Mice as an Animal Model for the Study of Adipose Tissue and Obesity

Ratones como Modelo Animal para el Estudio del Tejido Adiposo y la Obesidad

Carlos Alberto Mandarim-de-Lacerda¹; Mariano del Sol²; Béllica Vásquez³ & Marcia Barbosa Aguilá¹


SUMMARY: The study of adipose tissue has gained increasing importance in the biomedical area due to its implications for health and obesity. Obesity is a situation of great concern mainly in the Western world due to its high prevalence and morbidity. Experimental studies on obesity often need a model where it is possible to carry out experiments, drug testing, and other therapeutic procedures, which are typically not possible in humans. Although several animals are used for obesity studies, rodents are by far the most used animals, and among rodents, mice are particularly indicated for this investigation. This mini review will introduce the challenging classification of obesity in rodents, paralleling human obesity, defining and classifying what an obese mouse is. The text will differentiate between white adipose tissue (WAT, aimed at endocrine secretion and lipogenesis) and brown adipose tissue (BAT, aimed at thermogenesis) and describe the browning process of white adipocytes in an adaptation to increase thermogenesis. The text will also describe the various types of body fat in mice with their differences and indications for investigation and teach how to recognize and dissect these fats. At the end of this introductory reading, the young researcher is expected to have acquired sufficient knowledge to start an experimental investigative project on obesity.

KEYWORDS: Obesity; White adipose tissue; Brown adipose tissue; Fat pads; Mice.

INTRODUCTION

Obesity occurs with a positive energy balance, i.e., excess caloric intake linked to a low caloric expenditure (Bluher, 2019). Overweight and obesity are identified as abnormal or excessive growth of fat that can harm health. Hormones produced in adipose tissue, intestine, liver, and other target organs, and appetite regulation and satiety controlled by the hypothalamus, are relevant in obesity (Spezani et al., 2020). Furthermore, parental obesity can affect the health and longevity of children (Armitage et al., 2008), with inflammation in the hypothalamus and hyperleptinemia, culminating in food hyperphagia in offspring (Ornellas et al., 2016).

World Health Organization counted more than 1.9 billion people overweight in 2016 (39 % of the world’s adult population), whose were considered obese more than 650 million (13 % of the world’s adult population). Worldwide, the number of obese people has nearly tripled since 1975 (WHO, 2021).

White adipose tissue (WAT) is an endocrine organ that affects energy metabolism via lipolysis, lipogenesis, and energy storage by triacylglycerol (Fonseca-Alaniz et al., 2007). In addition, WAT secretes hormones, such as leptin and adiponectin, which act in different metabolic pathways of homeostasis. Animals “silenced” for the leptin gene, for example, develop obesity and associated comorbidities such as non-alcoholic fatty liver disease (Martins et al., 2021). Moreover, brown adipose tissue (BAT) shows an endocrine function more linked to thermogenesis (Villarroya et al., 2017). Table I summarizes the secretions of WAT and BAT.

In rodents, there are several models for studying obesity. The most used monogenic mutations in the leptin pathways (ob/ob mouse, db/db mouse. Zucker rat) and other monogenic models (Otsuka Long Evans Tokushima fat rat – OLETF). Also, polygenic models, diet-induced obesity (DIO), maternal overfeeding, and others.

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DIO animals mimic better the state of common obesity in humans and may be the best choice for testing future therapeutics. In addition, transgenic models may be used to explore the role of specific molecular targets and pathways in the physiology of food intake and their potential role in obesity (Lutz & Woods, 2012). DIO C57BL/6 mice triggers standard features of human metabolic syndrome. After 16 weeks on a high-fat diet (60% fat) (Aguila et al., 2021), mice showed more significant BW gain and visceral fat pads. Moreover, impairment of glucose clearance and insulin resistance are installed. The mice showed pancreas and liver masses increase with large pancreatic islet size and significantly intense alpha and beta-cell immunodensities (Fraulob et al., 2010).

**WAT and lipogenesis. BAT and thermogenesis**

The primary function of adipose tissue is to maintain energy balance, which involves the development of obesity. In addition, adipocytes are specialized in regulatory functions in homeostasis. Both types of adipose tissue, WAT and BAT, are described in mammals and exhibit distinct characteristics (Fig. 1) (Gesta et al., 2007).

WAT adipocytes are unilocular (reserve of a single macro droplet of fat), distributed throughout the body in greater quantity. However, BAT exists in smaller quantities, in specific places, whose adipocytes are multinuclear with the fat reserve in microdroplets and abundant mitochondria in the cytoplasm (Langin, 2010).

