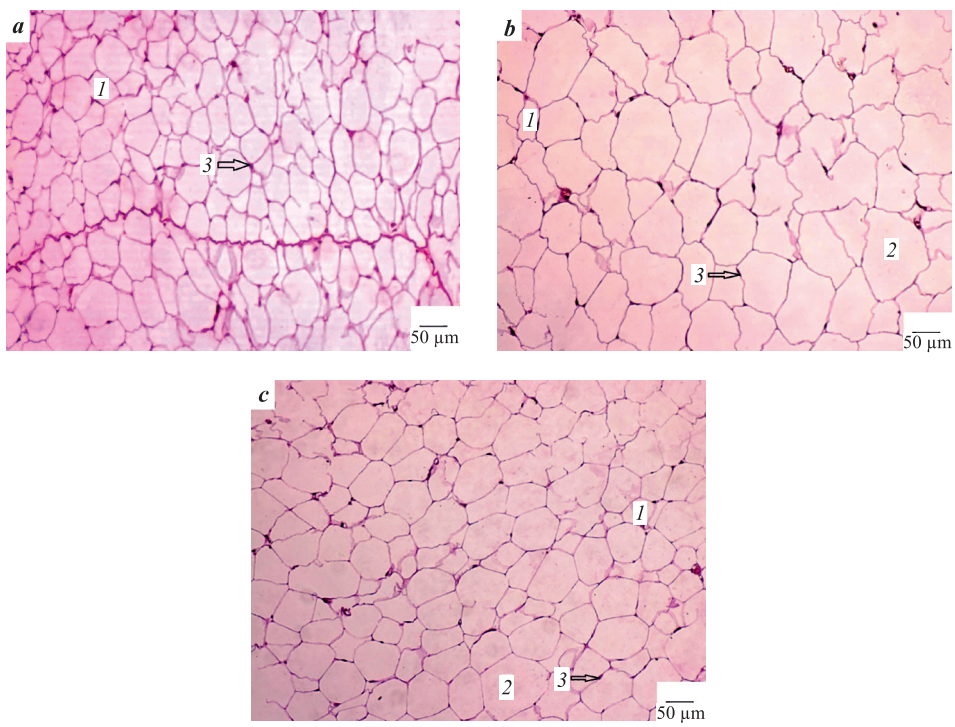

Fig. 1. Micrograph of the visceral white adipose tissue in control rat (*a*), after exposure to a high-calorie diet (*b*) and simultaneous exposure to a high-calorie diet and *L*-tryptophan (*c*). Van Gieson staining, ×200

Note: *1* – adipocyte in diameter <50 µm; *2* – adipocyte in diameter >100 µm; *3* – adipocyte nucleus

Table 2

Morphometry of white adipose tissue (Mean ±SD)

Indicators	Control (Group I)	High-calorie diet (Group II)	High-calorie diet + *L*-tryptophan (Group III)
Mean diameter of adipocyte [μm]	49.7 ±1.3	67.7 ±1.1*	52.2 ±1.8$^{\#}$
Area of adipocyte [μm^2]	2408 ±124	3608 ±164*	2765 ±150*$^{\#}$
Area of adipocyte nucleus [μm^2]	17.5 ±1.1	19.1 ±1.0	25.9 ±1.4*$^{\#}$
Number of adipocytes [pcs] (on an area of 0.35 mm^2)	100.5 ±6.9	81.7 ±12.4*	87.4 ±2.6*
Density of placement of adipocytes [pcs./mm^2]	287 ±19	233 ±35*	250 ±25*
Distribution of adipocytes by diameter [%]			
<50 μm	64.2 ±2.1	46.3 ±1.7*	51.6 ±1.7*
50–100 μm	34.7 ±1.1	45.0 ±1.5*	45.5 ±1.4*
>100 μm	1.1 ±0.4	8.7 ±0.6*	2.9 ±0.6*$^{\#}$
Relative area [%]:			
– parenchyma	91.3 ±1.4	90.7 ±1.8	91.8 ±0.7
– connective tissue	4.4 ±0.8	5.6 ±0.9*	4.5 ±0.3$^{\#}$
– vessels	4.3 ±0.5	3.7 ±0.3*	3.7 ±0.7*
Stromal-parenchymal index	0.095 ±0.017	0.10 ±0.01	0.090 ±0.009
Trophic index	0.047 ±0.007	0.041 ±0.007*	0.040 ±0.006*

