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CHANGES OF STRUCTURAL COMPONENTS AND BLOOD VESSELS IN LYMPH NODES AT EXPERIMENTAL OBESITY

Abstract. *Obesity is a chronic heterogeneous disease. Has a tendency to recurrent leakage.*
The purpose of the work was to study changes of structural components and blood vessels of rat mesenteric lymph nodes through different terms of abolition of a high-calorie diet with the prior induction of experimental obesity. Materials and methods. We carried out the study on 66 white rats of reproductive age (5.0-7.0 months) weighing 220-240 g. Microanatomy of the mesenteric lymph nodes structural components in white rats under conditions of physiological norm was studied on 10 intact animals. Experimental animals were divided into 4 groups. With the aid of biochemical blood analysis, it has been investigated determine the glucose, ALT, AST, cholesterol and triglycerides. Results. The level of glucose in the blood of white male rats gradually increases within six weeks after the abolition of a high-calorie diet, but eight weeks after the abolition of a high-calorie diet returns to the level of intact animals. The level of glucose in the blood of white female rats fluctuates throughout the experiment and eight weeks after the abolition of a high-calorie diet by 13% exceeds the rate of intact animals. As a result of our study, we found that two weeks after the abolition of a high-calorie diet in both male and female rats, the number of secondary lymphoid nodules in the cortical substance of the lymph nodes of white rats increases, the hematological centers of which are enlarged, enlightened, arteries and veins are deformed, extended, full-blooded. Six weeks after the abolition of a high-calorie diet, the amount of adipose tissue in the thickness of the capsule and around the organ does not decrease, medullar and cortical lymph sinus remain enlarged and deformed. Conclusions. With the increase in the duration of abolition of a high calorie diet in both male and female rats, the number of secondary lymphoid nodules in the parenchyma of the mesenteric lymph nodes decreases somewhat, around the luminous center there is no clear mantle, medullar, cortical, and marginal lymph sinus are slightly less extensive.
Key words*: obesity, experiment, lymphocytes, lymph nodes, arteries, veins.*

Introduction. Obesity is a chronic heterogeneous disease. Has a tendency to recurrent leakage [6, 16]. Obesity is part of a metabolic syndrome as a complex of humoral and metabolic disorders in the body [5, 7-10, 12-15]. It leads to a delay in water and sodium, which leads to hypervolemia, an increased amount of sodium in the vessel walls. It has a vasoconstrictor effect [2, 4]. An increase in the volume of circulating blood and peripheral vascular resistance adversely affects blood pressure, which is a risk factor for cardiovascular disease [1, 4, 11].

Influence of obesity as a concomitant disease on the structure of myocardium in patients with hypertension is studied. It has been found that with increasing body mass index, it increases systolic blood pressure, diastolic blood pressure and heart rate, the average value of the mass of the left ventricular myocardium, the absolute value of the thickness of the walls of the left ventricle, as well as the relative thickness of the walls [1]. The influence of obesity on the structure of the kidneys in patients with hypothyroidism has been analyzed. A decrease in the velocity of glomerular filtration was found [2].

The urgent task of modern medicine is to study the effect of obesity on the structure of the lymph nodes, which belong to the secondary lymphoid (immune) organs. They are biological "filters" in which antigens are neutralized, there is antigen-dependent proliferation and differentiation of T- and B-lymphocytes [13].

The purpose of the work was to study changes of structural components and blood vessels of rat mesenteric lymph nodes through different terms of abolition of a high-calorie diet with the prior induction of experimental obesity.

Materials and methods. We carried out the study on 66 white rats of reproductive age (5.0-

7.0 months) weighing 220-240 g.

