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DIETA RICA EM GORDURA INDUZ ALTERAÇÕES COMPORTAMENTAIS EM PEIXE-ZEBRA ADULTO

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Dedico este trabalho aos meus pais, com quem compartilho o mérito de minhas conquistas.

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RESUMO

A obesidade é uma doença de alta prevalência na população mundial (13% da população adulta em 2016), definida pelo acúmulo excessivo de gordura corporal. Várias comorbidades estão associadas, incluindo algumas que afetam o sistema nervoso central (SNC), como algumas doenças neurodegenerativas, o déficit cognitivo e distúrbios psicocomportamentais. O peixe-zebra surgiu como um modelo versátil e barato amplamente usado para estudar doenças humanas, incluindo obesidade e doenças neurológicas. Portanto, nosso objetivo foi verificar o impacto de uma dieta hiperlipídica no sistema nervoso central (SNC) do peixe-zebra, por meio de testes comportamentais bem estabelecidos. Os animais foram alimentados de acordo com três grupos dietéticos. O grupo de dieta padrão (SD) recebeu apenas 7,5 mg/ peixe de ração comercial para peixe, enquanto os grupos de dieta rica em gordura receberam 5 mg/peixe de ração + 7,5 (HFD-7,5) ou 15 mg/ peixe (HFD-15) de gema de ovo de galinha. O teor de gordura dietética (p/p) foi de aproximadamente 6,5%, 16,9% e 21,1%, respectivamente. Após duas semanas de dieta, os comportamentos foram avaliados. Ambos os grupos HFD apresentaram efeitos obesogênicos, indicados pelo aumento no IMC, comprimento abdominal e peso corporal em comparação com o grupo SD. demonstramos um comportamento agressivo e tipo ansioso induzido por ingestão de HFD em peixes-zebra, conforme medido pelo teste de agressão induzida por espelho e teste de tanque novo, respectivamente. Além disso, a maior concentração de HFD (HFD-15) causou déficit cognitivo no teste de esquiva inibitória enquanto a sociabilidade não foi afetada, conforme determinado pelo teste de preferência social. Nossos resultados estão de acordo com evidências em modelos humanos e roedores obesos, sugerindo efeitos semelhantes da ingestão de gordura. Portanto, destacamos o potencial inexplorado do peixe-zebra para elucidar este campo de estudo.

Palavras-chave: Obesidade, Distúrbios Psico-comportamentais, Peixe-zebra, Disfunção Cognitiva, Comportamento Tipo Ansioso.

ABSTRACT

Obesity is a disease with high prevalence in the world population (13% of adult population in 2016), defined by an excessive body fat accumulation. Several comorbidities are associated, including some affecting central nervous system (CNS), i.e. some neurodegenerative diseases, the cognitive deficit and psychobehavioral disturbs. Zebrafish has raised as a versatile and cheap model widely used to study human diseases, including obesity and neurological diseases. Therefore, our objective was to verify the impact of a high-fat diet on zebrafish central nervous system (CNS) using well- stablished behavioral tests. Animals were feed according with three dietary groups. The standard diet group (SD) received only 7.5 mg/ fish of commercial fish food, while the high-fat diet groups received 5 mg/fish of commercial fish food + 7.5 (HFD-7.5) or 15 mg/fish (HFD-15) of chicken egg yolk. Dietary fat content (w/w) was approximately 6.5%, 16.9% and 21.1%, respectively. After two weeks of diets ingestion, behaviors were assessed. Both HFD groups had obesogenic effects, indicated by increase on BMI, abdominal length and body weight compared with SD group. We show a HFD ingestion induced aggressive and anxiety-like behavior on zebrafish, as measured by mirror-induced aggression and novel tank diving test, respectively. Also, the higher concentration of HFD (HFD-15) elicited cognitive deficit on inhibitory avoidance test while sociability was unaffected, as determined by the social preference test. Our results are in accordance with evidences in obese human and rodent models, suggesting similar effects of fat intake. Therefore, we highlight the unexplored potential of zebrafish to elucidate this study field.

Keywords: Obesity, Psychobehavioral disturb, Zebrafish, Cognitive dysfunction, Anxiety-like behavior.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AL	Comprimento abdominal
BBB	Barreira hemato-encefálica
BF	Gordura corporal
BMI	Índice de massa corporal
BW	Peso corporal
CAL	Caloria
CH	Colesterol
CNS	Sistema nervoso central
DIO	Obesidade induzida por dieta
DPF	Dias após a fertilização
GC	Glicose
GMO	Modelo genético de obesidade
HFD	Dieta hiperlipídica
HSD	Dieta rica em açúcar
IAT	Teste de esquiva inibitória
MIAT	Teste de agressividade induzida por espelho
MPF	Meses após a fertilização
NNT	Teste do tanque novo
NPY	Neuropeptídeo Y
NR	*Nile Red*
ORO	*Oil Red O*
POMC	Pró-opiomelanocortina
SL	Comprimento padrão
SPT	Teste de preferência social
sWAT	Tecido adiposo branco subcutâneo
TG	Triglicerídeos
vWAT	Tecido adiposo branco visceral
WAT	Tecido adiposo branco

SUMÁRIO

1. INTRODUCTION

According to the World Health Organization (WHO), obesity is defined by an excessive body fat accumulation and is diagnosed by a body mass index (BMI) higher than 30 kg/m^2. Since 1975, obesity prevalence has tripled, reaching 13% of adult population worldwide in 2016, while 39% were overweight (WORLD HEALTH ORGANIZATION, 2016). Obesity increases risk of several comorbidities, such as, type 2 diabetes mellitus, cardiovascular diseases, musculoskeletal diseases, neurodegenerative and psychiatric diseases, cancer and reduces life expectancy by 5-20 years (BERRINGTON DE GONZALEZ *et al.*, 2010; FONTAINE *et al.*, 2003; MACMAHON *et al.*, 2009). Accordingly, 4 million deaths were associated with high BMI between 1990 and 2015 (AFSHIN *et al.*, 2017).

Obesity is resulted by a complex interaction of environmental and genetic factors, leading to a persistent imbalance of consumption and expenditure of calories and further pathological and excessive body fat accumulation (HILL; WYATT; PETERS, 2012). Therefore, changes on nutritional habits, including the consumption of disbalanced diets rich in calories is recognized as the main factor behind obesity pandemic and aforementioned comorbidities (VANDEVIJVERE *et al.*, 2015). Among these diets, the high-fat (HFD) diet is widely applied on the research field leading to obesity and comorbidities, including those affecting the CNS, i.e., memory loss, neurodegenerative diseases and psychobehavioral disorders (CAI, H. *et al.*, 2012; FREEMAN *et al.*, 2014; PROCACCINI *et al.*, 2016).

Zebrafish is a viable model for several human diseases, since they share several similarity with mammals, including organs anatomy and physiology (OKA *et al.*, 2010; ZANG; MADDISON; CHEN, 2018) and conservation of several genes involved in human diseases (HOWE *et al.*, 2013) (**Fig. 1**). Further, their use on obesity research is supported by similarities on lipid transportation/metabolism and control of energetic metabolism. Importantly, obesity pathogenesis in zebrafish is similar to humans on the anatomical, molecular, genetical and endocrine levels (GUT *et al.*, 2017; OKA *et al.*, 2010). The short lifespan and generation time allow to evaluate the long-term and intergenerational effects of obesity (SCHLEGEL; STAINIER, 2007; SHEN; YUE; PARK, 2018; SMITH, W. W. *et al.*, 2014). The relatively lower degree of complexity implies less ethical concern and can be useful to evaluate basic neural mechanisms. Plus, the reduced corporal size allow to work with animals numbers with less expenditure of budget and time (KLEINERT *et al.*, 2018; SCHLEGEL; STAINIER, 2007).

