Diabetes, obesity and metabolic syndrome models studied in zebrafish - further relevance for the neuropsychiatric disorders-related manifestations

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ABSTRACT

In the recent decades, the incidence of cardiovascular diseases, as well as endocrine and metabolic diseases, which are affecting the quality of human life, is constantly increasing. Currently, given that the prevalence of metabolic diseases, obesity and diabetes is constantly increasing worldwide, there is an increased interest in developing new therapies and in creating better experimental models for mimicking the complex human symptomatology. This could result in a faster diagnosis with lower cost, as well as in advances of the so-called personalized medicine. Among the animals that could be experimental models for these diseases, there is an increased interest in the zebrafish (*Danio rerio*), giving that the qualities that recommend it are its small size, the need for a small living space, relatively low costs of use, high homology to the human genome, high fertility and mostly the formation of the major organs at 24 hours after fertilization. In this way, the presence in zebrafish of all the key organs that are important and necessary for the complex metabolic control, as well as the similarities in lipid metabolism and the adipogenic pathway between zebrafish and mammals, could make this small organism an ideal tool for the study and the better understanding of adipogenesis, diabetes, obesity and the complex pathological processes which are

characterizing the metabolic diseases. This could have an increased relevance in the context of our previous work describing neuropsychiatric models in zebrafish (focusing for example on dementia), considering also the important connections that might exist between the general metabolic disorders and most of the neuropsychiatric-related manifestations.

KEYWORDS:

Metabolic syndrome, obesity, diabetes, zebrafish.

INTRODUCTION

The metabolic syndrome, generically called at the beginning by Gerald Reaven's, syndrome X in 1988, was then defined as an accumulation of abnormal disorders (1, 2). Syndrome X, later becoming the subject of several researchers, was finally defined as a combination of 4 disorders (diabetes, blood pressure, obesity, hypertriglyceridemia) closely related to each other (3).

Regarding the endocrine and metabolic diseases, zebrafish is considered to have great potential in the investigation of thyroid diseases, thyroid hormones and defects related to their receptors, helped by the fact that the zebrafish, like the mammals, has molecular mechanisms and components related to thyroid axis (4). Moreover, knowing that the organ systems of zebrafish resemble those of humans, especially those related to metabolic control to regulate body energy homeostasis, which results in obesity, zebrafish has been used to study various diseases in the field of metabolic diseases such as obesity, diabetes, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), atherosclerosis and liver cancer (5) (6).

Thus, it was lately suggested that zebrafish can be used successfully in modeling these diseases because it has all the key organs to balance energy homeostasis and metabolism in mammals, as well as appetite regulation, insulin and lipid storage. (5) (6) (7) (8).

Diabetes, known as a syndrome with high blood glucose levels and as a consequence of metabolic imbalance, also affects the quality of human life and requires the creation of an

experimental model on which this disease can be modeled. (8).

METABOLIC SYNDROME

As mentioned, the initially called Syndrome X, went through several definitions: obesity, along with a fasting blood glucose > 100 mg/dl, accompanied by high blood pressure, high triglycerides > 150 mg / dl and an increase in lipoproteins (Lower HDL) (9). Reaching another definition, practically an association of increased glucose with at least two other health problems: high blood pressure, increased plasma triglycerides, abdominal BMI > 30 kg / m² and microalbuminuria (9).

Finally, the European Insulin Resistance Study Group (EGIR) defined X syndrome as insulin resistance syndrome, along with 2 other factors: high plasma glucose, but not necessarily a person with diabetes, high triglycerides, low HDL, blood pressure. All definitions assigned to syndrome X show that the 4 disorders (diabetes, blood pressure, obesity and hypertriglyceridaemia) are related to each other (9).

Underlying the body's survival and functioning is the control of energy homeostasis between cells and tissues. When this homeostasis is disrupted in the body, the toxic accumulation of carbohydrates, proteins and lipids takes place. In diabetes, high blood sugar causes both micro- and macrovascular diseases (10), which in turn can cause strokes, heart attack, kidney failure, blindness and neuropathy (11) (12) (13). In conclusion, the metabolic syndrome must be understood at the system level precisely because of its multi-organ effects.

Zebrafish has great potential for investigating thyroid diseases and everything related to them, because it has all the components related to the thyroid axis, similar to mammals; thus, by using transgenic lines, this model is suitable for developing new therapeutic approaches (4). It has also been observed that long-term exposure to environmental chemicals causes disorders similar to those caused by endocrine disorders. Chemicals that interfere with the components of the endocrine system are called endocrine disrupting components (EDC). Zebrafish can be used to understand related mechanisms and to detect EDC by applying biomarkers, specific genes and a transcriptomic platform (14).