Microanatomy of the mesenteric lymph nodes structural components in white rats under conditions of physiological norm was studied on 10 intact animals. Experimental animals were divided into 4 groups: the first group (10 animals), being fed a high-calorie diet for eight week, whereupon by a two weeks fed a standard diet of vivarium instead of a high-calorie diet; the second group (10 animals), being fed a high-calorie diet for eight week, whereupon by a four weeks fed a standard diet of vivarium instead of a high-calorie diet; the third group (10 animals), being fed a high-calorie diet for eight week, whereupon by a six weeks fed a standard diet of vivarium instead of a high-calorie diet; the fourth group (10 animals), being fed a high-calorie diet for eight week, whereupon by a eight weeks fed a standard diet of vivarium instead of a high-calorie diet. Each group included 5 male and 5 female rats. High-calorie diet was achieved due to the fact that glutamate sodium was added into food in a dose of 0.07 g / kg of rat body weight, and fructose solution was added into water.

Control was provided by 16 white rats, fed a standard diet of vivarium instead of a high-calorie diet.

All experimental animals were kept under the vivarium of the Danylo Halytsky Lviv National Medical University. The study was performed in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), Council of Europe Directives 86/609 / EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty", the general ethical principles of experiments on animals adopted by the First National Congress of Ukraine on Bioethics (2001).

Images from the histological preparations of the club-shaped and mesenteric lymph nodes in the computer monitor were displayed from the MICROmed SEO SCAN microscope by means of the Vision CCD Camera. The studies were carried out within the established schedule of the trial in samples stained with hematoxylin, eosin and azane.

Results. After 2 weeks of abolition of fed a high-calorie diet, both in male and in female rats the medullary lymph sinuses of the mesentery lymph nodes are enlarged, tortuous. The number of lymphocytes in medullary lymph sinuses gap to decrease, the number of the proportion of reticular and connective tissue to grow. B-lymphocytes, plasmacytes and macrophages are densely located in the medullary lymph cords. «Empty» blood capillaries have thickened walls. The number of postcapillaries venules with high endothelium in the paracortical region of the mesenteric lymph nodes grows both (fig. 1A).

The number of secondary lymphoid follicles in the cortical substance of the mesentery lymph nodes continues to grow both in male and in female rats, the germinal center is cleared, enlarged (fig. 1B). Arteries and veins are deformed, varicose, full-blooded, there are signs of adhesion and aggregation of platelets in the lumen. The amount of adipose tissue grows around the nodes and in capsule.

The level of glucose in the blood of white male rats gradually increases within six weeks after the abolition of a high-calorie diet, but after eight weeks after the abolition of a high-calorie diet returns to the level of the intact animals. The level of glucose in the blood of white female rats during the experiment it fluctuates, and eight weeks after the abolition of a high-calorie diet exceeds by 13.0% the intact animals' index (table 1). The ALT level in the blood of white male rats gradually decreases to its minimum after six weeks after the abolition of a high-calorie diet, it is by 29.5% less than that of intact animals. During the next two weeks of experiment, it increases somewhat and it is by 18.6% less than the rate of intact animals. The ALT level in the blood of white female rats during the experiment it fluctuates, and after eight weeks after the abolition of a high-calorie diet it is by 8.0% less the intact animals' index (table 2).

The AST level in the blood of white male and female rats during the experiment it fluctuates, and after eight weeks after the abolition of a high-calorie diet it is by 31.5% and 34.6% less than that of intact animals (table 2).

The level of cholesterol in the blood of white male rats grows to its maximum after two weeks after the abolition of a high-calorie diet, which is 2.7 times correspondingly higher than the rate of intact animals. Then it is gradually decreases and returns to the level of intact animals. The level of

