Chapter

Zebrafish as an Experimental Model for the Study of Obesity

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Abstract

Obesity is considered a silent global pandemic, with a steady increase in adults and children. It is a complex disease that involves the interaction of genetic and environmental factors and predisposes individuals to severe chronic complications such as various types of cancer, dyslipidemia, hyperglycemia, hypercholesterolemia, nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis. To help elucidate the physiological mechanisms of this comorbidity in order to identify and develop effective treatments, the use of animal models is indispensable. Zebrafish is emerging as an important model for studying obesity and related metabolic diseases. In addition to being a small animal, with high genetic similarity when compared to humans and easy to handle, zebrafish also has the main well-conserved metabolism-related functions such as appetite regulation, insulin regulation, and lipid storage. Zebrafish is also suitable for the identification of new targets associated with the risk and treatment of obesity in humans. In this review, we highlight the studies that use zebrafish to study metabolic diseases demonstrating their important contribution in this area of research.

Keywords: metabolic syndrome, *Danio rerio*, transgenerationality, adiposity, inflammation

1. Introduction

The prevalence of obesity has increased significantly in the last three decades, being considered a pandemic that affects 1.9 billion people worldwide. In developed and developing countries, overweight and obesity are considered public health problems and have a high mortality rate due to associated risk factors [1].

Obesity is a risk factor for the development of type II diabetes mellitus (DM2), cardiovascular disease, hypertension, nonalcoholic liver disease, and cancer. Therefore, there is growing concern that it is estimated that the overall mortality rate from these non-transmissible diseases will increase in the coming decades [2].

Although it is a well-studied subject, many underlying biological mechanisms that mediate the effects of obesity are still unclear. Thus, animal models fill many of these gaps [3].

Zebrafish appears to be an excellent model for investigating the development of obesity, as it is a small fish, easy to maintain, and economical for breeding and has an easily observable and quantifiable behavior in a controlled environment [4]. In addition to these advantages of using zebrafish as a model, it still presents

interesting features for studies of metabolic diseases such as organ conservation and physiology of energy metabolism [5].

Due to this ascendant use of zebrafish in this area of research, the objective of this work was to carry out a bibliographical review involving as a theme the use of zebrafish as an experimental model associated with obesity, demonstrating the advantages of using this model to study the mechanisms of metabolic diseases and characterization for new therapeutic targets in recent years.

2. The etiology of obesity

Obesity is defined as a heterogeneous biological disorder, having as one of the main contributors to the disease an unbalanced diet with the excessive intake of hypercaloric foods [6].

The extra calories ingested lead to the storage of excess nutrients in adipose cells, where they accumulate as triglycerides or neutral lipids [7]. This condition leads to an increase in adipose tissue, which can result in resistance to anorectic hormones such as insulin and leptin, and a change in the energy balance at the central level, characterized by decreased energy expenditure and increased food consumption [8].

One of the globally accepted indicators for diagnosing obesity is the body mass index (BMI) (weight/height^2). According to the most recent classification, an adult with a BMI value >25 kg/m^2 is considered overweight, and when this value is >30 kg/m^2, it is already treated as a degree of obesity [9].

With data reported in the last decades, many authors have already considered obesity as a global pandemic. This can be observed, for example, in a study conducted in 2015 showing that 107.7 million children and 603.7 million adults were obese worldwide. Among adults, the prevalence of obesity in 195 countries from 1980 to 2015 revealed a higher rate of obese women in all age groups, and for both sexes, the rate of increase in obesity was higher at the beginning of adulthood [10].

Obesity is not only a singular disorder but a multifactorial disease whose consequences are beyond the presentation of a characteristic phenotype [11]. The occurrence of obesity reflects the interaction of biological, behavioral, genetic, and environmental factors [12], which are associated with the development of severe chronic complications such as various types of cancer, dyslipidemia, hyperglycemia, hypercholesterolemia, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis [6].

Increased adiposity also causes a condition known as the metabolic syndrome, defined as a set of interrelated metabolic characteristics that are linked to the development of cardiovascular disease and diabetes [13]. To be considered as a syndrome, three of the following five factors are necessary for each individual: abdominal obesity (waist circumference ≥102 cm for men and ≥88 cm for women), high triglycerides, reduced high-density lipoprotein cholesterol, high arterial blood pressure, and altered fasting glycemia [14].

All these consequences of obesity occur, since the adipose tissue besides having the function of energy stock is also the largest endocrine organ of the human body [15]. Adipocytes are responsible for the production of substances that regulate various organic functions such as energy balance, secretion of peptides, bioactive proteins, and adipokines that are responsible for a series of metabolic alterations, such as in the control of food intake, in the control of insulin sensitivity, and in inflammatory processes [16].