The organ systems of zebrafish are similar to humans in terms of metabolic control for regulating body energy homeostasis, and for this reason zebrafish has been used to model metabolic diseases such as obesity, diabetes, NAFLD, NASH, atherosclerosis and liver cancer (5) (6). The basic metabolic mechanisms of zebrafish are similar to those of humans (15). Other similarities of zebrafish with humans consist in the development of the peripheral nervous system (16), and the development of adipose tissue takes place in both species, as well as the existence of similar genetic factors (17).

Metabolic disease is a consequence of the interaction of lifestyle, socio-economic factors and behavioral factors (18) (19) (20). Both energy metabolism by maintaining energy homeostasis and the microbiome can certainly affect metabolism (18) (19) (20). In zebrafish, organs such as the thyroid and endocrine pancreas develop within 48 hours dpf (days after fertilization) and have the role of secreting hormones essential for maintaining energy homeostasis. (21) (22) (23) (24), and the maintenance of metabolic homeostasis begins by regulating glucose. Returning to the influence of the microbiome on metabolism, a basic microbiome has been observed in zebrafish, and researchers have begun to study how infection naturally or induced alters the intestinal microbiota by disrupting carbohydrate and lipid homeostasis

(25) (26) (27) (28) (29) (30) (31) (32). Over time, zebrafish have been used in the study of many metabolic diseases, innate metabolic errors (33) (34) hyper and hypothyroidism (35) (36), metabolic cancer (37), and in this review I will bring in discuss diabetes, obesity and dyslipidemia as the most common metabolic tubing (38) (39). So zebrafish can be a model for studying metabolic diseases due to functional preservation in lipid metabolism, adipose tissue biology, glucose structure and glucose homeostasis (40).

OBESITY

Obesity, a global health problem, is a disease that results from the interaction of genetic and environmental factors, characterized by increased fat storage in WAT (white adipose tissue). Causes of obesity include the quantity and quality of food consumed by individuals, as well as exposure to obesogens, a class of chemicals that disrupt the endocrine system, and their presence in early life affects adipogenesis and lipid metabolism (41) (42). Obesity, the result of a positive balance when the energy ratio is higher than its expenditure, causes the organs involved in maintaining balance, mean the brain, liver and adipose tissue to interact to regulate this aspect. Due to the similarities with the human body, the zebrafish is used to study obesity (43) (44). Zebrafish is a suitable model to study metabolic dysfunction because it has digestive organs, adipose tissue and skeletal muscle, organs that regulate energy homeostasis and metabolism in mammals (45) (46).

In mammals there are 2 main classes of adipose tissue: brown adipose tissue (BAT) which dissipates energy and generates heat, and white adipose tissue (WAT) with the role of energy storage and regulation of energy balance (47). BAT consists of several drops of microvesicular lipids as well as many mitochondria that have the role of providing heat using lipids. WAT is a large unilocular lipid drop formed by the union of small lipid droplets that will be stored as a small energy reserve (48). Adult zebrafish contain WAT in the pancreatic, visceral, esophageal, mandibular, cranial, and tail fins (49). Being poikilothermic animals, they do not contain

BAT, although they have been shown to have an approved coupling protein 1 (PCU 1) that can be associated with BAT in other species (50).

To better understand the physiological changes of an organism affected by obesity, zebrafish are used to monitor adipogenesis, from egg fertilization to larval stage, precisely because zebrafish larvae develop outside the mother's body and due to the optical transparency of them. Thus, with the help of appropriate tools, defects in the absorption, transport and arrangement of lipids can be detected (51) (52). Zebrafish also contain cells that specialize in the absorption and processing of lipids (eg, intestinal enterocytes, fat-storing adipocytes, liver hepatocytes, and pancreatic acinar cells) (53) (54). Adipocytes that form in fish larvae can be observed with the help of dyes: Sudanophilic dye Oil Red O (ORO), Sudan Nile Red, or Lipid Green (55), and to observe the transport of fatty acids are used BODIPY lipophilic fluorophore by injection yolk, from fish food, the latter reaching the circulatory system very quickly, lasts about 3 hours (56).