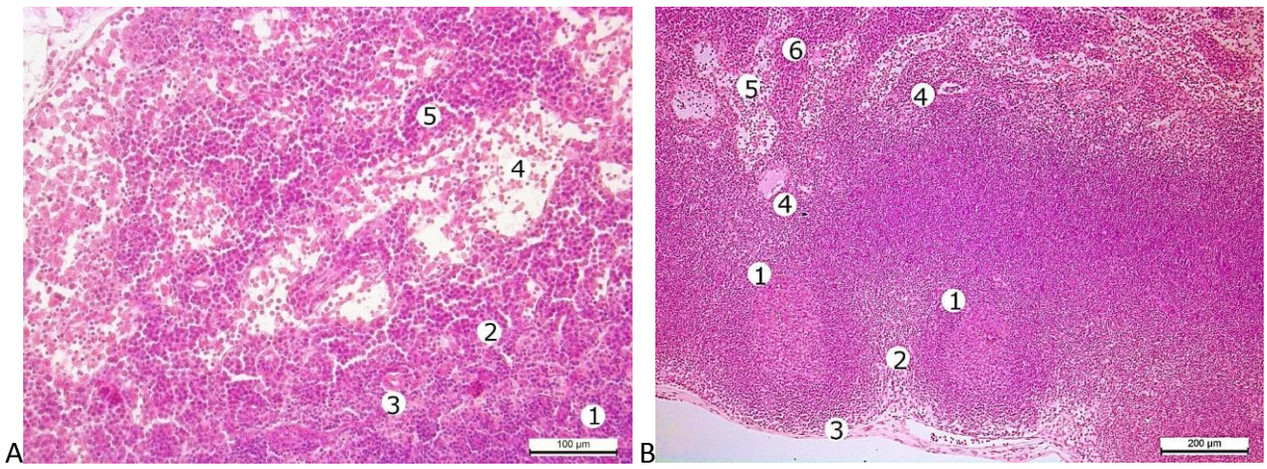


Fig. 1. A mesenteric lymph node of an intact white rat male (A) and white female rats' (B) after two weeks of abolition of fed a high-calorie diet. Stained with hematoxylin and eosin.
Magnif.: A. obj. × 20, ocul. × 10. Designations: 1 – cortical substance; 2 – paracortical region; 3 – venules in the paracortical region; 4 – medullary lymph sinus; 5 – medullary lymph cord.
Magnif.: B. obj. × 10, ocul. × 10. Designations: 1 – secondary lymphoid follicles with the cleared, enlarged germinal center; 2 – cortical lymph sinus; 3 – capsule; 4 – varicose and full-blooded venules with high endothelium in the paracortical region; 5 – medullary lymph sinus; 6 – medullary lymph cord.

Table 1

Indices of blood glucose levels in white rats, mmol/l (M ± m)

Group name	White male rat	White female rat
Intact animals	5.9±0.07	6.0±0.08
Group I	8.41±0.09	7.57±0.1
Group II	7.72±0.1	8.09±0.11
Group III	7.96±0.07	8.22±0.06
Group IV	5.82±0.1	6.78±0.08

Table 2

Indices of ALT and AST levels in the blood of white rats, U/L (M ± m)

Group name	White male rat	White female rat	White male rat	White female rat
	ALT		AST	
Intact animals	76.5±0.2	67.5±0.18	175.7±0.35	168±0.49
Group I	86.2±0.3	58.8±0.21	120.7±0.39	114.3±0.7
Group II	84.7±0.31	75.8±0.29	173.3±0.33	189.6±0.67
Group III	53.9±0.4	63.6±0.22	119.0±0.77	125.7±0.56
Group IV	62.3±0.08	47.1±0.09	120.4±0.45	109.8±0.44

cholesterol in the blood of white female rats grows to its maximum after six weeks after the abolition of a high-calorie diet, which is 4.5 times higher than the rate of intact animals. During the next two weeks, it decreases somewhat and is 2.2 times higher than the rate of intact animals (табл. 3).

The level of triglycerides in the blood of white male and female rats at the beginning of the experiment decreases, and after two weeks after the abolition of a high-calorie diet it is 44.8% and 17.9% correspondingly less than the rate of intact animals. By the end of the experiment, the level of triglycerides gradually increases and it is correspondingly lower by 5.8% та 19.4% than in the intact animals (table 3).