The adipokines, the adipose tissue distribution, and the metabolism of carbohydrates are significantly influenced by the lipid content in the diet than by the absolute amount of lipids. In an experiment with a high-fat diet (60% fat), different formulations were produced with different fat compositions, such as lard, olive oil, sunflower oil, and canola oil. As a result, leptin was more expressed, whereas adiponectin was less expressed with high-fat diets containing lard and olive oil. Also, the subcutaneous to visceral fat ratio was significantly lower with lard and olive oil than the other high-fat diets (Catta-Preta et al., 2012).

Batokines are adipokines secreted by BAT with autocrine and paracrine effects. Signaling molecules are released by adipocytes and target sympathetic nerve endings, vascular cells, and immune cells. In addition, BAT also has an endocrine function since batokines can actin other organs, such as the recognition of fibroblast growth factor 21 and myostatin, secreted by BAT, which targets the heart and skeletal muscle (Villarroya et al., 2019).

Unlike paraffin embedding and hematoxylin and eosin stain, which does not preserve the adipocyte content (extracted when sections are deparaffined), the oil red technique preserves the entire adipocyte and stains adipocyte cytoplasm. Fresh adipose tissue fragments should be embedded in Tissue-Tek OCT (Finetechnical Sakura, Tokyo, Japan) in aluminum molds, frozen quickly in liquid nitrogen, and stored at -80 °C until microtomy. Frozen sections with

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**Fig. 1. The adipocytes. (A) White adipose tissue (WAT) with unilocular white adipocytes (hematoxylin and eosin stain). Insert shows the ordinary white adipocyte structure characterized by a big central lipid droplet and eccentric (peripheral) nucleus and cytoplasm; (B) Brown adipose tissue (BAT) with multinuclear brown adipocytes (hematoxylin and eosin stain). Insert shows the ordinary brown adipocyte structure characterized by abundant mitochondria and several tiny lipid droplets. Between A and B, the insert shows an intermediary “brite” or beige adipocyte.**
10 μm thickness should be obtained in a cryostat, dried at room temperature for 60 minutes, fixed in 10% formaldehyde for 10 minutes, and then frozen and again dehydrated for 60 minutes. Afterward, the sections should be placed in 100% propylene glycol for 3 minutes, stained with a solution of Oil Red pre-heated for 8 minutes at 60 °C, differentiated in 85% propylene glycol for 3 minutes, washed in tap water for another 3 minutes, and mounted with glycerin (Fig. 2).

In some situations, the white adipocyte may undergo modification and adaptations to work similarly to the brown adipocyte, a process known as “browning,” when the white adipocyte is now called “beige” (or brite). For example, the beige adipocyte acquires more mitochondria and starts to express UCP1 (uncoupled protein 1, located in the inner mitochondrial membrane) and expend heat like brown adipocytes (Bargut et al., 2017). Thus, in addition to regulating thermogenesis, BAT improves lipid and carbohydrate homeostasis and contributes to weight loss mediated by rapid heat generation and macronutrient metabolism related to UCP1 function (Bargut et al., 2016; Velickovic et al., 2019).

Thus, the relevant aspects of obesity and adipose tissue should be studied, many of which are experimental research. However, this type of research, which has the potential to unravel mechanisms of action and behavior of adipocytes and fat pads, cannot be carried out initially in humans but must be parameterized in animal research. Therefore, we are

### Table I. Adipose tissue secretion (Fonseca-Alaniz et al., 2007; Villarroya et al., 2017).

<table>
<thead>
<tr>
<th><strong>White adipose tissue secretion</strong></th>
<th><strong>Brown adipose tissue secretion</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylation stimulating protein</td>
<td>Bone morphogenetic protein 8b</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Member of the TGF-beta superfamily</td>
</tr>
<tr>
<td>Adipsin</td>
<td>Fibroblast growth factor 21</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>Cell growth, morphogenesis, tissue repair, tumor growth, and invasion</td>
</tr>
<tr>
<td>Apelin</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>Apolipoprotein-E ‡</td>
<td>Molecular similar in structure to insulin, shows anabolic effects</td>
</tr>
<tr>
<td>Cholesterol ester transfer protein ‡</td>
<td>Transport protein for insulin-like growth factor 1</td>
</tr>
<tr>
<td>Estrogens †</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>Glucorticoids †</td>
<td>Proinflammatory, lipolytic, reduces sensitivity to insulin</td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td>Macrophage migration inhibitor factor</td>
</tr>
<tr>
<td>Insulin-like growth factor-1</td>
<td>Immunoregulator with paracrine action in WAT</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Monobutylin †</td>
</tr>
<tr>
<td>Leptin</td>
<td>Vasodilator and inducer of vascular neoformation</td>
</tr>
<tr>
<td>Lipoprotein lipase ‡</td>
<td>Plasminogen activation inhibitor 1</td>
</tr>
<tr>
<td>Macrophage migration inhibitor</td>
<td>Inhibits plasminogen activation blocking fibrinolyis</td>
</tr>
<tr>
<td>factor</td>
<td>Prostaglandins †</td>
</tr>
<tr>
<td>Monobutylin †</td>
<td>Regulator of various cellular processes, active during inflammation</td>
</tr>
<tr>
<td>Resistin</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Starts the coagulation cascade</td>
</tr>
<tr>
<td>Transforming growth factor-beta</td>
<td>Proliferation of preadipocytes and differentiation, development and apoptosis of adipocytes</td>
</tr>
<tr>
<td>Tumor necrosis factor-alpha</td>
<td>Lipolytic enhances energy intake and lessens sensitivity to insulin</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>Stimulates vascular proliferation in WAT</td>
</tr>
<tr>
<td>Visfatin</td>
<td>Insulinomimetic mainly produced by visceral fat</td>
</tr>
</tbody>
</table>