* Different from Group I at $P < 0.05$, $^{\#}$Different from Group II at $P < 0.05$ (One way ANOVA followed by Bonferroni post hoc test, $N = 10$ rats/group)

Rats in the Group II had a lower number of adipocytes with a diameter of <50 μm (by 28%, $p < 0.05$), a higher number of cells with a size of 50–100 μm (by 30%, $p < 0.05$), and a number of adipocytes with a diameter of >100 μm (by 690%, $p < 0.05$) compared to controls (Table 2).

The stromal component of WAT, including microcirculatory vessels, lymphatic capillaries, nerve fibers, and connective tissue fibers, was present in both control and obese rats. However, the relative area of connective tissue was 27% higher ($p < 0.05$), and the relative area of vessels and the trophic index were 14% and 13% lower ($p < 0.05$), respectively, in the WAT of obese rats from the Group II (Table 2). These changes in the stromal component of WAT in obese rats indicate the deterioration blood supply to adipocytes.

In WAT of rats that received HCD and *L*-tryptophan (Group III) the area of adipocytes, the number of adipocytes with diameter >100 μm and the relative area of connective tissue were 23%, 97% and 20% ($p < 0.05$)

smaller, respectively, and the nuclear area was 36% ($p < 0.05$) larger than in rats of Group II (Table 2).

In the blood serum of rats in the Group II, the concentrations of lipids, triglycerides, and cholesterol were higher ($p < 0.05$) by 54%, 72%, and 29%, respectively, compared to control animals. Conversely, the concentration of high-density lipoproteins was 54% lower ($p < 0.05$) in the II group. Rats in the Group III showed smaller concentrations of triglycerides, cholesterol, and lipids (by 40%, 21%, and 43%, respectively, $p < 0.05$) in their blood serum compared to the Group II. Moreover, the concentration of high-density lipoproteins was 112% larger ($p < 0.05$) in the Group III compared to rats that received only HCD (Figure 2).

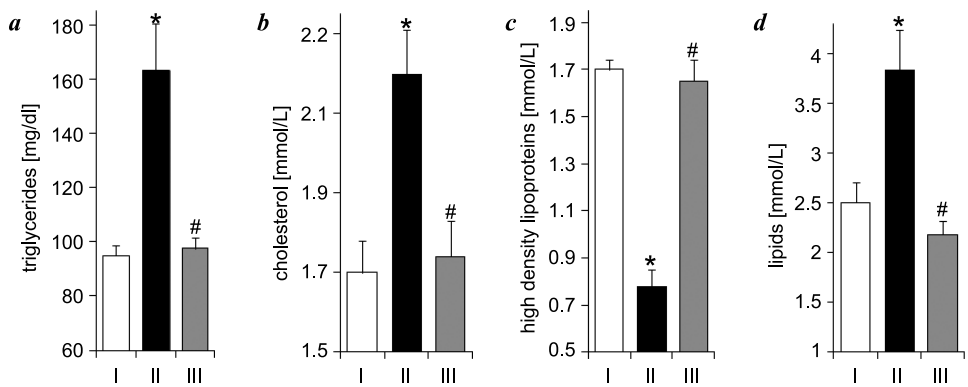

Fig. 2. Effect of *L*-tryptophan on lipid metabolism in blood serum: *L*-tryptophan reduced the concentration triglycerides (*a*); cholesterol (*b*); and total lipid (*d*) in rat's blood serum accumulated due to HCD. *Different from Group I at $P < 0.05$; #different from Group II at $P < 0.05$ (One way ANOVA followed by Bonferroni post hoc test, $N = 10$ rats/group)

Significant changes in BIA indicators were observed in rats with induced VO. The impedance values increased at both low and high frequencies of the testing current. At a frequency of 10^4 Hz, the impedance was 67% higher ($p < 0.05$) than the control, while at a frequency of 10^6 Hz, the increase was 70% ($p < 0.05$). Consequently, rats with VO exhibited a decrease in the impedance dispersion coefficient of WAT to 1.27 units compared to the control's value of 1.34 units. Rats which received HCD and *L*-tryptophan, showed lower impedance values at frequencies of 10^4 Hz and 10^6 Hz by 26% and 28% ($p < 0.05$), respectively, compared to the Group II (Figure 3).