In addition to being composed of adipocytes, adipose tissue is also composed of other types of cells, including lymphocytes, macrophages, fibroblasts, and vascular cells, which also play important roles in functional tissue control. Obesity generates

large changes in the cellular composition of adipose tissue and also modulates the individual cell phenotype within this tissue [17]. Macrophages present two classes of known subpopulations: those with the capacity to produce pro-inflammatory cytokines, such as TNF-α, called M1, and those involved in anti-inflammatory reactions, expressing cytokines such as IL-10, called M2 [18]. In healthy individuals, macrophages are dispersed among adipocytes and, for the most part, constitute the M2 subtype. However, in obese individuals, monocytes tend to differentiate into M1 macrophages [19].

The exacerbated production of inflammatory signs by adipocytes that occurs in obese individuals stimulates the differentiation of M1 macrophages that in turn release nitric oxide, TNF-α, IL-6, and IL-1 [20]. These substances further stimulate the inflammatory activity of adipocytes. Thus, macrophages and adipocytes contribute, individually, to the inflammation state in adipose tissue, mutually stimulate each other's inflammatory activity, and contribute significantly to a chronic systemic inflammation of the obese individual [21].

In addition to metabolic disorders in the peripheral organs, inflammatory changes related to obesity also disrupt brain functions, especially affecting areas of the brain that regulate energy homeostasis and systemic metabolism [22].

The concern with obesity is increased by the fact that some data show that genes play an important role in predisposing individuals to obesity, showing that having one or both of the obese parents, especially the mother, increases the risk of obesity in the offspring [23]. Women who are overweight or obese when they enter pregnancy are more likely to have children more likely to develop obesity during childhood, adolescence, and adulthood [24]. This observed modulation not only happens with the maternal diet; researchers have identified epigenetic changes related to altered metabolism in the offspring resulting from variations in the father's diet, indicating that paternal behaviors may also put offspring at risk for obesity [25].

All these consequences show that nutritional status and diet are important factors related to the promotion and maintenance of good health. Obesity is one of the main causes of chronic non-transmissible diseases.

3. Zebrafish as a model for obesity

The regulation of energy expenditure and consumption involves many organs, including the brain, intestines, skeletal muscle, and adipose tissue. Therefore, the use of animal models is essential for a better understanding of the development and progression of metabolic dysfunction, such as obesity. Zebrafish is an excellent model for studying this type of disease because it has the major organs that are important for the regulation of energy homeostasis and metabolism in mammals including digestive organs, adipose tissue, and skeletal muscle. In addition, zebrafish has the main well-conserved functions, such as appetite regulation, insulin regulation, and lipid storage [5].

A primary characteristic of obesity is adipocyte hypertrophy and hyperplasia. Similar to mammalian white adipose tissue, adipocytes from the early-stage zebrafish contain several small lipid droplets, whereas mature adipocytes have a single large lipid droplet [26]. In zebrafish, lipids are also stored in visceral, intramuscular, and subcutaneous adipocytes [27], providing the opportunity to understand the regulation of body fat distribution in cases such as obesity.

The first diet-induced obese zebrafish model (DIO) was reported in 2010 through overfeeding with *Artemia* saline for 8 weeks. It has been shown through a comparative transcriptome analysis that the visceral adipose tissue between zebrafish, rat, mouse, and human has a similarity in lipid metabolism [28]. After

the publication of Oka et al., several other methods of inducing obesity in zebrafish were applied as an induction by overfeeding with *Artemia* saline or commercial feed or through a hypercaloric diet with corn oil or commercial oil-enriched feed for different purposes of the study on obesity [7, 28–30].

Metabolic pathways that control body weight in mammals are conserved in the zebrafish. Therefore, zebrafish is often used as a transgenic model for obesity [5]. A note of caution is that not all genes of lipid metabolism are highly conserved in sequence and function in zebrafish. For example, zebrafish leptin protein is only 19% identical to human protein. In mice and humans, leptin is an adipostatic hormone that regulates adipose mass, and failure in leptin signaling results in hyperphagia and obesity [31]. Unlike mammals, leptin and leptin receptor are not expressed in adipose tissue in zebrafish. The zebrafish deficient in the leptin receptor has mainly interrupted glucose homeostasis [32], which is different from the phenotypes seen in mouse models such as severe hyperphagia, hyperlipidemia, and morbid obesity [33].

The zebrafish model presents important advantages over other models because it is a small fish, easy to maintain, and economical for breeding and has an easily observable and quantifiable behavior in a controlled environment [5]. The fact that it also has its sequenced genome and an extensive number of data in the literature facilitates the study of this model in the area on metabolic disorders such as obesity [34–36].

3.1 Modulating the processes of obesity

Obesity is dynamic; both its nature and its consequences are complex. This fact limits the decision-making process for the management of therapeutic options and prevention of the disease on a large scale, so the identification of the different pathways of induction to obesity is essential for subsequent studies.

As an example, there is a worrying fact that is gaining the attention of several researchers: the fact that obesity has a high prevalence in lower socioeconomic populations. Evidence shows that although genetic predisposition and a positive energy balance are implicated in obesity, environmental factors, including exposure to pesticides, heavy metals, and other contaminants, are widely suspected to have obesogenic activity, which are correlated with lower socioeconomic status [37, 38].