Zebrafish can be considered a suitable model for human lipid metabolism because functions such as regulating appetite (by the presence in the hypothalamus of appetite circuits) of insulin (through the pancreas) and lipid storage are well preserved (57) (58) (59). Excess nutrients increase plasma TGL levels and the appearance of hepatic steatosis (60) as well as the presence in zebrafish of hormones that regulate lipid metabolism: SREBF 1, PPAR α , NR 1 N 3, LEP (60) make it a suitable model.

The preservation of these metabolic pathways that intervene in the differentiation of adipocytes energy homeostasis (61) and cholesterol metabolism make zebrafish an ideal model for human lipid metabolism (62). Obesity caused by the creation of a prolonged imbalance between calories entered and insufficient energy expenditure, is characterized by adipose tissue hypertrophy and hyperplasia. Obesity occurs practically when the excess energy, stored in the form of

lipid droplets, is deposited in both adipose and non-adipose tissues (63) (64).

Excess nutrients cause adipocyte hypertrophy and hyperplasia in adipose tissue; Among the cells that inflame adipose tissue are macrophages and pro-inflammatory molecules: tumor necrosis factor (TNF- α), interleukin-6 (IL-6), monocyte chemoattractant protein (MCP-1) (65) (66) (67). Adipocytes and macrophages interact and thus inflammatory adipocytokines decrease, leading to complications associated with obesity. In many organs it all causes some metabolic disorders, insulin resistance, diabetes, cardiovascular disease, stroke and even some cancers (68) (69). It has been shown that in order to assess the development of comorbidities related to obesity, the distribution of fat in a body is more important than the total amount of fat (70) (71). When adipose tissue grows, plasma free fatty acids (FAA) are released and in obese patients it forms a link between obesity and metabolic syndrome by promoting lipotoxicity in the liver and muscles, by altering the action of insulin and causing non-alcoholic fatty liver disease (NAFLD) (72).

Knowing that the hypothalamus is the site of appetite circuits (73), leptin receptors and melanocortin system proteins (74) (75) we can highlight the similarities of fish with humans in this regard. Intracerebroventricular administration of neuroactive peptides (NPY) has also been shown to definitely stimulate food intake, and administration of CART (regulated cocaine and amphetamine-transcription) of melanocortin peptides and CRF (corticotropin-releasing factor) inhibits feeding (76). A zebrafish model for obesity can be created by chromosomal translocation, which suppresses central melanocortin receptors, knowing that the central melanocortin system (CMS) controls and regulates energy homeostasis. It contains pro-melanocortin (POMC), peptides and agouti-bound peptide (AgRP). Zebrafish following this chromosomal translocation increase in weight due to the high content of triglycerides (TGL), due to increased expression of AgRP, an endogenous

antagonist of melanocortin and due to visceral fat accumulation (36) (77).

Leptin, the main adipostatic factor, is a hormone that informs the brain about the total energy stored as TGL in fat cells; and by regulating the amount of adipose tissue in the body, by inhibiting food intake, by stimulating energy expenditure, reducing body weight and fat percentage intervenes to maintain euglycemic status (78). Leptin leads to the onset of hepatic steatosis and the onset of insulin resistance (79). Deficiency of this hormone causes the onset of deep hyperphagia and obesity, infertility and diabetes. The difference is that in zebrafish the leptin receptor is expressed in the liver and other tissues, and not in adipose tissue as in humans, and the deficiency of receptors in zebrafish larvae causes an increase in the number of beta cells and insulin mRNA levels, and larval zebrafish in response to a fatty diet may have beta cell hyperplasia or peripheral insulin resistance, so it can be said that leptin has a major role in maintaining glucose homeostasis and a secondary role as an adipostatic factor (80).

Zebrafish, like mammals, have white adipose tissue, initially made up of several small drops of lipids, and as they develop, they turn into a single large drop of lipids (81). Visceral adiposity is considered to be a critical risk factor for other metabolic diseases and T2DM (82). Zebrafish contain lipid deposits in visceral, intramuscular and subcutaneous adipocytes (83) and can assess the degree of metabolic disorders related to obesity. In humans, these quantitative measures consist of the calculation of body mass index and quantitative computed tomography (CT), and in fish lipophilic dyes are used: red Nile, oil Red O and Sudan Black B (84) (85). With zebra larvae having optical transparency, the researchers developed screens based on live imaging and fluorescence to study digestive physiology or lipid metabolism. Nile red has been used to quantify intracellular neutral lipid droplets and for vivid imaging (86) as well as to purify adipose tissue (87). In addition, a variety of fatty acid analogs and fluorescent lipid tracers are available,

including BODIPY cholesterol analogs and fluorescence reports such as PED 6 for monitoring the metabolism and distribution of exogenous lipids in live zebrafish (88) (89). Micro CT 3D is also available for this small animal and allows the measurement of total adipocyte tissue volume as well as various fat deposits (90). Recently, Landgrof et al., compared the methodology for quantifying the body fat mass of zebrafish using MR imaging (MRI) and 4 in 1 Echo MRI (Echo MRI tm) (91). These methods provide accurate measurements of zebrafish adiposition and provide means for longitudinal monitoring.