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|†, non-protein molecule; ‡, protein without hormonal action. |
motivated to present this article with morphological data to
guide research on obesity and adipose tissue in the mouse
model.

The challenging diagnosis of obesity in rodents

We have difficulty defining obesity in rodents
(Fenton, 1956). Hence, let us start with a parallel with
humans. The most straightforward and epidemiologically
viable indicator for diagnosing obesity in humans is
calculating the body mass index (BMI), the “Quetelet Index
” described in 1832. Adolphe Quetelet (1796-1874) was a
Belgian scientist who developed a keen attention to
probability calculus to study human physical characteristics
and social aptitudes (Eknoyan, 2008). The BMI is the body
weight (BW, kilograms) to square body height (BH, meters)
ratio, expressed in kg/m$^2$.

$$BMI = \frac{BW}{(BH)^2}$$

Several measurements and ratios have been proposed,
and are used, to determine how obese a subject is. Currently,
the waist circumference and waist-to-hip ratio are
recommended (Tutunchi et al., 2020), adjusting target
population different biotypes (Western, Asian, male, female,
others) (Andracech et al., 2021). Also, it should be
considered the body adiposity index (BAI =
((hip circumference)/(height×1.5)–18)), which can be used
in the clinical setting to estimate adiposity directly (Bergman
et al., 2011).

The World Health Organization agreement on the
standardized classification of overweight and obese, based
on BMI, allows a comparative analysis of prevalence rates
worldwide for the first time (James et al., 2001). The
classification generally used for Western adults considers
those with BMI < 18.5 to be underweight. Normal people
have BMI between 18.5 and 24.9. Overweight is people with
BMI between 25.0 and 29.9. Obese are people with BMI
above 30 (Class I, between 30.0 and 34.9; Class II, between
35.0 and 39.9; Class III, above 40.0) (WHO, 2000).

An index was formulated to determine the surface
area of rodents, making a parallel between rodents and
humans (Lee, 1929). The “Lee index” (generally expressed
in percentage) is calculated as the cubic root of BW (in
grams) divided by the nasoanal length (NAL, in millimeters)
and was proposed to rodents with the same purpose of the
human BMI.

$$Lee\ index = \left(\frac{\sqrt[3]{BW}}{NAL}\right)$$

$$Lee\ index = \left(\frac{BW^{0.33}}{NAL}\right)$$

Fig. 2. Oil red preparation. Hepatic steatosis (accumulation of fat drops in hepatocytes, typical of non-alcoholic fatty liver disease). (A) Photomicrographs of frozen sections stained by Oil Red-O showing macro- and microvesicular steatosis. The fat droplets identified by
numbers correlate with the segmented image in B; (B) Image analysis of the same microscopic field after image segmentation
(transformation to pure black and white image, white assigned to fat vesicles and black assigned to remaining liver tissue).
However, the Lee index is unappropriated to define obesity in rodents because it is not perfectly correlated with animal body fat, and there are no defined standards for rodent obesity as there are for BMI and human obesity (Stephens, 1980).

Figure 3 shows how the BMI varies in a 1.85 m tall person and how the Lee index varies in a 120 mm nasoanal mouse as their BW varies. For example, in humans, the BW increases from 75 kg to 115 kg (+ 50%), corresponding to going from BMI 21.9 (normal) to BMI 33.6 (obese, Class I), a BMI change of 53.4 %. However, mice growth from 20 g to 30 g (+ 50%) parallels a variation of the Lee index from 2.24 % to 2.56 % (in other words, an increase of only 14 %).

In a population of regular and obese mice, the weight to length ratio estimates body fat more reliable than the Lee Index (because the Lee index did not correlate well enough with body fat to be used as a method of obesity estimation), as well as the carcass analysis. Therefore, the proportional weight of the gonadal fat pad is now recommended as a simple, consistent estimate of body fat in normal or obese mice (Rogers & Webb, 1980). Moreover, a suitable but not always accessible alternative for analyzing fat distribution in rodents is DEXA.