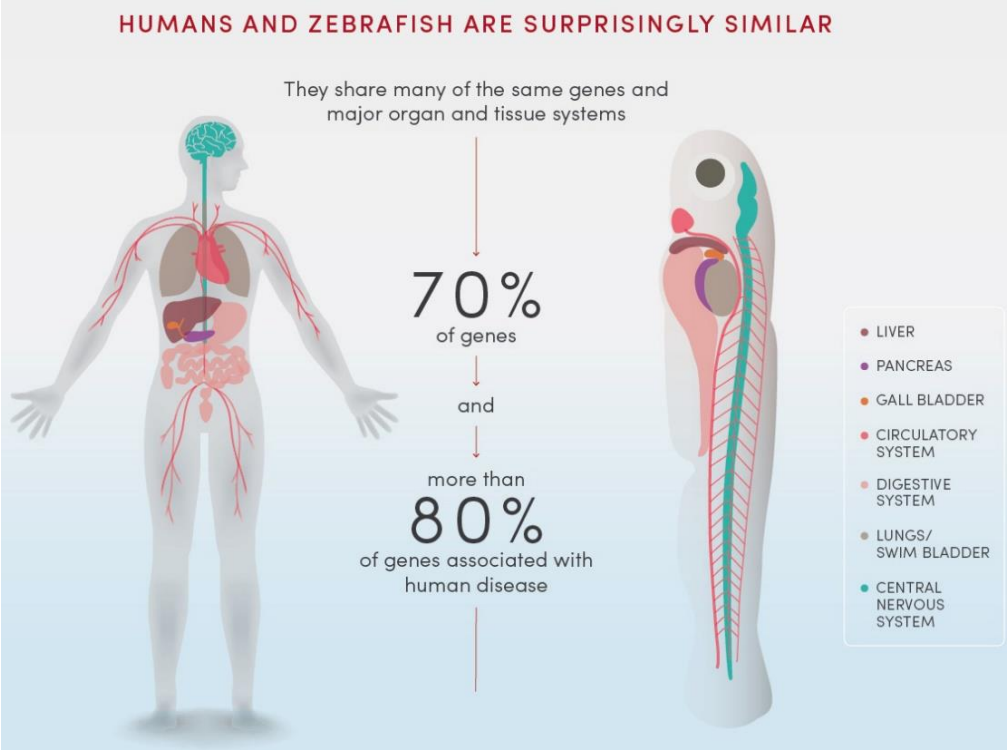

Fig. 1. Infographic showing the high degree of similarity of zebrafish and human. Extracted from https://animal.research.utah.edu/faqs.php, © 2021 THE UNIVERSITY OF UTAH

Several models of obesity are already described for zebrafish, including diet-induced obesity (DIO) and genetic models (ZANG; MADDISON; CHEN, 2018). DIO can be achieved in adult zebrafish using diets rich in fat, sugar and/or cholesterol leading to increased body weight, body mass index, visceral and subcutaneous fat content, steatosis and metabolic alteration, such as, increased fasting blood glucose, cholesterol, and triglyceride levels (LANDGRAF *et al.*, 2017; MEGURO; HASUMURA; HASE, 2015; OKA *et al.*, 2010). DIO zebrafish larvae also increased lipogenesis, steatosis and higher levels of triglycerides and cholesterol (MA *et al.*, 2019; ZHOU *et al.*, 2015)

Neuroscience is also benefited by zebrafish use, specially to the assessment of basic neural process, neuroactive drug screening and evaluation the effect of compounds on the CNS (KALUEFF; STEWART; GERLAI, 2014). This success is supported by a conservation of zebrafish brain morphology and physiology (KALUEFF; STEWART; GERLAI, 2014; WULLIMANN; RUPP; REICHERT, 1996), including all main neurotransmitter, receptors, brain structures and neuroendocrine hormones (ALSOP; VIJAYAN, 2009; PANULA *et al.*, 2006). Further, several models of neurological disorders is described in adult and larval zebrafish using genetic and pharmacological approaches, including anxiety disorder (MAXIMINO *et al.*, 2010), aggressive behavior (NORTON; BALLY-CUIF, 2010), memory

impairment (MICHAEL STEWART; V. KALUEFF, 2012; YU *et al.*, 2006) and impaired social behavior (MILLER, N.; GERLAI, 2012; MILLER, N. Y.; GERLAI, 2011).

Similarities are also highlighted by a conservation of neurological substrate underlying several behavioral domains. Those behaviors can be easily assayed using well-characterized test which are applied on neurological diseases models and for drug screening (KALUEFF; STEWART; GERLAI, 2014). For example, is possible to evaluate anxiety-like behavior (FONTANA *et al.*, 2019), sociability (SAVERINO; GERLAI, 2008), aggressiveness (GERLAI *et al.*, 2000), and memory (BERTONCELLO *et al.*, 2019; BLANK *et al.*, 2009; FRANSCESCON *et al.*, 2020) through the novel tank test (NTT), social preference test (SPT), mirror-induced aggressive test and inhibitory avoidance test (IAT), respectively. Importantly, these tests have undergone pharmacological validation and yeld cross-species results (BENCAN; SLEDGE; LEVIN, 2009; BLANK *et al.*, 2009; GERLAI *et al.*, 2000).

The association of obesity and unbalanced diets with neurological disorders and neurobehavioral alterations in human is well known and it is widely explored in other animals models (O'BRIEN *et al.*, 2017; PROCACCINI *et al.*, 2016). However, the same is not observed with zebrafish and only one article focused on this subject so far. Meguro *et. al.* (2019) described a memory impairment after feeding adult zebrafish with a lard-based HFD for 8 weeks. This alteration was associated with modulation of genes known as regulator of neuronal function, oxidative response, and blood-brain barrier integrity, supporting a conserved basis of HFD-induced neuropathogenesis (MEGURO; HOSOI; HASUMURA, 2019).

Therefore, our objective was to characterize the effect of a short-term HFD on a wide range of behaviors. For this, we fed zebrafish with two different amount of chicken egg yolk (7.5 or 15 mg/fish/day) or a standard diet for two weeks. After the dietary protocol, we evaluated obesity- (i.e., body weight and body mass index) and metabolic- (i.e., abdominal length) related endpoints as well as behavioral alteration (i.e., memory, sociability, aggressiveness, and anxiety-like behavior). This proof-of-concept study will support the use of this novel zebrafish model and emphasize the transactional potential of zebrafish on obesity and neuroscience field.

1.1 ANIMAL MODELS ON OBESITY RESEARCH

Animal models gave great contributions on obesity and metabolic research fields along the years. Also, the use of animals represent a high-valuable solution for the limitations of human and epidemiological studies (HARIRI; THIBAULT, 2010). On the beginning of XX century, pioneer studies achieved important finding using dog as animal model, such as the discovery of insulin in 1922 awarded by the 1923 Nobel prize (BANTING *et al.*, 1922). Non-human primates were also used on research taking advantage of the highest phylogenetic proximity with human which reflects on a more conserved genetic, physiology and anatomy (KLEINERT *et al.*, 2018). These model lead to important findings, such as, the role of parasympathetic nervous system on insulin (D'ALESSIO *et al.*, 2001) and glucagon secretion (HAVEL; VALVERDE, 1996). However, the big size, limitation on the number animals available and difficulty/expense of the maintenance makes these model less suitable for most laboratories.