It was also showed that a high-fat diet given to zebrafish larvae increases adiposity, and in adult zebrafish it causes hepatosteatosis, hypertriglyceridemia, and high BMI (39) (92). One of the methods of inducing obesity is to administer excess fat immediately after 5 days after fertilization. Early larvae do not have WAT (93), in which case lipid droplets accumulate in the bloodstream, and the amount of whole larval triacylglycerol is used as an indicator to quantify the progression of obesity (94) (95). A high-fat diet consists of administering egg yolk (95).

Zebrafish larvae begin to eat 5 days after fertilization (dpf), until then they absorb the essential fats, cholesterol and triacylglycerol provided by a sac of maternal yolk (96). At 5 dpf the lipids become visible in several tissues, with the formation of a small deposit in hepatocytes; instead, the first signs of adipogenesis can be seen at 8 dpf in the visceral cavity, close to the pancreas. Adipocytes appear in the pancreas of larvae at 12 dpf (96). To highlight adipocytes, at 15 dpf, red Nile dye is used; 15 dpf being the day when the adipocytes in the visceral area (internal intra-abdominal and surrounding organs become visible (97); a location associated in humans with risk factors for the development of type 2 diabetes mellitus (T2DM) (98) (99). The number of adipocytes correlates with the size of the larvae, rather than with their age; thus, it was observed that at 17 dpf the larvae have WAT in the pancreatic and visceral area, and at 20-22 dpf,

subcutaneous and cranial adipocytes develop, depending on the size (93).

There is also an increase in microsomal triglyceride transfer protein (MTP) mRNA levels in the proximal intestine and liver, following a high-fat diet (90). MTP has a 54% identity with human MTP and is involved in the breakdown of lipoproteins in the born ApoB, and these ApoB-MTP complexes can prevent lipid degradation by proteasomes and increase plasma lipid levels. ApoB levels increased in response to overeating (100) (101).

The high degree of conservation in the distribution and formation of adipose tissue in zebrafish compared to mammals make it a suitable model to study obesity.

DIABETES

Diabetes is a syndrome characterized by elevated blood glucose levels (hyperglycemia) and metabolic imbalance (102).

Chronic blood glucose is the major biochemical diagnostic parameter observed in the two major forms of diabetes: type 1 or insulin-dependent diabetes, caused by autoimmune destruction of insulin-producing pancreatic beta cells, and T2DM or non-insulin-dependent characterized by insulin insensitivity (102).

The development of the pancreas in the organs of vertebrates often involves a two-stage process. First, the formation of a functionally immature organ during embryogenesis, which is followed by differentiation into mature form (103) (104). The second step most often occurs during the postembryonic / postnatal period, when the concentration of plasma thyroid hormone (TH) becomes high. In addition, mammalian weaning, frog metamorphosis, and the larval-juvenile transition of the zebrafish have been proposed to be functionally equivalent events during postembryonic development, based on cellular and molecular events in their digestive organs, including the pancreas, and increase in plasma TH concentration during these periods (103) (104).

To understand exactly when and where TH works in the pancreas, the thyroid is ablated when treated with metronidazole. Thyroid ablation leads to loss of endogenous TH (105). A transgenic line can be used to perform conditioned TH during the transition from larvae to the juvenile transition. After thyroid ablation, insulin expression was decreased and glucagon expression was loaded, followed by increased blood glucose during the transition from larval to juvenile. Decreased insulin secretion and increased glucagon secretion and the resulting increase in blood glucose continued in adult fish. The TH treatment of adult thyroid fish increased insulin secretion and reduced glucagon secretion and reduced blood glucose levels. These results suggest that TH is important to generate α and β cells and to maintain their maturation. In addition, glucose sensitivity was found to be improved in both α and β cells after T3 treatment (106). Thus, TH can regulate α and β cells in cell maturation by increasing glucose sensitivity (105).