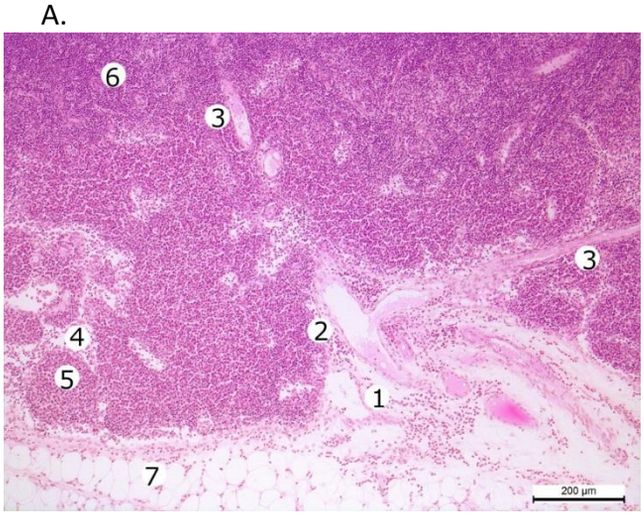
After 4 weeks of abolition of fed a high-calorie diet, both in male and in female rats the number of secondary lymphoid follicles in the cortical substance are not decrease. The medullary and cortical lymph sinuses are enlarged, tortuous. Arteries and veins are deformed, varicose and full-blooded in the hilum of mesenteric lymph nodes (fig. 2 A). The number of B-lymphocytes in B-dependent zones and in the lumen of the vessels grows (fig . 2 B).

Table 3

Indices of cholesterol (mmol/l) and triglycerides (mmol/l) in blood of white rats (M ± m)

Group name	White male rat	White female rat	White male rat	White female rat
	cholesterol		triglycerides	
Intact animals	0.9±0.01	0.87±.,02	0.69±0.009	0.67±0.01
Group I	2.45±0.011	0.93±0.011	0.37±0.006	0.55±0.01
Group II	1.29±0.013	1.34±0.01	0.51±0.09	0.6±0.011
Group III	1.86±0.01	3.92±0.013	0.59±0.008	0.67±0.009
Group IV	0.93±0.01	1.93±0.011	0.65±0.007	0.54±0.01

A.



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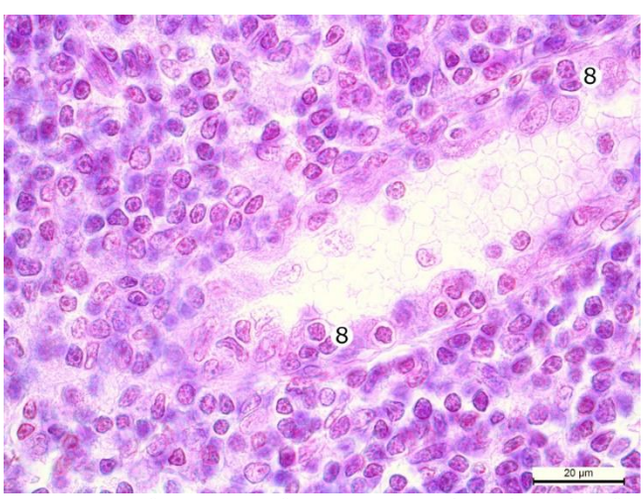

Fig. 2. A fragment of a mesenteric lymph node of a white male rat after four weeks of abolition of fed a high-calorie diet. Stained with hematoxylin and eosin. Magnif: A - obj. × 10, ocul. × 10 (A); B - obj. × 100, ocul. × 10 (B). Designation: 1 – hilum of a lymph node; 2 – varicose and full-blooded artery in the hilum; 3 – varicose artery in parenchyma; 4 – medullary lymph sinus; 5 – medullary lymph cord; 6 – paracortical region; 7 – adipose tissue around the nodes and in capsule; 8 – the output of lymphocytes through the wall of an enlarged venule.