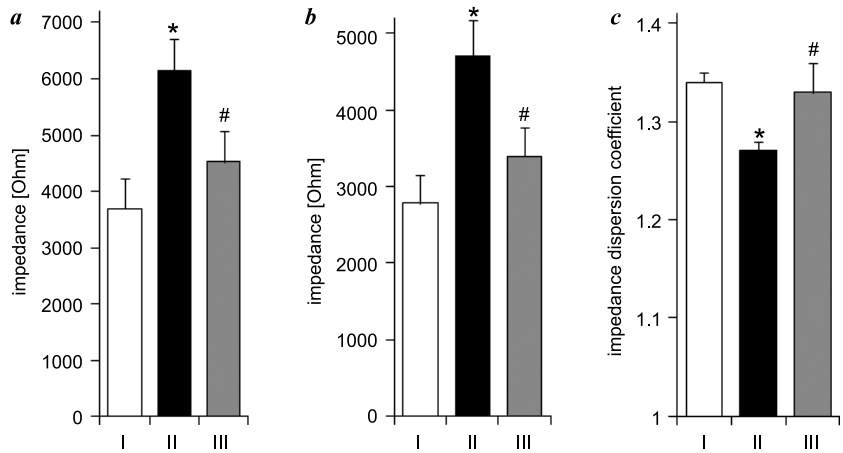

Fig. 3. Effects of *L*-tryptophan on bioimpedancemetry indicators: *L*-tryptophan reduced the impedance on 10^4 Hz (*a*) and 10^6 Hz (*b*) in rat's visceral fat increased due to HCD *Different from Group I at $P < 0.05$; #different from Group II at $P < 0.05$ (One way ANOVA followed by Bonferroni post hoc test, $N = 10$ rats/group)

Discussion

The diagnosis and prevention of VO are critical tasks in modern medicine. Clinicians place special emphasis on detecting and treating the disease in its early stages, where morpho-functional changes in visceral adipose tissue of varying severity are observed. During the initial stages of VO, there are no significant disruptions to the morpho-functional state of WAT that are typically associated with the development of inflammatory processes. Consequently, the effectiveness of treatment measures is highest during this phase. Therefore, it is crucial to identify reliable methods for preventing the progression of VO.

Tryptophan, an essential amino acid, plays a role in numerous metabolic functions. It serves as a unique building block for proteins (RICHARD et al. 2009). Tryptophan is also a precursor to essential endogenous indolamines such as serotonin and melatonin, which function as neurotransmitters, neuromodulators, and neurohormones (TORDJMAN et al. 2017). Increased synthesis of these signaling molecules can enhance overall health and quality of life (POEGGELER et al. 2022). Tryptophan is employed in the treatment of various conditions, including depression, sleep disorders, cognitive impairments, anxiety, and neurodegenerative diseases (KALUZNA-CZAPLINSKA et al. 2019). Conversely, decreased tryptophan secretion has been associated with obesity, anorexia, bulimia nervosa, and other diseases (SHIPELIN et al. 2021).

Our studies confirmed a significant increase in both absolute and relative visceral fat weight in an induced visceral obesity rat model. This increase was primarily due to hypertrophy of WAT adipocytes. Additionally, a decrease in blood saturation of adipose tissue was observed. The concentration of total lipids, triglycerides, and cholesterol increased in the blood serum of these rats.

In rats that simultaneously received HCD and *L*-tryptophan morphological, biochemical, and biophysical changes of WAT were manifested to a much lesser extent, and in some parameters did not differ from the control. The weight gain of visceral fat was less pronounced, and the adipocytes were smaller compared to the experimental group that did not receive *L*-tryptophan. Additionally, the lipid metabolism parameters were similar to the control values. These findings suggest that *L*-tryptophan reduces the manifestations of VO and attenuates fat accumulation in WAT.

