THE STUDY AND METHODS USED TO INDUCE OBESITY IN LABORATORY ANIMALS: THEORETICAL AND PRACTICAL CONCERNS

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Abstract. Obesity is defined as a body mass index of over 30 kg / m^2 and represents a significant health risk because it increases the risk of diabetes, cardiovascular disorders, stroke and colon cancer. The study of the mechanisms by which obesity induces physiological dysfunctions can be facilitated by the use of an animal experimental model. Induction of obesity in laboratory animals can be accomplished by administering monosodium glutamate that causes lesion of the ventromedial hypothalamic nucleus, by administering hypercaloric diets and using genetically modified rodents. The animal model should reproduce the genesis of obesity and its pathophysiological mechanisms encountered in humans. In the obesity study, hormonal parameters such as leptin and adiponectin can be quantified, also the degree of differentiation of adipose tissue in brown or white and anthropometric measurements, such as: Body Mass Index (BMI), Obesity Index (OI), and Specific Weight Gain Rates (g / kg) are performed.

Key words: obesity, hypercaloric diets, anthropometric measurements, obesity index

INTRODUCTION

Obesity is a major health problem in the world due to its morbidity and mortality. In recent years, obesity has seen a growing proportion in segments of population from developed or developing countries. Studies have shown that excess of fat can cause metabolic abnormalities such as dyslipidemia, insulin resistance, and may also increase the risk of cardiovascular complications such as coronary artery disease and hypertension (Rosini et al., 2012 Ezzati et al., 2006). Normally, adipose tissue normally falls between 10 and 25% of body weight. When this body mass index (BMI) exceeds 30 kg / m^2 represents a significant risk to health (Kanasaki et al., 2011; Van den Berg et al., 2016). Some researchers argue that obesity is determined by 50 to 90% of genetic factors, while environmental factors determine its phenotypic expression. Environmental factors that contribute directly to global obesity are inadequate food (high fat and sugary foods) and sedentary lifestyle (Rosini et al., 2012).

THE PATHOPHYSIOLOGY OF OBESITY

Lipogenesis is the process of lipid synthesis, with the increase of adipose tissue mass in the case of increased lipogenesis, by the process of hypertrophy of the adipocytes by excessive intracytoplasmic accumulation of triglycerides and cholesterol esters. The process of fat tissue development takes place in two successive stages. In the initial phase, also called the lipogenesis phase, accumulation of triglycerides in the adipocytes is achieved, so the size of the adipocytes increases considerably. In the second stage of adipogenesis, following the increased lipid storage needs, the recruitment and differentiation in the perivascular stromal compartment of the preadipocytes in the adipocytes is performed, thus supplementing the adipocytes already existing in the tissue. In recent obesity studies, it has been observed that the differentiation of adipocyte precursors (preadipocytes) into adipocytes is done through a

network of transcription factors. The number of adipocytes in a body fat volume increases in obesity by preexisting hypertrophy of the adipocytes and hyperplasia due to the formation of new adipocytes in existing precursor cells (adipogenesis).

Adipose tissue is a connective tissue specialized in maintaining homeostasis and is mainly composed of adipocytes, pre-adipocytes, macrophages, endothelial cells, fibroblasts and leukocytes. In mammals, adipose tissue is multilocular, consisting of subcutaneous and visceral deposits. There are two major types of adipose tissue: brown and white fat. The two types of tissues have antagonistic roles, the brown produces energy, while the white one stores it (Luo et al., 2016, Svensson et al., 2014). The brown adipose tissue is composed of brown fat cells with a high cytochrome content in the cytoplasmic mitochondria, and due to the rich vascularity it is brown. Brown tissue is well represented in the embryo and newborn, then gradually replaced with white fat. The largest amount of brown tissue in adults is found in the abdomen, mediastinum, perineal area, around the aorta and the neck. White adipose tissue is not as richly vascularized as brown adipose tissue, but each adipocyte is in contact with a blood capillary, approximately 60-85% of the mass white fat is made up of lipids, of which 90-99% are triglycerides, the rest are small amounts of free fatty acids, diglycerides, cholesterol, phospholipids, etc. The rest of the body's white fat is represented by water and protein (Ross et al., 2011). It has been observed from animal experiments that white adipose tissue has the ability to turn into brown adipose tissue (Li Qiang et al., 2012).