The pancreas is an organ with two sets of functions: those related to the digestive system (acinar cells and ductal cells), and those of the endocrine system (islets). Several phylogenetic analyzes suggest that the digestive system and the endocrine system evolved differently (107) and that the single unified pancreas developed as a new organ through the fusion of endocrine and digestive parts during fish evolution (107).

The zebrafish pancreas comes from two progenitor domains called the dorsal bud (DB) and the ventral bud (VB). However, unlike dorsal and ventral pancreatic buds in mammals, DB and VB in zebrafish have distinct differentiation potentials (108). DB is formed within 24 hours after fertilization (HPF) and consists of early endocrine cells grouped to form the main islet. VB arises from the intestinal tract after 34 dpf and these cells migrate to swallow the main islet (108). The endocrine compartment consists of alpha cells that secrete glucagon, beta cells that produce insulin, gamma cells that produce somatostatin, epsilon cells that produce ghrelin, and pancreatic polypeptides that

produce PP (109) (110). In addition to the pancreas, the development and function of other organ systems involved in glucose homeostasis, including the brain, liver, adipose tissue and skeletal muscle are also preserved (111). Preservation of pancreatic structure and system glucose homeostasis makes zebrafish useful for identifying new targets in pancreatic-related diseases such as diabetes.

Pancreatic β cells secrete insulin in response to high glucose levels. In β cells in mammals under high glucose conditions, this one moves into β cells through the glucose transporter, GLUT2. Beta cells then produce more ATP, which increases the ATP / ADP ratio through glucose metabolism. Increasing the ATP / ADP ratio stimulates the closure of the ATP-dependent potassium channel (K_{Atp}), subsequently evoking the depolarization of β cells that leads to the activation of 2⁺ channels that stimulate insulin secretion. Like mammals, GLUT 2 is expressed in pancreatic zebrafish β cells. In addition, K_{Atp} and Ca²⁺ channels regulate insulin secretion according to blood glucose levels (112),(113). These indicate that the pancreatic β cells of zebrafish function in the same way as other vertebrates. On the other hand, these investigations were performed using main islets, but not with secondary islets. The molecular mechanisms underlying the secondary development of islets in zebrafish is similar to that of the development of mammalian islets. Thus, determining the functional differences between the main islets and the secondary islets could become important to understand how vertebrates acquired functional islets.

T1DM is an organ-specific autoimmune disease caused by the destruction of insulin-producing pancreatic beta cells by T cells (114). T2 DM is a metabolic disease associated with insulin resistance. Hyperglycemia in T2DM is initially accompanied by compensatory hyperinsulinemia, but as the disease progresses oxidative stress triggers beta cell apoptosis, and the amount of insulin produced by the pancreas gradually decreases (115). T2 DM is an often associated lifestyle disease.

with obesity and considered part of the metabolic syndrome (116). T2DM is accompanied by chronic inflammation and oxidative stress (117) and patients often develop non-alcoholic fatty liver disease (118). Insulin is the cause of hyperglycemia in T1 DM. The most common treatment for this form of the disease is insulin injection. Antidiabetic drugs for T2DM can directly target hyperglycemia by promoting the release of insulin from beta cells in the early stages of the disease by improving the insulin sensitivity of target cells in various ways or by slowing the digestion of carbohydrates after meals. T2DM patients can also improve their health by reducing obesity, inflammation and oxidative stress.

Deficiency of glucose, the main fuel of the brain, leads to coma, convulsions causing permanent damage to the brain. Glucose reaches the brain through CBF (119) cerebral blood flow through endothelial cells, to the blood-brain barrier (BBB) (120) and eventually to glial cells through facilitated diffusion. Glucose is transported to glial cells and neurons by GLUT 7 carriers. Although insulin alters glucose metabolism in the CNS (121) it is not the main mechanism in the brain for neuronal absorption. But insulin influences activities such as neuronal growth and survival, synaptic plasticity, neurotransmitter release, tubulin activity, cognitive improvement of memory, learning (123).

Destruction of pancreatic beta cells is the leading cause of T1 DM and a consequence of T2DM progression. The toxic glucose analogue of alloxan is well known for its use in rodent diabetes models and has been used successfully in wild zebrafish larvae along with staining of the fluorescent analog 2-NBDG for one hour and using a fluorescent microscope (124) a green fluorescence is obtained. For wild-type larvae, an alternative method of visualizing the pancreas is to use fluorescent conjugated antibodies against insulin or glucagon (125).