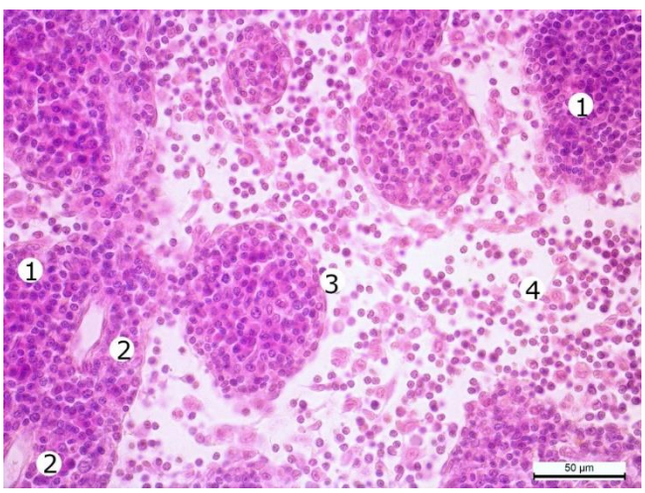

Fig. 3. A fragment of medullary substance of a mesenteric lymph node of a white female rat after six weeks of abolition of fed a high-calorie diet. Stained with hematoxylin and eosin. Magnif: obj. × 40, ocul. × 10. Designation: 1 – B-lymphocytes, plasmacytes and macrophages are densely located in the medullary lymph cords; 2 – varicose and deformed venule; 3 – reticuloendotheliocytes; 4 – varicose and deformed medullary lymph sinus.

After 6 weeks of abolition of fed a high-calorie diet, the amount of adipose tissue around the nodes and in capsule does not decrease, cortical and medullary lymph sinuses stay varicose and deformed (fig. 3). The inclusion of a yellow-brown color in the cortical substance observed, the vast majority of which are concentrated in the germinal center of secondary lymphoid follicles. The number of the plasmacytes and active macrophages grows (fig. 4).

After eight weeks of abolition of fed a high-calorie diet, the number of secondary lymphoid follicles in the cortical substance of the mesentery lymph nodes somewhat decreases, the enlightened germinal centeres without a clear mantle zone around him (fig. 5). The medullary, cortical and marginal lymph sinuses somewhat

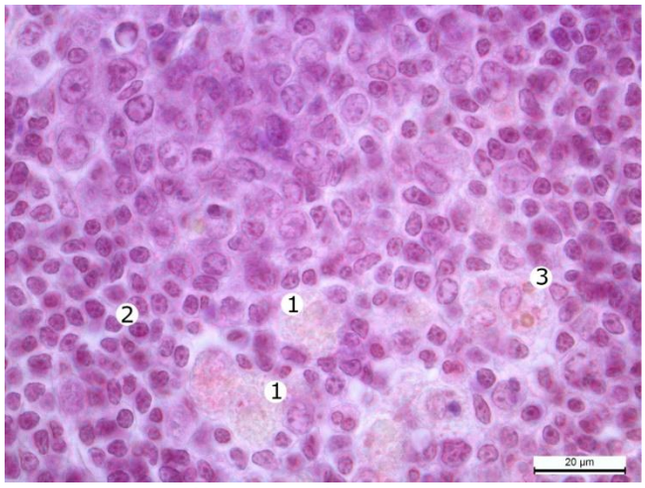

Fig. 4. A fragment of cortical substance of a mesenteric lymph node of a white female rat after six weeks of abolition of fed a high-calorie diet. Stained with hematoxylin and eosin. Magnif: obj. × 100, ocul. × 10. Designation: 1 – the inclusion of a yellow-brown color in the cortical substance; 2 – B-lymphocytes; 3 – macrophages.