CHRONIC INFLAMMATORY RESPONSE IN OBESITY

Inflammation in obesity differs from classical inflammation, defined by cardinal signs of tumor, rubor, calor, dolor. The classic response to inflammation is associated with increased basal metabolism and is the rapid response of the immune response to an infection. Once the infection or injury is neutralized, the inflammation disappears. However, the inflammatory response we encounter in obesity is of a different nature (Baba et al., 2004; Hotamisligil, 2006).

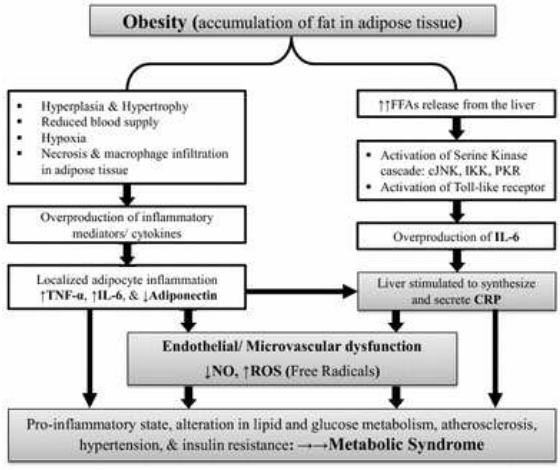

Figure 1 Mechanisms linking abdominal obesity and metabolic syndrome via inflammatory mediators. Abbreviations: TNF-a, tumor necrosis factor alpha; IL-6, interleukin 6; NO, nitric oxide; ROS, reactive oxygen species; JNK, c-jun N-terminal kinase; IKK, inhibitor of k kinase; PKR, protein kinase R. (Ellulu et al. 2016)

In obesity, primarily the inflammatory trigger factor is metabolic. Inflammation is caused by excessive nutrition. Metabolic cells transmit metabolic signals to initiate metabolic response and damage metabolic homeostasis (Fig.1). An increased level of cytokine TNF-α was observed in adipose tissue of obese mice as compared to control mice (Hotamisligil et al., 1995). At present, it is known that not only TNF-α is elevated, but a number of inflammatory cytokines have increased obesity values, including interleukin-6 (IL-6), IL-1β, CCL2 (Berg et al., 2005). Recent studies have shown that other tissues express proinflammatory cytokines (liver, pancreas, brain and muscle tissue), which in turn enhances the expression of inflammation in obesity (Shoelson et al., 2006).

Recent studies have suggested that adipose tissue hypoxia is an important cause of chronic obesity inflammation, as a result of reduced blood flow in adipose tissue (Larsen et al., 1966). Hypoxia induces inflammatory response by direct and indirect effects; the direct effect is achieved by activating multiple signaling pathways in adipocytes and macrophages due to hypoxic stress. Transcription factors (HIF-1α and NF-kB) are important signaling molecules for hypoxia, which induce inflammation. The indirect effect of hypoxia is observed by the death of adipocytes (apoptosis and necrosis) and lipolysis. Adipocyte death induces macrophage infiltration in adipose tissue to exert its phagocytic function (Cinti et al., 2005). This can help increase the amount of free fatty acids in circulation, as dead cells release them into blood circulation. Also, plasma levels of free fatty acids are associated with lipolysis in adipose tissue. Lipolysis is the result of reduced insulin action in adipocytes, which occurs under fasting conditions or in insulin resistance. The induction of lipolysis in adipocytes by hypoxia occurs by inhibiting insulin receptor proteins in adipocytes and by reducing signaling activity of insulin receptors, as a consequence the signaling pathways for insulin are inhibited (Yin et al., 2009).

ADIPOCITARY FACTORS

Adipose tissue secretes a number of factors influenced by metabolic disorders. For a long time adipocytes have not been recognized as endocrine cells until some important factors that are highly secreted by adipocytes such as leptin and adiponectin have been discovered. Adipocyte secretory factors are called adipokine. Adipokines have a great influence on systemic functions and interact with many organs (Shimabukuro et al., 1997).