Nowadays, small rodents have become the most used animal model and is one of the most applied on metabolic disorders and obesity research (REES; ALCOLADO, 2005). The effect of leptin and ghrelin on central nervous system for the energy balance control was discovered in rats and mice (KOJIMA *et al.*, 1999; ZHANG *et al.*, 1994). Also, several genetic tools and strains are available. For example, the classical ob/ob mice possess a mutation of leptin gene and exhibit early-onset obesity (INGALLS; DICKIE; SNELL, 1950). And it is often used on preclinical trials of anti-obesity drugs (ZANG; MADDISON; CHEN, 2018). Plus, different protocols of HFD and HSD are described for diet-induced obesity (DIO). DIO is widely used and yielded good results, even though it may be more time-consuming and expensive and several rising factors difficult the results interpretation and comparation between studies (HARIRI; THIBAULT, 2010; KLEINERT *et al.*, 2018).

Non-mammalian model organism, such as Zebrafish, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, had become a valuable tool on obesity research. Their use is supported by similarities to mammals, including lipid transportation/metabolism and control of energetic metabolism, and the short lifespan and generation time allowing to evaluate the long-term and intergenerational effects of obesity (SCHLEGEL; STAINIER, 2007; SHEN; YUE; PARK, 2018; SMITH, W. W. *et al.*, 2014). The relatively lower degree of complexity imposes less ethical barrier and can be useful to evaluate more detailed mechanism. The reduced corporal size make possible to work with a higher number of animals with less expenditure of budget and time (KLEINERT *et al.*, 2018; SCHLEGEL; STAINIER, 2007).

1.2 ZEBRAFISH MODELS OF HUMAN DISEASE

Zebrafish (*Danio rerio*) was firstly used in research field in 1960s, but only gained popularity in 1980s when George Streisinge demonstrated the ease and high-throughput of genetic manipulations in zebrafish (Streisinger et al., 1981). Since then, zebrafish received great attention on laboratories of genetic and embryonic development, due to its naked eye-visible extrauterine development, transparency on the first months of life, easy and low-cost maintenance and quickly maturation and reproduction (Bradford et al., 2017). Plus, a pair of zebrafish can lay ~200 egg facilitating the investigation of rare genetic events and experiments that requires a high experimental number (GUT *et al.*, 2017). Due to reduced size is possible to realize transcriptomics and other 'omics analysis of whole-organ, whole-tissue and whole-organism, yelling a more representative result (CAO *et al.*, 2016; NOLTE *et al.*, 2015). Zebrafish possess orthologous of 70% of human genes and 82% of those related with human diseases (HOWE *et al.*, 2013) (**Fig. 1**). All these advantages have made zebrafish an important model to understand the role of genes in human diseases and to develop genetic therapies (LIESCHKE; CURRIE, 2007; PHILLIPS; WESTERFIELD, 2014). The ease and high-throughput of laboratorial experiments using zebrafish in addition to a great facility to perform pharmacological exposure on the tank water compel this fish to become an excellent model to drug discovery and screening, been applied in preclinical trials and to evaluate the impact of compounds to human health and to the environment (YOGANANTHARJAH; GIBERT, 2017).

Specifically on obesity research, zebrafish has received special attention among non-mammal models due to several advantages. The neuroendocrine system responsible for lipid storage control and energetic balance is more well-stablished compared with *C. elegans* and *D. Melanogaster*. For example, the Agouti-Related Protein (AGRP), ghrelin and leptin was shown to play roles similar to mammals counterparts (CRUZ *et al.*, 2010; GORISSEN *et al.*, 2009; SONG *et al.*, 2003). Zebrafish is a vertebrate and therefore display a relatively higher similarity degree to humans on genetic, anatomy and physiology manners (GUT *et al.*, 2017; ZANG; MADDISON; CHEN, 2018). Unlike other vertebrate model, zebrafish has a fast embryonic development displaying a complete body plan and main organs already formed within 48 hours of life (KIMMEL *et al.*, 1995). Neuronal and endocrinal processes responsible for homeostasis regulation is also developed early in life (GUT *et al.*, 2017).

Further, zebrafish have all the organs essential for understanding obesity and lipid metabolism, including those of digestive tract, white adipose tissue (WAT), liver, and skeletal muscles, and the small size facilitate a whole-body analysis allowing a holistic comprehension

of process underlying obesity and comorbidities (LIESCHKE; CURRIE, 2007; SCHLEGEL; STAINIER, 2007). Zebrafish display conserved biochemicals pathways, including those involved in energy homeostasis (DEN BROEDER, M. J. *et al.*, 2015), appetite control (GUT *et al.*, 2017; NISHIO *et al.*, 2012), glucose (ELO *et al.*, 2007), triglycerides and cholesterol metabolism (OKA *et al.*, 2010), as well as adipocyte function and regulation (FLYNN *et al.*, 2009). Besides, zebrafish display similarities with mammals counterpart on obesity pathogenesis, lipid metabolism, energy homeostasis and response to anti-diabetic/ anti-obesity drugs (OKA *et al.*, 2010; ZANG; MADDISON; CHEN, 2018).

These characteristics made zebrafish a viable model for obesity-related comorbidities involving metabolic alterations, such as, diabetes, metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, hepatic steatosis, stroke and hearth diseases (GUT *et al.*, 2017) (**Fig. 2**).

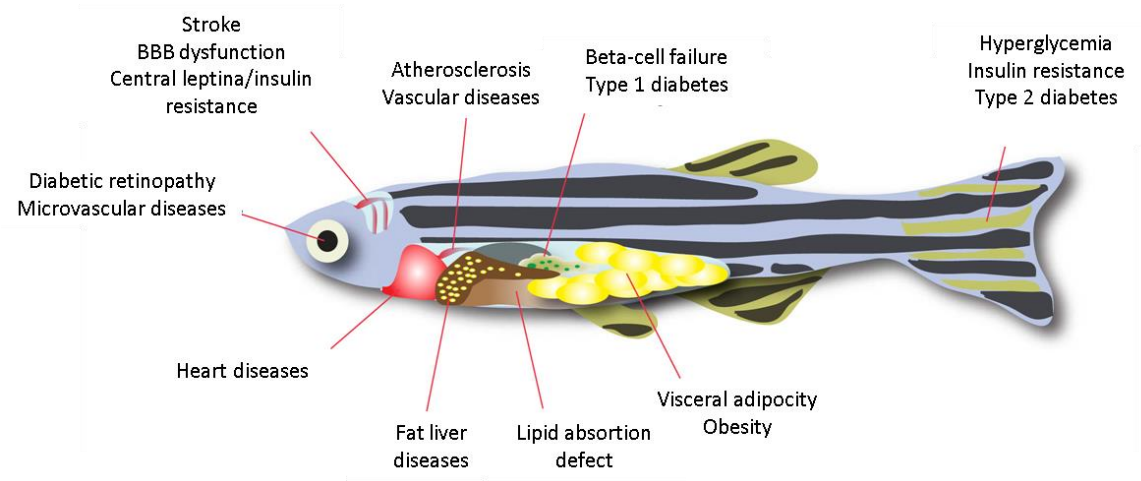

Figure 2. Zebrafish as a model of obesity-related comorbidities. Zebrafish is an useful models of obesity and its comorbidities, including those affecting the brain, vascular and digestive systems, hormonal function and steatosis. Modified from GUT et. a., 2017, Copyright © 2017 the American Physiological Society. (License number: 501652138).