Zebrafish is also an excellent model for toxicological studies, and its use in this field of study has already demonstrated that the presence of cadmium in maternal blood during pregnancy is associated with an increased risk of juvenile obesity in offspring [37]. It has also been observed using zebrafish larvae that exposure to substances such as benzopyrene and ethanol may induce hepatotoxicity in vivo via membrane remodeling, leading to the condition of nonalcoholic fatty liver disease (NAFLD) that is closely linked to obesity [39].

In addition to uncovering this association between exposure to heavy metals and a higher incidence of obesity, zebrafish has been used to elucidate the modulation pathways of the influence of the microbiota on obesity. In recent decades it has been shown that intestinal microorganisms and diet represent attractive targets for controlling the absorption of dietary lipids and energy balance. Although the microbiota may influence nutrient uptake, diet may also affect the composition and function of the microbial community [40]. Recently, the profile of the intestinal microbiome in zebrafish with type 2 diabetes mellitus induced by obesity was performed. This work revealed functional similarities in intestinal bacterial environments between humans and zebrafish affected with DM2 [41].

Zebrafish is thus considered as an alternative model organism to study bacterial-host interactions in human obesity and related diseases. Thus, the zebrafish model

becomes useful to uncover mechanisms that may respond, for example, to the efficiency of probiotics in modulating the microbiota, demonstrating whether they are capable of modifying host nutrient metabolism and energy homeostasis [42].

3.2 Drug screening and treatment

There is a recognized efficacy of the natural compounds combined with pharmacological treatment for the treatment of obesity, and thus there is a need to continually explore new anti-obesity compounds [43].

Within this scope, the zebrafish is a model with upward relevance for the discovery of new drugs for metabolic disturbances. The advantage of the use of this model for the screening of new drugs is due to the fact that zebrafish is already consecrated for preclinical tests.

In the larval stage, zebrafish is used to identify nontoxic molecules to treat obesity, since at that stage they already have those of signal transduction pathways that regulate lipid metabolism [44]. Another example is the use of zebrafish transgenic larvae expressing GFP on neutrophils, which allows visualization of recruitment of neutrophils to a specific site injured, in vivo and in real time. This model has been widely characterized in the last 10 years, demonstrating the conservation of cellular and molecular mechanisms with inflammatory processes in mammals and allowing the discovery and testing of anti-inflammatory compounds that have mechanisms closely related to obesity [45].

About work performed in adulthood, when used to study the development of type 2 diabetes mellitus through overfeeding, zebrafish has been shown to have human-like responses to antidiabetic drugs such as metformin and glibenclamide. In this way, the zebrafish was shown to be a suitable model also for identifying therapeutic targets and chemical screening in that area [29].

Research has also been conducted using the zebrafish model to evidence the effect of green tea extract, *Camellia sinensis*, in inhibiting lipid accumulation, decreasing the volume of visceral adipose tissue, and altering the expression of catabolic lipid genes [30]. On the other hand, other studies have demonstrated the effect and mechanisms of β-glucan derived from the edible fungus, *Agaricus bisporus*, regulating lipid metabolism and preventing lipid deposition and providing experimental data for its use in diet and food dependence [46]. It is also observed that the use of a natural polyphenol, resveratrol, has anti-obesity effects via regulation of lipid metabolism [47].

In recent studies the use of zebrafish for the study of secondary metabolites with bioactivities relevant to various metabolic functions produced by marine cyanobacteria was observed and some substances with activities of significant reduction of lipids [48, 49].

In addition to studies for the identification of new drugs, zebrafish is also used to elucidate the mechanisms underlying the effect of certain known substances. As an example, it has been reported that the substance tanshinone IIA has anti-adipogenic and anti-obesity effects on 3T3-L1 cells in obese mice induced by hypercaloric diet; however the mechanisms for this condition were little known. With the use of zebrafish that demonstrated the same response to the substance tanshinone IIA, it was observed that the anti-adipogenic effect of tanshinone IIA in 3T3-L1 cells is mediated by the control of the expression and/or phosphorylation levels of C/EBP-α, PPAR-γ, FAS, perilipin A, and STAT-3/5 [50].

As the consolidation of zebrafish for the study of obesity was recently performed, it is still a poorly studied model and has great potential to be explored in several areas of study on the metabolic syndrome and discovery of new therapeutic targets.

4. Conclusions

Knowing the problems caused by obesity and its alarming increase, it was observed in this review that many mechanisms underlying this condition have not yet been elucidated. It has also been shown that zebrafish is emerging as a relevant model in the study of obesity and related metabolic disorders. The advantages that make zebrafish a more adequate model when compared to other animals, such as rodents, are associated with its good efficacy to characterize the mechanisms and pathways involved in diseases, its ability to test new pharmacological and therapeutic approaches, the need for small amounts of compost for drug testing, low cost of maintenance, and conservation of the main metabolic pathways related to energy status. In this sense, zebrafish appears as a relevant model for preclinical trials and shows to be a model with great potential to help solve problems related to obesity.

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Conflict of interest

None of the authors have any conflicts of interest.

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