LEPTIN

Leptin is a well-known product of the *ob* gene, synthesized mainly by the adipose cells and released into the bloodstream. The first time was discovered in rodents with genetic obesity (*ob / ob* mice). The role of leptin is to control food intake and energy metabolism, thus correcting leptin deficiency in animals causes a marked reduction in food intake and consequently a normalization of obesity syndrome (Harris, 2000; Ahima et al., 2000). Leptin has many structural similarities with the cytokine family. The leptin receptors are part of the class I cytokine receptors. These receptors have been discovered at the hypothalamic level, the brain center for satiety, yet the respective receptors are not limited to the brain; they can also be identified in the liver, spleen, cardia, renal, intestinal and adipose tissue. The role of leptin is to control food intake and energy metabolism (Ioffe et al., 1998).

ADIPONECTIN

Circulating adiponectin forms several different complexes in adipocytes before it is released into circulation. The most common stereochemical form of adiponectin is the trimer, secreted by adipocytes and released into circulation. The trimer function is not fully

known. In addition to trimers, adiponectin forms two other structures by covalently linking 2 trimesters or 6 trimesters (Shimabukuro et al., 1997). The low level of adiponectin has been associated with cardiovascular disease, diabetes and insulin resistance. Adiponectin was administered to rodents with promising results. It is believed that the metabolic changes induced by this hormone would reduce the markers of metabolic syndrome by inhibiting phagocytic phagocytic activity and producing TNF-α, thus reducing the occurrence of cardiovascular disease and diabetes mellitus (Yokota et al., 2000).

ASSESSMENT OF OBESITY INSTALLATION

There are some tests that can determine obesity, some of which are more accurate than others:

- Hydrostatic measurement of the percentage of body fat
- Dual Energy X-ray Absortiometry (DEXA)
- Bioelectric Impedance BIA
- Weight / height charts
- Index of body mass

Calculation of body mass index (BMI) is one of the most widespread methods of obesity analysis. Only data on weight and height is required and the result is obtained on the spot. BMI is often used by large insurance companies to determine the risk of cardiovascular disease in patients over 65 years of age.

The BMI calculation is as follows: The BMI is calculated by dividing the weight expressed in kg at the height expressed in square meters: BMI = m / (h^2). The classification of overweight or obese risk after BMI is by abdominal circumference and BMI comorbidity (kg / m ^{2}). To assess the degree of obesity in animals, measurements of body length, abdominal circumference and body weight were performed. The obtained values were introduced into an extrapolated formula of human medicine, a formula that can appreciate the obesity scale: OI = (Bm + Ac) / Bl, where OI = obesity index; Bm = body mass (g); Ac = abdominal circumference (cm); Bl = body length (cm). The body length was measured from the bottoms to the base of the tail, and the abdominal circumference was measured in the umbilicus (Lean et al., 1995).

Body Mass Index (BMI): Bodyweight (g) / Length ^{2} (cm ^{2}). Specific weight gain ratio (g / kg): dM / Mdt, where dM is the body weight gain during dt = t2-t1 and M is the rat body weight at t1. (Novelli et al., 2007).

ANIMAL MODELS TO INDUCE OBESITY

The study of the mechanisms by which obesity induces physiological dysfunctions can be facilitated by using an animal model in research. There are different types of animal models, made on rodents, which develop obesity due to genetic mutations. However, given that the model should be as close as possible to the genesis of obesity in humans, inducing this state by eating high calorie foods is the most appropriate. Obesity is determined by genetic factors from 50% to 90% of cases, and the environment only determines phenotypic expression. There is a consensus that the genetic factor itself is not the cause of obesity. Cases of genetic mutations (such as the removal of genes regulating the production of leptin, satiety hormone) are rare. However, cases of polymorphism that modify hormone production, which regulates food intake and energy consumption, are detected in the

population, and polymorphism associated with environmental factors such as sedentary lifestyle, excess carbohydrate and fat intake saturated, increase the risk of developing obesity. Unlike humans, the genesis of obesity in laboratory animals is mostly linked to genetic changes that can alter or suppress secretion of neuropeptides and the hormones responsible for satiety. Moreover, according to the modified gene, animals will develop obesity sooner or later along with other disorders such as insulin resistance, diabetes, hypercholesterolemia, hypertension, with the pathophysiological investigation of obesity and its comorbidities. Currently, animal models have been used to investigate candidate genes and to confirm the cause of obesity and other diseases. This is based on investigating the genetic sequence of diseased individuals as compared to healthy animals (Rosini et al., 2012).