On the other hand, zebrafish have some disadvantages. The small size can be considered a limitation for some experiment considering the difficulty to collect enough amount of biological material. Morphology of some organs is significant different from those of human and brown adipose tissue is inexistent. Some techniques and methods applied on classical models are still troublesome with zebrafish, for example there are not some zebrafish-specific antibodies for histochemistry (GUT *et al.*, 2017). Also, the dietary treatment and the results may be hard to translate to human due to essential differences in micro- and macronutrients requirement between zebrafish and mammals (SICCARDI *et al.*, 2009; SMITH, D. L. *et al.*, 2013). Another problematic point is that environmental parameter, such as the amount of food, quantity of

animals in the same tank and temperature has a great impact on growth and adipogenesis (LEIBOLD; HAMMERSCHMIDT, 2015; VERGAUWEN *et al.*, 2013).

1.3 METHODS TO EVALUATE THE METABOLIC AND OBESITY-RELATED PARAMETERS

The obesity-related parameters can be easily assayed in adult zebrafish by morphometric analysis. Body weight can be measured using a precision balance and a beaker filled with water. The standard length (SL), the distance between the tip of the mouth to the base of the tail, can be measured in euthanized or anesthetized animals using millimeter paper or computational software, such as the ImageJ (LEIBOLD; HAMMERSCHMIDT, 2015). Following the same procedure, it is possible to calculate the abdominal length, which is an important diagnostic criteria for metabolic syndrome, indicating higher visceral adiposity such as observed in humans (BIGAARD *et al.*, 2005). The BMI can be determined dividing the bodyweight (g) by the square of the SL (cm^2) (OKA *et al.*, 2010).

Other methods allow to specially evaluate adipogenesis in a more reliable and expressive manner in detriment of been more time and/or cost consuming. A 3D micro-computed tomography analysis permits to calculate the body fat volume of DIO zebrafish (HASUMURA *et al.*, 2012; MEGURO; HASUMURA; HASE, 2015). Importantly, this method allow to calculate visceral (vWAT) and subcutaneous(sWAT) fat volume separately which is important to better evaluate the obesity pathogenesis since vWAT is more associated with metabolic disorder and mortality risk (IBRAHIM, 2010). Similarly, MRI and EchoMRI can be performed to determine total fat volume, expressing a high correlation between both methods (LANDGRAF *et al.*, 2017). Total lipid content of larvae and adult zebrafish can be extracted by Folch method (FOLCH; LEES; SLOANE STANLEY, 1957) and afterward quantified by weighting (FLYNN *et al.*, 2009) or by high performance thin layer chromatography when in small amounts (MARTÍNEZ *et al.*, 2020).

Lipophilic dyes, such as Oil Red O (ORO), Nile Red (NR) and BODIPY is specially efficient for fat quantification in larvae and juvenile zebrafish up to 39 days of life (FLYNN *et al.*, 2009) since the corporal transparency allow to easily quantify the whole-body adiposity and circulating lipids without need to section the tissue (TINGAUD-SEQUEIRA; OUADAH; BABIN, 2011; ZHOU *et al.*, 2015). NR can be simply added to husbandry water before anesthetizing the larvae and further perform whole-body in vivo analysis on microscope. The

procedure can be repeated with the same individual after some days, allowing to evaluate the adiposity before and after a treatment or perform a longitudinal analysis avoiding the interference of inter-individual variation of adiposity (TINGAUD-SEQUEIRA; OUADAH; BABIN, 2011). ORO application requires euthanizing and fixation of fish, but it is still a high-throughput method allowing to stain lipid on adipose cell or vases, such as circulating triglycerides (ZHOU *et al.*, 2015). It is possible to combine different dyes to perform a more specific analysis of lipids deposits (KOOPMAN; SCHAART; HESSELINK, 2001; MINCHIN; RAWLS, 2011). ORO staining of liver cryosection allow to evaluate hepatic steatosis in adult zebrafish while vWAT and sWAT can be quantified by HE staining of cross-sectioned zebrafish (LANDGRAF *et al.*, 2017).

Due to reduced size, routine assay in mammals for assessment of metabolic parameters are still cumbersome in zebrafish, for example the difficulty to obtain enough blood sample. Whole-blood blood collection can be performed by decapitation, resulting in 5-10 uL of sample while a smaller amount is obtained from the dorsal artery without necessity of euthanizing the animal. Afterwards, glucose levels can be measured by a glycosometer for human use (EAMES *et al.*, 2010) while circulating triglycerides and cholesterol levels can be assessed using the appropriated kit (LANDGRAF *et al.*, 2017). Larvae metabolic parameters can be determined using dyes specific to cholesterol and triglycerides (MINCHIN; RAWLS, 2011).

1.4 ZEBRAFISH MODELS OF OBESITY

1.4.1 DIET-INDUCED OBESITY

Diet-induced obesity (DIO) can be achieved in adult and larvae of zebrafish, using diets rich in fat/sugar, western diet and overfeeding with standard diet (ZANG; MADDISON; CHEN, 2018) (table 1). Landgraf *et. al.* fed 3-6 months post fertilization (mpf) male fish with an egg yolk based HFD for 8 weeks. Dietary fat content was 53.7% compared with 22% of standard diet. HFD animals developed obesity-related characteristics, including higher body weight, BMI and subcutaneous/visceral fat content. Also, metabolic parameters were affected *i.e.*, levels of fasting glucose, triglycerides, and cholesterol and belly enlargement (LANDGRAF *et al.*, 2017). In another study, 4-6 mpf male zebrafish were fed with a similar HFD with 16.9% of fat, compared with 6.5% of control. Increasing of body weight was observed after 1 week of diet while higher BMI and belly enlargement occur within 2 weeks (PICOLO *et al.*, 2021). Meguro *et. al.* used corn oil and lard to produce two HFD, both with

24% of fat against 4% of control diet. After 6 weeks of any HFD, 5-7 mpf female zebrafish display increased visceral and subcutaneous adiposity (MEGURO; HASUMURA; HASE, 2015). Interestingly, 3 mpf zebrafish were fed with a lard based HFD for 11 weeks had body weight unaffected even though the diet altered neurobehavioral parameters (MEGURO; HOSOI; HASUMURA, 2019). Overfeeding with *Artemia*, a live food rich in fat is suitable and easy obesogenic protocol widely applied. 3-6 mpf male zebrafish were overfeed with 60 mg of *Artemia*, instead of 5 mg offered to control group. The dietary treatment lead to high body weight, BMI and adiposity after 8 weeks of diet (LANDGRAF *et al*., 2017). Interestingly, only 14 days of overfeeding lead to body weight gain on 3.5-4.5 mpf male and female zebrafish (HASUMURA *et al*., 2012). In other studies, BMI increased after 1 week of overfeeding in 3.5 mpf zebrafish while steatosis and increased triglycerides level was observed within 8 weeks (OKA *et al.*, 2010; TAINAKA *et al.*, 2011). Montalbano *et. al.* analyzed brain and gut tissue and showed that overfeeding with *Artemia* is associated with modulation of genes involved on homeostasis regulation and obesity phenotype, such as, leptin, ghrelin, orexin, NPY and POMC (MONTALBANO *et al.*, 2018).