The bioelectrical impedance analysis (BIA) method has been shown to be highly informative for assessing body composition and diagnosing the degree of obesity (BRUNANI et al. 2021). Our studies using the BIA method revealed significant changes in the bioelectrical properties of WAT in experimental rats that developed VO due to the HCD. These changes were attributed to the histologically confirmed hypertrophy of adipocytes and the significant accumulation of free lipids within them. Lipids are known to have low hydration levels and high electrical resistance. Furthermore, an increase in connective tissue elements and a decrease in blood supply to adipose tissue further contributed to these effects.

The increase in electrical impedance at both low and high frequencies of the test current, along with a decrease in the coefficient of frequency dispersion of impedance, indicates a decline in the polarization processes, functional activity, and metabolic activity of adipose tissue. However, it's important to note that changes in bioelectrical parameters can be influenced not only by increased fat content in adipocytes but also by alterations in its physical and chemical properties. Previous research by other authors has indicated a connection between BIA indicators and the properties and structure of adipose tissue (YUKEN et al. 2004).

In rats that received *L*-tryptophan, the degree of changes in impedance and the coefficient of frequency dispersion were significantly lower, suggesting a reduction in the manifestation of VO. This indicates that tryptophan has protective properties and contributes to the preservation of normal bioelectrical properties of WAT in the case of VO.

The lipolytic effect of tryptophan has also been observed by other researchers. For example, in rats on an HCD, the concentration of triglycerides in the blood serum increased. However, when the rats received

tryptophan (at a dose of 250 mg/kg), the triglyceride levels approached the control values (SHIPELIN et al. 2021). In experiments on mice with alimentary and genetic obesity, orally administered *L*-tryptophan at a dose of 1 mg/ml of water led to a decrease in body and adipose tissue weight, and serum cholesterol levels. These changes were accompanied by a reduction in inflammation markers (SIVAPRAKASAM et al. 2021). Similarly, in piglets receiving tryptophan with drinking water at concentrations of 0.4% and 0.8%, researchers observed a decrease in food intake, triglyceride levels, hepatic lipogenesis, gluconeogenesis, and an increase in glycolysis and lipolysis intensity (GOODARZI et al. 2021).

The positive effects of tryptophan on the state of WAT in VO can be attributed to the influence of its metabolites, such as serotonin and melatonin (MANGGE et al. 2014, NAMKUNG et al. 2015). Increased levels of serotonin in the central nervous system have been shown to contribute to a reduction in food consumption and body weight, as well as an increase in energy expenditure through the activation of brown adipose tissue by the sympathetic nervous system. Serotonin also regulates the metabolism of carbohydrates and fats and helps alleviate stress, which further affects calorie consumption (BLUNDELL and LAWTON 1995, BUWALDA et al. 2001). The researchers investigated the association between increased visceral fat weight and elevated tryptophan catabolism through the kynurenine pathway, resulting in reduced serotonin production in individuals with severe obesity. Increased levels of kynurenine, as a consequence, may contribute to metabolic disorders in obesity (GELPI et al. 2022, LISCKA et al. 2022). Furthermore, melatonin exerts significant effects on WAT by promoting the formation of beige adipocytes, enhancing mitochondrial function, and reducing oxidative stress (JIMENÉZ-ARANDA et al. 2014).

Conclusions

Our study using an induced visceral obesity rat model revealed that obesity development is accompanied by an increase in the weight of visceral adipose tissue and significant morphological, biochemical, and biophysical changes.

The administration of *L*-tryptophan at a dose of 80 mg/kg, in conjunction with a high-calorie diet, mitigated the accumulation of visceral fat and improved lipid metabolism by reducing the elevated levels of total lipids, cholesterol, and triglycerides in the blood serum.

Furthermore, *L*-tryptophan attenuated the severity of morpho-functional and bioimpedance changes in white adipose tissue caused by obesity.

These findings have both theoretical and practical implications, particularly in the clinical application of tryptophan for comprehensive prevention of visceral obesity development. Future research should focus on elucidating the mechanisms by which tryptophan and its metabolites contribute to the correction of visceral obesity.

Translated by Roman Yanko and Mikhail Levashov

Accepted for print 28.07.2023

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