Glucose transporters (GLUT) in zebrafish have a structure and tissue distribution similar

to that of mammals (126) (127). Fluorescent analogues of glucose 2-NBDG and GB-2 Cy 3 are both substrates known for GLUT and have been used to monitor glucose uptake in zebrafish larvae. Increased uptake of 2 NBDG could be inhibited by the glucose transporter inhibitor () and reduced in the presence of excess D-Glucose to illustrate that uptake of 2 NBDG in zebrafish occurs through GLUTs in similar to that of mammalian glucose (129).

The pancreas of the zebrafish is very similar to that of humans in terms of cell types, as well as the secretion of hormones and digestive enzymes. Regulation of zebrafish genes for insulin, glucagon, and other proteins involved in glucose metabolism has also been shown to be similar in the two species (130). Increased endogenous glucose levels in zebrafish larvae are associated with a increased expression of the gluconeogenic regulatory enzyme PEPCK (131). The major regulatory mechanism for this enzyme is the activation of expression by cortisone and glucagon and inhibition of glucagon receptor signaling lead to a reduction in PEPCK which in turn improved insulin sensitivity and reduced blood glucose in models with severe insulin resistance (132). In addition, hyperglycemia and excessive gluconeogenesis are present in all forms of diabetes. (133).

Direct measurement of in vivo glucose levels in larvae can also be used as an indicator of glucose homeostasis reflecting the net activity of glycolysis and gluconeogenesis (134). Inflammation is triggered by numerous stimuli (microbiological, physical and chemical) which activates the immune system and recruits immune cells. It is accompanied by the production of inflammatory mediators such as proinflammatory cytokines (interleukin -6, IL-6, IL-8, IL-1beta, macrophage granulocyte colony stimulating factor and tumor necrosis factor alpha (135).

Inflammation and oxidative pathways are interconnected to form a positive feedback loop. Immune cell recruitment and the secretion of cytokines and chemokines lead to the production of reactive oxygen species (ROS) and reactive nitrogen species (NOS).

During diabetes, inflammation in wild zebrafish larvae was achieved by exposure to high concentrations of glucose or fructose (136).

In fact, this mini-review of ours could open the discussion on the obvious connections that might exist between the digestive and gastrointestinal-metabolic component that is associated with the neuropsychiatric one (137, 138), considering the latest expertise of our group in this area (139), as well as the social implications in this context (140, 141), and of course the neurological and psychiatric manifestations related to the general metabolic disorders, in the context of the oxidative stress (another important expertise and perspective of our group -142) or not (143-146).

We could also mention in this context, the developing of the techniques for glucose homeostasis in zebrafish, as many transgenic strains of zebrafish with fluorescent protein expression have been used to study pancreatic development and glucose homeostasis in living vertebrates (148).

Thus, methods for zebrafish pancreatic function have been established, including postprandial blood glucose measurement and glucose tolerance tests as well as pancreatic dissection techniques and islet cell culture (150).

In larvae, blood collection for glucose measurement is not a viable methodology, but free glucose in whole larvae can be measured by a coupled enzyme fluorescent test (151). For adult zebrafish, the small size (3-4 cm) makes blood collection difficult. However, several protocols have been developed for blood collection: lateral incision in the dorsal aorta, decapitation and tail ablation, although these methods require animal sacrifice (153).

Measuring insulin function was a major challenge in this context. As surrogate indicators, insulin mRNA levels can be determined directly by qPCR and insulin promoter activity can be determined indirectly by measuring EGFP signal strength in Tg (-

l.oins.EGFP) over zebra (154). Insulin sensitivity can also be assessed by intraperitoneal injection of insulin into hyperglycemic zebrafish (156). Also, young fish (4-11 months) acclimatize more easily to the glucose exposure protocol compared to older fish (1-3 years) so they showed a

transient hyperglycemia, but persistent hyperglycemia can be achieved in young zebrafish by gradually increasing glucose concentration (157).

CONCLUSIONS

In this way, the presence in zebrafish of all the key organs that are important and necessary for the complex metabolic control, as well as the similarities in lipid metabolism and the adipogenic pathway between zebrafish and mammals, could make this small organism an ideal tool for the study and the better understanding of adipogenesis, diabetes, obesity and the complex pathological processes which are characterizing the metabolic diseases. This could have an increased relevance in the context of our previous work describing neuropsychiatric models in zebrafish (focusing for example on dementia), considering also the important connections that might exist between the general metabolic disorders and most of the neuropsychiatric-related manifestations.

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