Zebrafish larvae are also a valuable tool for obesity research and screening of anti-obesity drugs since it has a quick response to obesogenic diet and the body transparency allow to easily quantify the whole-body adiposity and circulating lipids using lipophilic dyes, as mentioned before. For example, Tingaud-Sequeira et. al. fed ~15 days post fertilization (dpf) larvae with chicken egg yolk for just one day and applied NR to *in vivo* quantify the effect of several anti-obesogenic drugs on WAT area (TINGAUD-SEQUEIRA; OUADAH; BABIN, 2011). Similarly, Zhou et.al. evaluated the effect of several hypolipidemic drug on the circulating lipids of zebrafish larvae fed with chicken egg yolk for 2 days using the Oil red O (ZHOU *et al.*, 2015). This HFD diet applied form 9 to15 dpf also lead to increased triglycerides levels and higher adipogenesis (KOPP *et al.*, 2016). Interestingly, Broeder *et. al.* fed zebrafish larvae with an egg-yolk based HFD and high-glucose diet from 6 to 15 dpf. Only HFD-fed animals display increased adipogenesis which was accompanied by modulation of several genes involved in the control of lipid metabolism (DEN BROEDER, M. *et al.*, 2017). 8 dpf larvae fed with a high-cholesterol diet developed steatosis, increased adiposity, and had high levels of total cholesterol and triglycerides. Also, lipid accumulation was diminished by treatment with bezafibrate and pioglitazone, two lipid-lowering drug for human use (MA *et al.*, 2019).

1.4.2 GENETIC MODELS OF OBESITY

DIO experiments have a variety of influencing factor, such as, type and duration of diet, nutritional variation of ingredients and difficulty to determine the amount of food eat by each animal. Therefore, genetic models of obesity (GMO) are an option to reach more reproductible and comparable results and allow to evaluate the role of specific genes on obesity pathogenesis. Further, GMO dispense the need of an obesogenic treatment, being less time and cost consuming (GUT *et al.*, 2017; ZANG; MADDISON; CHEN, 2018).

Several biochemical pathways and physiological system are associated with lipid metabolism and obesity pathogenesis, being a potential target for obesity research. The AgRP and melanocortin system is conserved in zebrafish and plays a important role on regulation of fat accumulation (RINGHOLM *et al.*, 2002; SONG *et al.*, 2003). Tg(b-actin:AgRP) zebrafish lineage overexpresses AgRP and display increased body weight, visceral adipocyte hypertrophy and hypertriglyceridemia within 1 year of life (SONG; CONE, 2007). The overexpression of Akt 1, a key gene enrolled on adipogenesis, lead to obesogenic phenotype observed in the tg(krt4:Hsa.myrAkt1)^{cy18} zebrafish lineage. Fish display increased adiposity from 21 dpf onward and increased body weight, BMI, triglycerides levels and ectopic fat accumulation within 3-5 mpf (CHU *et al.*, 2012). The mice lineages (*ob*/*ob*) and (*db*/*db*) is a classical GMO due to deficient is leptin (INGALLS; DICKIE; SNELL, 1950) and leptin receptor (CHEN *et al.*, 1996), respectively. Interestingly, both leptin and leptin receptors are conserved between fish and mammals (PROKOP *et al.*, 2012) and leptin knockout lead to obesogenic phenotype with increased body weight and length (AUDIRA *et al.*, 2018). The dwarf *vizzini* zebrafish has a mutation on growth hormone 1 gene (*GH1*) leading to an increase of visceral and subcutaneous adiposity and resembling phenotype of humans GH deficiency (MCMENAMIN *et al.*, 2013). Targeting microRNAs (miRs) is a suitable method for the developing obesity models since many of them plays an important role on regulation of homeostasis and lipid metabolism (VICKERS *et al.*, 2013). The miR-27b shows a key role in inhibiting adipogenesis in mammals and the down-regulation lead to a higher fat accumulation (JI *et al.*, 2009). Similarly, the miR-27b-SP zebrafish lineage expressing a “sponge” that disrupt miR-27b activity display obesity-related features. 10 dpf zebrafish larvae fed with HFD developed steatosis and increased circulating lipid while adult zebrafish display hepatic steatosis, increased body weight, fat mass and higher cholesterol and triglycerides levels (HSU *et al.*, 2018).

Table 1. Models of diet-induced obesity in adult and larvae zebrafish

Type and time of treatment	Age and sex	Parameters affected	Reference
Adult			
HFD			
Egg yolk, 53,7% fat, 8 weeks	3-6 mpf; Male	BW, BMI, vWAT, sWAT, BF, AL, GC, TG, CH, lipid metabolism genes	Landgraf et al., 2017
Corn oil or lard 24% fat, 6 weeks	5-7 mpf; Male and female	BW, vWAT, sWAT, BF, lipid metabolism proteins	Meguro et al., 2015
Overfeeding			
Artemia 60 mg, 8 weeks	3-6 mpf; Male	BW, BMI, BF, vWAT, sWAT, lipid metabolism genes	Landgraf et al., 2017
Artemia 60 mg, 5-6 weeks	3.5-5.5 mpf; Male and female	BW, BF, vWAT, sWAT,	Hasumura et al., 2012
Artemia 60 mg, 8 weeks	3.5 mpf; Male and female	BMI, TG, hepatic steatosis, lipid metabolism genes	Oka et al., 2010
Artemia 60 mg, 4 weeks	3.5 mpf	BW, TG, lipid metabolism genes	Tainaka et al., 2011
Artemia 60 mg, 5 weeks	3-9 mpf; Male	BW, BMI, vWAT, sWAT, hormones	Montalbano et al., 2018
Type and time of treatment	**Age**	**Effect**	**Reference**
Larvae			
HFD			
Egg yolk, 1 day	15 dpf	BF	Tingaud-Sequeira et al., 2011
Egg yolk, 2 days	5 dpf	BF	Zhou et al., 2015
Egg yolk, 6 days	9 dpf	BF, TG	Kopp et al., 2016
Egg yolk, 9 days	6 dpf	BF, lipid metabolism genes	den Broeder et al., 2017
HCD			
Egg yolk, 21 days	8 dpf	BF, AL, TG, CH, hepatic steatosis, oxidrive stress, lipid metabolism genes, survival	Ma et al., 2019

AL: abdominal length; BF: body fat; BMI: body mass index; CH: cholesterol; GC: glucose; sWAT: subcutaneous white adipose tissue; vWAT: visceral white adipose tissue; BW: body weight; TG: triglycerides.

1.5 ASSOCIATION OF OBESITY/UNBALANCED DIET IN NEUROLOGICAL DISEASES

The worldwide increasing of obesity prevalence is accompanied by the increasing number neuropathology incidence. In fact, a growing body of evident demonstrate the impact of obesity and disbalanced diet on the central nervous system (O'BRIEN *et al.*, 2017; PROCACCINI *et al.*, 2016). However, this relationship is not totally clear since several mechanism are involved, including neuroinflammation induced by microglia activation (CAI, D., 2013; VALDEARCOS *et al.*, 2014), mitochondrial dysregulation, oxidative stress (TAN; NORHAIZAN, 2019) and blood-brain barrier (BBB) dysfunction (FREEMAN; GRANHOLM, 2012). Recent studies point to a pivotal role of metabolic alteration on brain health, increasing susceptible to comorbidities (BLÜHER, 2020), including those affecting the CNS (CADENAS-SANCHEZ *et al.*, 2020).

Obesity and HFD are considered independent factors for neurodegenerative diseases including dementia Huntington, Alzheimer, and Parkinson diseases (MAZON *et al.*, 2017). Interestingly, a meta-analysis of 15 prospective studies, involving more than 25000 participants showed that BMI is strongly correlated with dementia and that obese individuals has twice the change of developing Alzheimer disease (ANSTEY *et al.*, 2011). BMI also shows correlation with reduction of brain volume (WARD *et al.*, 2005) and gray matter density (PANNACCIULLI *et al.*, 2006), showing a severe and global impact to the CNS. Interestingly, incidence and progression of neurodegenerative disorders is clearly associated with metabolic disfunction (PROCACCINI *et al.*, 2016), while the control of metabolic parameters shown a beneficial effect to the symptoms (WATSON *et al.*, 2005). Importantly, short-term obesogenic diets can affect the brain independently of obesity onset (ATTUQUAYEFIO *et al.*, 2017). For example, exposition to HFD for just 5 days lead hippocampal-dependent learning and memory deficits in rats (BEILHARZ; MANIAM; MORRIS, 2014; KANOSKI; DAVIDSON, 2010) and human (ATTUQUAYEFIO *et al.*, 2017), while hippocampal-independent memory is not affected until ~30 days of diet, demonstrating hippocampus higher vulnerability to unbalance diets (BEILHARZ; MANIAM; MORRIS, 2014).