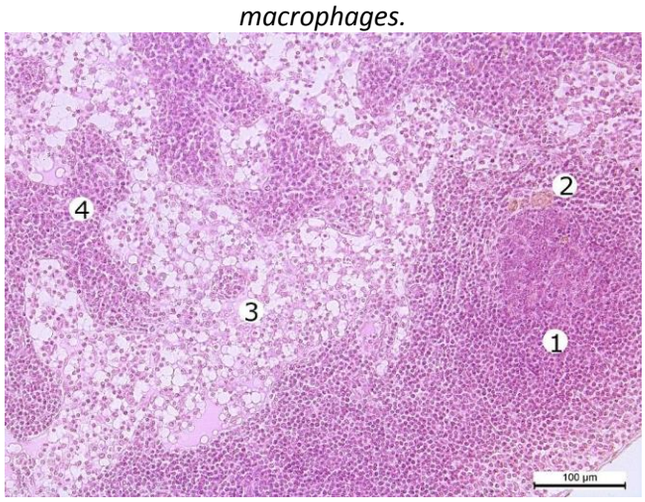

Fig. 5. A fragment of a mesenteric lymph node of a white female rat after eight weeks of abolition of fed a high-calorie diet. Stained with hematoxylin and eosin. Magnif: obj. × 20, ocul. × 10. Designation: 1 – the germinal center of secondary lymphoid follicles without a clear mantle zone around him; 2 – the inclusion; 3 – medullary lymph sinus; 4 – medullary lymph cords.

less advanced. Arteries with a thickened wall, dilated; veins and venules are dilated and full-blooded, mainly in hilum and in the medullary substance.

Discussion. As a result of the analysis of modern literature, it has been found that the development of obesity causes pathological changes in the lymphatic system, such as the weakening of the hematopoietic barrier, lymphedema and the progressive violation of the processes of recirculation of lymphoid and myeloid cells. In addition, obesity is accompanied by chronic inflammation of the white adipose tissue, which causes local and systemic inflammatory response from the immune system, associated with structural and functional changes in the lymphoid organs [10].

In lymph nodes there is development of both cellular and humoral immune response. However, due to microenvironmental features that promote the differentiation of T-lymphocytes in the direction of Tx1, the lymph nodes are more oriented towards the development of the cellular immune response. Investigated by the authors of the inguinal lymph nodes are regional organized lymphoid structures for antigen-expressing cells of the visceral adipose tissue and intestine. In female rats with obesity, there was a statistically insignificant increase in the relative weight of the inguinal lymph nodes with a simultaneous decrease in their cellularity compared with similar indices in intact animals, indicating the development of lymphedema. Also noted a significant individual variability of indicators in this group [3].

According to the results of our study, we showed an increase in the relative area of the B-dependent zone and a decrease in the T-dependent. Similar changes can lead to redistribution of activity in the direction of humoral immune response. There are signs of constant immune activity.

Conclusions: As a result of the study performed on male and female rats, we found that:

1. After 2 weeks of abolition of fed a high-calorie diet, both in male and in female rats the number of secondary lymphoid follicles in the cortical substance of the mesentery lymph nodes grows, the germinal center is cleared, enlarged; arteries and veins are deformed, varicose, full-blooded.

2. With an increase of terms of abolition of fed a high-calorie diet, both in male and in female rats the number of secondary lymphoid follicles in the cortical substance of the mesentery lymph nodes somewhat decreases, the enlightened germinal centeres without a clear mantle zone around him. The medullary, cortical and marginal lymph sinuses somewhat less advanced.

Prospects for further research in this direction related to the further study of morphometric and

submicroscopic changes in the structural components of lymph nodes of rats through different terms of experimental obesity and in conditions of its correction.

Relationship with scientific programs, plans and themes. This study is a part of complex projects: “Features of the lymphoid organs and vascular bed structural organization in the normal ontogenesis and their alteration regularities under the effect of antigens, chemical and physical factors on the organism” - state registration number 0115U003903 and “Structure of organs and their bloodstream in the ontogenesis, under the influence of laser irradiation and pharmaceuticals, with blood supply disorders, reconstructive operations and diabetes mellitus”- state registration number 0110U001854.