Table 1.

Different types of diet used (Rosini et al., 2012)

Obesity model	Achievement method	Bibliographic source
Hyperlipidic diet (added soy oil)	Food administration for 12 weeks, male 250 - 275 g	**Barnes et al. (2003)**
Hypercaloric diet (peanuts, chocolate with milk and corn biscuits	Food administration for 8 weeks, male 180 - 200 g	**Burneiko et al.(2006)**
Cafeteria diet (biscuits, salami, butter, cheese and ham)	Food administration for 20 weeks, male 200 g	**DeSchepper et al. (1998)**
Hyperlipidic diet (peanuts, milk chocolate and sugar cookies)	Food administration for 8 weeks, male 81 days	**Estadela et al.(2004)**
Cholesterol rich diet (1% cholesterol + 0.25% colic acid added)	Food administration for 8 weeks, males 210-230 g	**Guerra et al.(2007)**
Cafeteria Diet (pate, fries, chocolate, ham and biscuits)	8-week feed in 40-day-old males	**Lopez et al.(2003)**
Hypercalaric diet (AIN 93 plus condensed milk and sucrose)	Food administration for 8 weeks in males of 270-300 g	**Moraes et al.(2007)**
Hypercalaric diet (AIN 93 plus condensed milk and sucrose)	Food administration for 8 weeks in males of 295 - 310 g	**Moraes et al.(2008)**
Hypercalaric diet (sweetened condensed milk and sucrose)	Food administration for 12 weeks in females of 200 - 210 g	**Naderalli et al.(2001)**
Hypercaloric diet (sweetened condensed milk and sucrose)	Food administration for 15 weeks in males of 180-200 g	**Naderalli et al.(2003)**
Hypercaloric dietary (peanuts, casein, soybean oil, chocolate + corn biscuits or chips or instant noodles + race cheese or sweetened condensed milk + biscuits)	Food administration for 14 weeks in males of 105-120 g	**Nascimento et al. (2008)**
Hyperlipidic diet (corn starch, casein, sucrose, dextrin powder, lard, soybean oil, cellulose, mixture of minerals and vitamins, cystine, and choline)	Food administration for 12 weeks in 30-day-old males	**Silva et al. (2010)**
Hypercalaric diet (peanuts, milk chocolate, biscuits and corn starch)	Food for 3 weeks in male 225 g	**Zambon et al.(2009)**

Obesity animal model achieved by ventromedial hypothalamic nucleus by monosodium glutamate administration. Monosodium glutamate (MSG) is administered to newborn rats. As a result, animals lose their ability to regulate the amount of energy synthesized from food, as well as catabolic energy, by ventromedial hypothalamic nucleus necrosis. The hypothalamic-pituitary axis is destroyed and the disease is manifested after a single dose, the clinical signs consisting of hypoaesthesia, hypophagia, ovarian inactivity, delayed puberty, and elevated levels of corticosteroids in the blood. MSG can be administered subcutaneously or intraperitoneally at doses of 2-4 mg / g during the neonatal period (Novelli et al., 2007; Jaime, 2013).

Model of obesity through hypercaloric diet in rodents. Hypercalaric diets can be obtained by the administration of excess carbohydrates or by the addition of fat in the daily diet, several types of diets can be seen in Table 2. Regardless of the nature of the ingredients added, all diets bring extra calories between: 3.7 Kcal / g and 5.4 Kcal / g. An example of diet based on excess carbohydrate is 33% of rodent feed, 33% of Nestle powder, 7% of condensed milk, 7% of saccharin and 27% of water in adult rats for 15 weeks (Table 1). Another model is based on 48% standard food, 8% maize oil, 44% frozen milk for 12 weeks (Ha and Zernel, 2003; Nagaoka, 1996).