Another impact of obesity and unbalanced diet is on psychiatric disorders. Chronic consumption of high energy diets rich in fat and/or sugar can alter neurocircuitry involved on mood regulation and reward system, including prefrontal cortex and amygdala (BOITARD *et al.*, 2015; JOHNSON; KENNY, 2010). Obese individuals are more susceptible to develop mood disorders, such as depression/ anxiety and aggressiveness (CERNIGLIA *et al.*, 2018;

LINDBERG *et al*., 2020), also affecting obese child (PUDER; MUNSCH, 2010). Also, poor dietary habits show a correlation with major depression and anxiety disorders (JACKA *et al.*, 2010). Social/economical and psychological factors difficult human data interpretation and determination of casuistic relationship between neurobehavioral disorders and obesity/ bad diets. Therefore, animals models are a valuable tool resembling similar and helping to clarify this issue (BUCHENAUER *et al.,* 2009; DE NORONHA *et al.,* 2017). Rats fed with a HFD display aggressive and anxiety-like behavior in association with increased levels of glucocorticoids and estrogen levels (BUCHENAUER *et al.*, 2009; HILAKIVI-CLARKE; CHO; ONOJAFE, 1996). Accordingly, these hormones were shown to modulate neurocircuit related to mood regulation (BOITARD *et al.*, 2015). Another proposed mechanism is associated with increased levels of triglycerides which in turn can cross the BBB leading to central leptin resistance, culminating in reduced levels of neuropeptide Y and neurobehavioral alterations. Noteworthy, only 5 days of diet can induce central leptin resistance in rats (KARL; DUFFY; HERZOG, 2008; WIDDOWSON *et al.*, 1999).

1.6 ZEBRAFISH MODELS OF NEUROBEHAVIORAL DISORDERS

The use of zebrafish on neuroscience research rapidly increased on recent decades, reinforcing the translational potential of zebrafish. Given the relatively reduced complexity of zebrafish CNS, this model is especially useful for assessment of basic neural functions, evaluation of compounds effect on the CNS and screening of neuroactive drug (KALUEFF; STEWART; GERLAI, 2014). Further, several models of neurological disorders are described in adult and larval zebrafish using genetic and pharmacological approaches, including depression (KYZAR *et al.*, 2013; ZIV *et al*., 2013), anxiety disorder (MAXIMINO *et al.*, 2010), aggressive behavior (NORTON; BALLY-CUIF, 2010), epilepsy (STEWART *et al.*, 2012), neurodegenerative diseases (PANULA *et al.*, 2006), memory impairment (MICHAEL STEWART; V. KALUEFF, 2012; YU *et al.*, 2006), autism spectrum disorder (STEWART *et al.*, 2014), and impaired social behavior (MILLER, N.; GERLAI, 2012; MILLER, N. Y.; GERLAI, 2011).

This success is supported by a conservation of zebrafish brain morphology and physiology (KALUEFF; STEWART; GERLAI, 2014; WULLIMANN; RUPP; REICHERT, 1996), including all main neurotransmitter, receptors, brain structures and neuroendocrine hormones (ALSOP; VIJAYAN, 2009; PANULA *et al.*, 2006). Dorsal, medial, and lateral pallium area are

considered homologous of mammalian isocortex (MUELLER *et al.*, 2011), amygdala (BRAFORD, 1995) and hippocampus (NORTHCUTT, 2006; PORTAVELLA *et al.*, 2002) , respectively, and display conserved physiology and functionality. Similarity is also observed in the cellular level since microglia (PERI; NÜSSLEIN-VOLHARD, 2008), astrocytes (KAWAI; ARATA; NAKAYASU, 2001), oligodendrocytes (YOSHIDA; MACKLIN, 2005) and all major cell types are present and display conserved characteristics.

Major neurotransmitter systems share physiological and anatomical similarity with mammals holding association with neuropathologies and behavioral alteration (PANULA *et al.*, 2006). Zebrafish serotoninergic system is well described and HT-5 positive neurons is detected in hypothalamus, thalamus, posterior turbeculum, anterior raphe nucleus, pineal gland, cerebellum, among other areas (LILLESAAR, 2011). Thus, this system is associated with locomotion (BRUSTEIN *et al.*, 2003; GABRIEL *et al.*, 2009), fear /anxiety (BENCAN; SLEDGE; LEVIN, 2009; SACKERMAN *et al.*, 2010) and aggressive behavior (JONES; NORTON, 2015; TELES *et al.*, 2013). Dopaminergic system is also conserved in zebrafish (FLINN *et al.*, 2008) and lower levels of this neurotransmitter are associated with reduced locomotion (ANICHTCHIK *et al.*, 2004). The presence of cholinergic neurons is described on central, dorsal, lateral and subcommissural nucleus of the ventral telencephalic, hypothalamus, dorsal thalamus and optic tectum (CLEMENTE *et al.*, 2004; KASLIN *et al.*, 2004; MUELLER; VERNIER; WULLIMANN, 2004). Similarly to humans, nicotine modulate cholinergic system through activation of nicotinic receptor leading to modulation of memory formation and exerting anxiolytic effect (LEVIN; BENCAN; CERUTTI, 2007; LEVIN; CHEN, 2004). All three histamine receptor found on mammalian brain (H1, H2 and H3) are already described in zebrafish (PEITSARO *et al.*, 2007). Further, histaminergic neurons were detected on optic tectum, hypothalamus, amygdala and hippocampus and are associated with alertness, memory and anxiety-like behavior (ERIKSSON *et al.*, 1998; PEITSARO *et al.*, 2003; PEITSARO; ANICHTCHIK; PANULA, 2000). Glutamate is the main excitatory neurotransmitter in vertebrates and is found on cerebellum, optic tectum and telencephalon, which comprises the regions homologous to mammalian hippocampus and amygdala (RICO *et al.*, 2010). Memory formation in zebrafish is similar to mammals and highly dependent of glutamatergic system. Treatment with mk-801, a NMDA-receptor antagonist, lead to impaired memory formation (BLANK *et al.*, 2009). The inhibitory neurotransmitter GABA and glycine are described in zebrafish brain, displaying important functions on movement. GABAergic neurons are found on cerebellum, hypothalamus and telencephalon (DELGADO; SCHMACHTENBERG, 2008;

KIM *et al.*, 2004) and is targeted in models of seizure in zebrafish and rodents (BARABAN *et al.*, 2005)

Also, zebrafish response to a wide range of neurotropic drugs, such as, antidepressants, anxiolytic, antipsychotics, ethanol, antiepileptics and anesthetics/analgesics is similar to mammals, suggesting a conserved neural substrate (KALUEFF; STEWART; GERLAI, 2014).

1.7 BEHAVIORAL TESTS IN ZEBRAFISH

Zebrafish present a robust repertoire of behaviors and, likewise classical murine models, behavioral analysis demonstrate cross-species response to neurotropic drugs and can confirm phenotype of human diseases with a high degree of similarity. Thus, neurological substrate responsible to zebrafish behavior is well-characterized and resemble those in mammals (KALUEFF; STEWART; GERLAI, 2014). The aforementioned particularities of zebrafish makes behavioral test less-time and cost consuming and an excellent tool for neuroactive drug screening and discovery (NORTON; BALLY-CUIF, 2010).