References:

1. Kozhem’iaka, H.V., Kovalova, O.M., Ashcheulova, T.V., Honchar, O.V. (2016). Vplyv ozhyrinnia na formuvannia zmin heometrii miokarda u khvorykh na hipertonichnu khvorobu [Influence of obesity on the formation of changes in the geometry of myocardium in patients with hypertension]. *Problemy bezperervnoi medychnoi osvity ta nauky,* (4), 29-34.

2. Didushko, O.M. (2016). Vplyv ozhyrinnia na funktsiiu nyrok u khvorykh na hipotyreoz [The effect of obesity on the function of the kidneys in patients with hypothyroidism]. *Klinichna endokrynolohiia ta endokrynna khirurhiia*, 1 (53), 41-46.

3. Pozur, V.V., Svyatecz`ka, V.M., Usok, V.S., Potapenko, M.S., Dy`ment, G.S., Yankovs`ky`j, D.S., ta in. (2015). Reakciya limfoyidny`x organiv pry` zastosuvanni mul`ty`probioty`ka «Sy`mbiter acy`dofil`ny`j» u shhuriv z glutamatny`m ozhy`rinnyam [The reaction of lymphoid organs in the use of the multiprobiotic "Symbiter acidophilus" in rats with glutamate obesity] *Nauk. zap. Ternop. nacz. ped. un-tu. Ser. Biol.,* 2 (63), 50-58.

4. Alpert, M.A., Omran, B.P., Bostick, J. (2016). Effects of Obesity on Cardiovascular Hemodynamics, Cardiac Morphology, and Ventricular Function. *Curr. Obes. Rep*, (15), 156-171.

5. Blair, S.N., Archer, E., Hand, G.A. (2013). Commentary: Luke and Cooper are wrong: physical activity has a crucial role in weight management and determinants of obesity. *Int J Epidemiol,* (42), 1836-1838.

6. Zobel, E.H., Hansen, T.W., Rossing, P., von Scholten, Global, B.J. (2016). Changes in Food Supply and the Obesity Epidemic. *Curr Obes Rep*, (5), 449-455.

7. Wang, J., Zhang, Q., Zhao, L., Li, D., Fu, Z., Liang, L. (2014). Down-regulation of PPARalpha in the spinal cord contributes to augmented peripheral inflammation and inflammatory hyperalgesia in diet-induced obese rats. *Neuroscience*, (278), 165-178.

8. Franks, P.W., McCarthy, M.I. (2016). Exposing the exposures responsible for type 2 diabetes and obesity. *Science*, (354), 69-73

9. Seijkens, T., Kusters, P., Chadziqeorqiou, A., Chavakis, T., Lutqens, E. (2014). Immune cell crosstalk in obesity: a key role for costimulation? *Diabetes*, 63 (12). 3982-3991.

10. Jiang, S.Z., Lu, W., Zong, X.F. (2016). Obesity and hypertension. *Exp. Ther. Med*, 12 (4), 2395-2399.

11. Lasselin, J., Capuron, L. (2014). Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms. *Neuroimmunomodulation*, (21), 95-101.

12. Lumeng, C.N. (2013). Innate immune activation in obesity. *Mol Aspects Med*, (34), 12-29

13. Madden, C.J., Morrison, S.F. (2016). A high-fat diet impairs cooling-evoked brown adipose tissue activation via a vagal afferent mechanism. *Am J Physiol Endocrinol Metab*, (311), 287-292.

14. Hall, M., do Carmo, J., da Silva, A., Juncos, L., Wang, Z., Hall, J. (2014). Obesity, hypertension, and chronic kidney disease. *Int J Nephrol Renovasc Dis*, (7), 75-88.

15. deOliveira, C., Scarabelot, V.L., deSouza, deOliveira, C.M., Medeiros, L.F., deMacedo I.C., et al. (2014). Obesity and chronic stress are able to desynchronize the temporal pattern of serum levels of leptin and triglycerides. *Peptides*, (51), 46-53

Swinburn, B. (2013). Commentary: physical activity as a minor player in the obesity epidemic: what are the deep implications? *Int J Epidemiol*, (42), 1838-1840.