Rodent fat pads

Different fatty pads in rodents (as in humans) have distinct structural and functional characteristics. Fat pads are the places where adipocytes preferentially group and fat accumulates in rodents. Fat accumulation might occur by increasing the number of adipocytes (hyperplasia) and increasing the size of adipocytes (hypertrophy). The subcutaneous adipose tissue is the largest and least harmful adipose depot to store excess lipids. However, it has a limited ability to expand and recruit new cells. When the subcutaneous adipose cells become expanded (hypertrophic obesity), this leads to dysregulated and dysfunctional subcutaneous adipose tissue and ectopic fat accumulation in many depots (Gustafson & Smith, 2015). The visceral fat has a more significant potential to produce hormones and cytokines, including inflammatory ones, while subcutaneous fat can undergo the most intense browning process. Fig. 4. illustrates fat distribution in mice.

I. The inguinal fat pad is the subcutaneous fat (located between the lower part of the rib cage and the mid-thigh). The subcutaneous fat is where usually browning occurs when stimulated.

II. The intra-abdominal or visceral fat pad is composed of various compartments:
   a) the fat around de branches of the superior and inferior mesenteric arteries (situated between the leaflets of the mesentery). Here, the evaluation of cytokines and proinflammatory markers is suitable;
   b) the retroperitoneal fat (connected to the posterior abdominal wall near the kidneys). Here, fat remains even after weight loss;
   c) the genital (gonadal) fat (located in the lower part of the abdomen, connected to the epididymis in males or the ovaries and oviducts in females). This fat pad is suitable for cytokines and proinflammatory markers evaluation.

III. The interscapular and mediastinal fats are rich in BAT’s multilocular adipocytes.

The adiposity index is another possibility to evaluate...
The size of white adipocytes varies with obesity. Frequently, the white adipocyte diameter is measured to characterize its size variation. However, the white adipocyte diameter analysis is only valid to interpret the variations due to obesity if the section cuts the adipocyte in the equatorial plane because adipocytes are nearly spherical (sometimes adipocytes cut in the polar plane appear with a smaller diameter than the actual diameter). As we cannot be sure of this, the averaged adipocyte cross-sectional area might be estimated using the probabilistic statistics of stereology (Mandarim-de-Lacerda & del Sol, 2017): the ratio between the volume density of adipocytes ($V_{V\text{[adipocyte]}}$) and twice the numerical density of adipocyte per area or $Q_A[\text{adipocyte}]$ (Borges et al., 2020).

$$V_{V[\text{adipocyte}]} = \frac{P_{P[\text{adipocyte}]}}{P_T}$$

$$Q_A[\text{adipocyte}] = \frac{N[\text{adipocyte}]}{A_T}$$

$$\bar{a}[\text{adipocyte}] = \frac{V_{V[\text{adipocyte}]}}{2Q_A[\text{adipocyte}]}$$

Abbreviations: $\bar{a}$ [adipocyte] is the cross-sectional area of adipocytes; $A_T$ is the test area; $N$ [adipocyte] is the number of adipocytes; $P_{P[\text{adipocyte}]}$ is the point counting; $P_T$ is the total number of test-point in the frame; $Q_A[\text{adipocyte}]$ is the numerical density per area of adipocytes; $V_{V[\text{adipocyte}]}$ is the volume density of adipocytes (Mandarim-de-Lacerda, 2003).

Final remarks

Rodents, particularly mice, can contribute to the study of obesity with translation to human studies. First, however, the scientist needs to know and consider the particularities of the animals, such as the various types of fat pads, their location, and suitability for the study proposed. For example, the visceral fat fits hormone and adipokine secretion investigation, while the subcutaneous fat might be used to

Mice adipose tissue distribution

Subcutaneous white adipose tissue
1. Anterior
2. Flank

Visceral white adipose tissue
3. Mesenteric
4. Retroperitoneal
5. Perirenal
6. Perigonadal

Brown adipose tissue
7. Interscapular
8. Mediastinal (not shown)

Fig. 4. Mice adipose tissue distribution. In the dissected images, the various fat pads named in the left frame are indicated. (A) ventral view showing the anterior subcutaneous tissue (1) and the flank (2); (B) and (C) ventral view of the open abdominal cavity in a thin mouse (B) and an obese mouse (C) indicating mesenteric fat (3); (D) dissection of the posterior portion of the abdomen indicating the retroperitoneal (4), perirenal (5) and perigonadal (6) pads; (E) animal’s dorsal view of the interscapular fat (7, brown adipose tissue).
examine browning. Furthermore, heat production and thermogenesis are better analyzed in brown fat.

Moreover, there are different strains of rodents. For example, the Swiss (white) mouse is less susceptible to fattening by diet than the C57BL/6 mouse strain (a wild-type control for all possibilities of gene silencing).

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