Anxiety-like behavior in zebrafish is defined by erratic movement, reduced locomotion, higher exploration of bottom areas and corners, among other characteristics (KALUEFF *et al.*, 2013) and can be assessed by the novel tank test (NTT). This test is similar to the murine open field and is based on the natural tendency to display anxiety-like behavior in a new environment. The fish freely explore the new tank which is virtually divided into horizontal areas. Afterwards, exploratory behavior is evaluated in each area and anxiety-like behavior is associated with a lower tendency to explore the bottom areas (FONTANA *et al.*, 2019). This test is widely used and underwent pharmacological validation using anxiolytic (such as buspirone, diazepam and fluoxetine) and anxiogenic drugs (BENCAN; SLEDGE; LEVIN, 2009; EGAN *et al.*, 2009).

Zebrafish live in groups and display a natural preference to interact with conspecifics (SAVERINO; GERLAI, 2008). The social preference test (SPT) is widely applied for measuring social behavior in zebrafish and is based on the tendency of a solitary fish to spend more time closer to an adjacent tank containing conspecific. Notably, this behavioral task is also highly sensitive to various pharmacological treatments, which modulates the social preference as occurs in humans and rodent models (FONTANA *et al.*, 2018; GERLAI *et al.*, 2000; MÜLLER *et al.*, 2020).

In humans, aggressive behavior is associated with several neuropsychiatric disorders and impact life quality and social interaction (ZABEGALOV *et al.*, 2019). Therefore, this is another important parameter to be analyzed in zebrafish. The mirror-induced aggression test (MIAT) is widely applied to assess aggressive behavior of zebrafish. This behavioral task has been already characterized in zebrafish and is based on the tendency of fish to attack their reflection on the mirror when individually placed in a tank (GERLAI *et al.*, 2000). Importantly, the MIAT is sensitive to drugs that positively influences aggression in rodents and humans (GERLAI *et al.*, 2000; GUTIÉRREZ *et al.*, 2020; NORTON; BALLY-CUIF, 2010).

Long-term and short-term memory can be assessed by the inhibitory avoidance test (IAT). IAT is based on the avoidance of zebrafish to preferred places when a potentially dangerous stimulus (e.g., mild electric shock) is previously administered. The test is widely used and is pharmacologically validated, being sensible to mk-801 and alcohol administration (BERTONCELLO *et al.*, 2019; BLANK *et al.*, 2009; FRANSCESCON *et al.*, 2020).

1.8 EFFECT OF HFD ON ZEBRAFISH CNS

Despite few studies showing the effect of obesity or unbalanced diet on zebrafish CNS, some evidence suggests an effect similar to those that occur in classical models, such as rodents. It is well known that high fat intake can impair memory within 5 days in human and murine models (KARIMI *et al.*, 2013; O'BRIEN *et al.*, 2017). Similarly, adult zebrafish fed with a lard based HFD for 8 weeks also display cognitive decline. Interestingly, this was accompanied by modulation of genes known as regulator of neuronal function, oxidative response, and blood-brain barrier integrity, supporting a conserved basis of HFD-induced neuropathogenesis (MEGURO; HOSOI; HASUMURA, 2019).

Also, obesity is commonly accompanied with metabolic changes, making individuals more susceptible to neurological diseases (CADENAS-SANCHEZ *et al.*, 2020; HAMER; BATTY; KIVIMAKI, 2012; PROCACCINI *et al.*, 2016). High levels of glucose and triglycerides, and dysregulation of hormones such as leptin, insulin and ghrelin can impact brain metabolism and induce neuronal death (MAZON *et al.*, 2017). Similarly, zebrafish overfeed with artemia for 5 weeks display altered expression of leptin, ghrelin and orexin on the brain (MONTALBANO *et al.*, 2018). Hyperglycemia was associated with anxiety-like behavior and memory impairment in a model of diabetes mellitus. Normalization of glucose levels using diphenyl diselenide reversed the anxiety-like behavior (DOS SANTOS *et al.*, 2018) while memory impairment was associated with increasing levels of acetylcholinesterase (CAPIOTTI *et al.*,

2014). Knock-out of leptin lead to several behavioral changes in zebrafish, including anxiety-like behavior, reduced aggressiveness and fear besides obesity. This results were accompanied by decrease of several neurotransmitter, altered levels of leptin, insulin and ghrelin and oxidative stress on the brain (AUDIRA *et al.*, 2018).

2. JUSTIFICATION

Zebrafish is an interesting experimental animal model that is receiving lot of attention for supporting classical models and enabling new findings. However, its translational potential can be poorly explored in some areas and studies are necessary to validate the use of zebrafish for new applications.

Few studies focused on evaluate the effect of unbalanced diet on zebrafish CNS. There are few data exploring the effect of a short-term diet on memory impairment and anxiety in this model. Therefore, this study aims to perform a face validation of behavioral changes induced by HFD in zebrafish focusing on a wide range of behavior domains in order to determine the overall effect of the diet, and reinforce the cross-species conserved response to such diets. This effort will support zebrafish usage as a new model, potentially leading to a better understanding of how CNS is affected by a HFD and help developing new therapeutic approaches.

3. OBJECTIVE

3.1 GENERAL OBJECTIVE

Evaluate the effect of a HFD on obesity-related and neurobehavioral parameters in adult zebrafish.

3.2 SPECIFIC OBJECTIVES

Evaluate the effect of a short-term HFD in adult zebrafish on:

a) obesity and metabolic -related parameters (i.e. body weight, body mass index and abdominal length);

b) behavioral parameters (i.e. aggressiveness, anxiety-like behavior, memory formation and sociability) using well-stablished tests; and

d) validate the applicability of behavioral test with DIO zebrafish.

4. PUBLICATION

The work developed during the master's program generated an original article published in the journal: **Progress in Neuro-psychopharmacology and Biological Psychiatry**

Article title: **Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish.**

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Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish

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ABSTRACT

Obesity is a global health problem with high prevalence and defined by a high body mass index (BMI). Several comorbidities affecting the central nervous system (CNS) are associated with obesity (*e.g.*, neurodegenerative diseases, cognitive deficit, and psychobehavioral disturbs). The zebrafish (*Danio rerio*) has been considered a suitable model organism to investigate the neurobehavioral features of various human diseases. Here, we verify the impact of a high-fat diet (HFD) on the CNS by specifically assessing the effects of short-term HFD on anxiety-like responses, aggression, social preference, and memory, which are essential behaviors for survival and reproduction. Animals were separated in three experimental groups. The standard diet group (SD) received 7.5 mg/fish of dry food, while HFD groups received 5 mg/fish dry food plus 7.5 (HFD-7.5) or 15 mg/fish (HFD-15) of chicken egg yolk daily. Dietary fat content (*w*/w) was approximately 6.5%, 16.9%, and 21.1%, respectively. We performed behavioral tests and morphometric analyses after two weeks of HFD. In comparison to SD animals, HFD groups showed typical obesogenic responses with increases in BMI, abdominal length, and body weight. HFD individuals also showed increased aggression and anxiety-like behaviors in the mirror-induced aggression and novel tank diving tests, respectively. Interestingly, HFD did not change the social preference behavior, mean swimming speed or spontaneous activity levels, while the HFD-15 group showed cognitive deficits in the inhibitory avoidance test. Collectively, this "proof-of-concept" study is the first report to characterize the effects of short-term HFD on different behavioral domains of zebrafish with high degree of face validity. Moreover, our data reinforce the growing utility of zebrafish to explore the neurobehavioral basis of obesity, providing clinically translatable data, complementing the existing rodent models and supporting future mechanistic studies.