A fat-based model is: 17.4% carbohydrates, 42.9% protein and 39.7% animal fats or 55% fats, 21% carbohydrates and 24% protein. It is worth mentioning that there are pet food businesses that have a high percentage of carbohydrates, which can be used in diets for fattening. As an example, Purina Ltd contains 26.5% protein, 3.8% fat, 40% carbohydrate, 4.5% fiber in 100 grams of feed, quantified in 12.56 kj / g metabolisable energy. During experiments weighing animals at least once a week and measuring the amount of food received twice a day.

In most studies, obesity was induced in laboratory animals using hypercaloric diets (80%). Shin et al. have studied the excessive accumulation of visceral fat and insulin resistance through fat-rich diets because this diet model has a similarity to genesis and the metabolic syndrome that causes obesity in humans. Campos et al. have induced obesity by administering monosodium glutamate to study the reproductive parameters of obese Wistar rats and to determine the frequency of obese adult descendants (Campos et al., 2008). Martín-Cordero et al. have used the genetic model of the Zucker rat to study metabolic syndrome and determine inflammatory status and evaluate the effect of a physical exercise program (Martín-Cordero et al., 2011)

CONCLUSIONS

For the study of the pathophysiological mechanisms involved in the development of obesity, numerous experimental protocols on laboratory animals were developed. However, the phenotype of human obesity is caused by the interaction between genes and the environment; therefore the laboratory animal model that has the highest resemblance and mimics closely the human metabolic pathway is the polygenetic and/or the hypercaloric diet model. Recently, the hypercaloric diet (high in fructose or carbohydrate) is intensively used due to the similarity to the genesis and metabolic syndrome encountered in humans.

REFERENCES

1. Ahima RS, Flier J S. 2000. Leptin. Annu Rev Physiol. 62:413-437.
2. Amita Yadava, Megha A Kataria, Vandana Sainia, Anil Yadavb. 2013. Role of leptin and adiponectin in insulin resistance. Clinica chimica Acta Volume 417. Pag 80-84.
3. Baba A I, Cătoi C. 2004. Morfopatologie generală. Ed Academic Pres. Pag 20-28.
4. Berg A H, Scherer P E. 2005. Adipose tissue, inflammation and cardiovascular disease. Circ Res 96: 939-49.
5. Campos K.E., G.T. Volpato, I.M.P. Calderon, M.V.C. Rudge and D.C. Damasceno. 2008. Effect of obesity on rat reproduction and on the development of their adult offspring Brazilian Journal of Medical and Biological Research, 41: 122-125.
6. Cinti S, Mitchell G, Barbarelli G, Mueano I, Ceresi E, Faloia E. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 46:2347-55.
7. Ellulu MS, Khaza'ai H, Rahmat A, Patimah I, Abed Y (2016a) Obesity can predict and promote systemic inflammation in healthy adults. Int J Cardiol 215:318–324.
8. Ezzati M, Martin H, Skjold S, SV, Murray CJL. 2006. Trends in national and state-level obesity in the USA after correction for self-report bias: Analysis of health surveys. J. R. Soc. Med. 99: 250-257.
9. Ha E, Zernel MB, 2003. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people, J Nutr Biochem, 14:251-60;
10. Harris B S. 2000. Leptin-much more than a satiety signal. Annu Rev Nutr. 20:45-75.
11. Hotamisligil G S, Arner P, Caro J F, Atkinson R L, Spiegelman B M. 1995. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J clin Investig. 95: 2409-15.
12. Hotamisligil G S. 2006. Inflammation and metabolic disorders. Nature 444: 860-67.
13. Ioffe E, Moon B, Connolly E, Friedman J M. 1998. Abnormal regulation of the leptin gene in the pathogenesis of obesity. Proc Natl Acad Sci USA. 95:11852-11857.
14. Jaime E AF, 2013. Whey Protein Hydrolysate Increases Translocation of Glut-4 to the Plasma Membrane independent of Insulin in Winstar Rats, Plos One, 8(8): e 71134;
15. Kanasaki Keizo and Daisuke Koya. 2011. Biology of Obesity: Lessons from Animal Models of Obesity. Journal of Biomedicine and Biotechnology.1-11.
16. Larsen O A, Lassen N A, Quaade F. 1966. Blood flow through human adipose tissue determined with radioactive xenon. Acta Physiol Scand. 66:337-45.
17. Lean M E J, T S Han, C E Morrison (1995). Waist circumference as a measure for indicating need for weigh management. BMJ VOLUME 311, 158-161.
18. Li Qiang, Liheng Wang, Ning Kon, Wenhui Zhao, Sangkyu Lee, Yiying Zhang, Michael Rosenbaum, Yingming Zhao, Wei Gu, Stephen R Farmer, Domenico Accili. 2012. Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of PparϒCell; 150 (3).
19. Luo Liping and Meilian Liu. 2016. Adipose tissue in control of metabolism. Journal of Endocrinology. 231, 77–99.
20. Maggie S Strable, Janes M Ntambi. 2010. Genetic control of de novo lipogenesis: role in diet-induced obesity. Crit Rev Biochem Mol Biol; 45(3): 199-214.
21. Martín-Cordero Leticia, Juan J García, Maria D Hinchado and Eduardo Ortega. 2011. The interleukin-6 and noradrenaline mediated inflammation-stress feedback mechanism is dysregulated in metabolic syndrome: Effect of exercise. Cardiovascular Diabetology, 10:42.
22. Nagaoka S., 1996. Studies on regulation of cholesterol metabolism induced by dietary food constituents or xenobiotics, J Jpn Soc Nutr Food Sci, 49:303-313;
23. Novelli E L B, Y S Diniz, C M Galhardi, G M X Ebaid, H G Rodrigues, F Mani, A A H Fernandes, A C Cicogna and J L V B Novelli Filho. 2007. Anthropometrical parameters and markers of obesity in rats. Laboratory Animals 41, 111–119.

24. Rosini Tiago Campos, Adelino Sanchez Ramos da Silva, Camila de Moraes, 2012. Diet-induced obesity: rodent model for the study of obesity-related disorders. Rev Assoc Med Bras. 58 (3):383-387.
25. Ross H M, Pawlina Wojciech. 2011. Histology: a text and atlas: with correlated cell and molecular biology. 6th edition, Lippincott Williams&Wilkins, pag 254-263.
26. Shimabukuro M, Koyama K, Chen G, Wang M Y, Trieu F, Lee Y. 1997. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci USA. 94:4637-4641.
27. Shin Terada, Sayuri Yamamoto, Seiji Sekine, Toshiaki Aoyama. 2012. Dietary intake of medium- and long-chain triacylglycerols ameliorates insulin resistance in rats fed a high-fat diet. Nutrition 28 92–97.
28. Shoelson S, Lee J, Goldfine A. 2006. Inflammation and insulin resistance. J Clin Investig. 116:1793-801.
29. Svensson Per-Arne, Kristin Lindberg, Jenny M. Hoffmann, Magdalena Taube, Maria J. Pereira, Tala Mohsen-Kanson, Anne-Laure Hafner, Magnus Rizell, Jenny Palming, Christian Dani and Maria K. Svensson. 2014. Characterization of Brown Adipose Tissue in the Human Perirenal Depot. Obesity. 22:8. 1831-1837.
30. Van den Berg Susan M., Andrea D. van Dam, Patrick C.N. Rensen, Menno P.J. de Winther, Esther Lutgens. 2016. Immune modulation of brown(ing) adipose tissue in obesity. Endocrine Reviews. 1-38.
31. Yin J, Gao Z, He Q, Ye J. 2009. Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. Am J Physiol Endocrinol Metab. 296:E333-42.
32. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Nihara S, Funahashi T, Tenner A J, Tomiyama Y, Matsuzawa Y. 2000. Adiponectin a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood. 96:1723-1732.