1. Introduction

The increasing prevalence of obesity is a global health concern. Globally, ~13% of adults were obese and 39% were overweight in 2016 (WHO, 2016). Obesity is defined by a body mass index (BMI) higher than 30 kg/m^2, which reflects increased fat storage (Wang and Beydoun, 2007). This condition is associated with several pathologies, including diabetes, heart diseases, cancer, hypertension, non-alcoholic fatty liver disease, and arthritis, eliciting a higher mortality rate (Flegal et al., 2010; Malnick and Knobler, 2006). Unbalanced diets, such as Western diet and high-fat diet (HFD) are major causes for increasing obesity and the aforementioned comorbidities (Cordain et al., 2005; Vandevijvere

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et al., 2015). Furthermore, obesity and HFD consumption are associated with various central nervous system (CNS) disorders (O'Brien et al., 2017) and metabolic changes, including deleterious body fat distribution, increased inflammatory markers, endocrine alteration, and higher levels of blood glucose, triglycerides, and cholesterol. Obesity-induced metabolic disorders underlie several obesity-related comorbidities (Blüher, 2020), including CNS vulnerability and susceptibility to neurobehavioral disorders (Cadenas-Sanchez et al., 2020).

High fat intake and obesity are considered strong risk factors for neurodegenerative diseases including vascular dementia, Alzheimer, Huntington, and Parkinson diseases (Anstey et al., 2011; Mazon et al., 2017; Pedditizi et al., 2016; Procaccini et al., 2016). Accordingly, obese individuals are more susceptible to behavioral and psychological disorders such as aggression, anxiety/depression (Cerniglia et al., 2018; Lindberg et al., 2020), and cognitive deficits (Francis and Stevenson, 2011).

Because various mechanisms are involved (*i.e.*, blood-brain barrier dysfunction (Freeman and Granholm, 2012), oxidative stress, mitochondrial dysfunction (Tan and Norhaizan, 2019), central leptin/insulin resistance (Banks et al., 2018), and neuroinflammation (Cai, 2013), the specific pathways that link high-fat intake to altered CNS homeostasis are not well-understood. Animal models have been extensively used to elucidate how HFD and obesity affect the brain function. In rodents, HFD elicits cognitive deficits (Greenwood and Winocur, 1990) and similarly to what occurs in humans (O'Brien et al., 2017), impairments on the hippocampus function have been suggested as one of the mechanisms involved (Karimi et al., 2013; Stranahan et al., 2008). Interestingly, even short-term exposure to unbalanced diets can cause cognitive impairment, independently of obesity onset. For example, rats fed with a fat-rich diet show impairments on hippocampal-dependent learning and memory (HDLM) after 5 days, although effects on hippocampal-independent learning and memory (HILM) are not observed until after 30 days of diet (Beilharz et al., 2014). Accordingly, a fat-rich diet for 4 days reduces HDML, but not HIML in humans (Attuquayefio et al., 2017). Rodents fed with HFD also display increased aggressiveness (Buchenauer et al., 2009) and anxiety/depressive-like behaviors (Baker and Reichelt, 2016; de Noronha et al., 2017), suggesting the use of laboratory models to investigate the neurobehavioral effects of HFD.

The zebrafish (*Danio rerio*) has been considered a valuable model organism for obesity and lipid research (Gut et al., 2017). This species shows various similarities with mammals regarding the control and functioning of energy homeostasis, storage and transport of lipid, obesity pathogenesis, organs anatomy and physiology, and response to anti-diabetic drugs (Oka et al., 2010; Zang et al., 2018). Zebrafish fed with different HFD protocols show increased fasting blood glucose, cholesterol, and triglyceride levels, as well as increased visceral and subcutaneous fat content, BMI, and body weight (Landgraf et al., 2017; Meguro et al., 2015). Likewise, overfeeding with *Artemia*, a live food rich in fat, increases BMI, and triglycerides levels, promoting hepatic steatosis and higher lipid storage (Oka et al., 2010). Previously, it has been shown that fish fed with HFD for 8 weeks have worse performance in the active avoidance test (Meguro et al., 2019). However, there are no studies investigating the effects of short-term HFD on different behavioral domains in adult zebrafish. Because behavioral activity results from a complex interaction between organisms and environment (Tamashiro, 2015), the assessment of multiple behaviors may not only improve face validity of a certain model, but also suggest possible effects of experimental manipulations on the CNS homeostasis.

Here, we fed zebrafish with two HFD composed of different amounts of chicken egg yolk (7.5 or 15 mg/fish) for two weeks. The main goal was to characterize the short-term effects of HFD on various zebrafish behaviors (*i.e.,* anxiety, aggression, group preference, and memory) as well as on relevant obesity- (*i.e.,* body weight and body mass index) and metabolic- (*i.e.*, abdominal length) related endpoints. Our "proof-of-concept" study will support the validity of novel zebrafish models of obesity, as well as help to elucidate the evolutionarily conserved behavioral responses of HDF in vertebrates.

2. Materials and methods

2.1. Animals

Male wild-type, 4–6 months old short-fin phenotype zebrafish (*Danio rerio*) were acquired from a local supplier (Hobby Aquários, Brazil) and maintained on 40 L tanks at a maximum density of 2 fish/L of non-chlorinated water. These fish are expected to be genetically heterogeneous, which better represents natural populations, thereby decreasing the effects of arbitrary genetic drift inherited traits (Speedie and Gerlai, 2008). Importantly, experiments were performed using only male fish to avoid hormonal interference and ectopic fat accumulation (Landgraf et al., 2017). Fish were sexed based on the morphological differences between male and female zebrafish (*e.g.*, shape of the body and coloration) (Parichy et al., 2009) and further confirmed by gonadal extraction after euthanasia. Water temperature was set at 27 ± 2 °C, pH 7.0–7.2, and conductivity 1500–1600 $\mu S.cm^{-1}$). Animals were maintained on a natural light-dark photoperiod (~12/12 h light/dark cycle) and fed with a commercial fish flake food (Alcon BASIC™, Alcon, Brazil) twice daily. Two weeks following the acclimatization period, fish were randomly allocated in 2.5 L tanks filled with non-chlorinated water (10 fish per aquarium; 2 aquariums per group) and separated in groups according to the respective diet. All experiments were performed using animals obtained from two independent batches to ensure data reproducibility. The protocols applied were previously approved by Ethics Committee on the Use of Animals (CEUA) of the University of Brasilia (Process number: 040/2020).

2.2. Experimental diets

To assess the effect of the HFD diet on behavioral and morphological parameters, animals were fed once a day according to the three dietary groups. The standard diet group (SD) was daily fed with 7.5 mg/fish of dry food Tetra-ColorBits (6.5% fat, 47.5% proteins; 4.9 cal/mg). The high-fat diet groups (HFD) received 5 mg/fish dry food supplemented with egg yolk (26% fat, 16% proteins, 2% carbohydrates; 3.23 cal/mg) resulting in 7.5 mg/fish (HFD-7.5) or 15 mg/fish (HFD-15) per day. The SD used here reached the basal energy requirement of 30 cal/fish (Pannevis and Earle, 1994) with 6.5% fat content (*w*/w), while HFD-7.5 and HFD-15 received 48.7 and 73.0 cal/fish with 16.9% and 21.1% fat content (w/w), respectively. Chicken eggs were purchased from a local supplier of human food.

2.3. Morphometrical measurements

At days 0, 3, 7, 10, and 16, animals were individually transferred to a beaker filled with non-chlorinated water to estimate the effect of diet on average body weight using a precision balance (Fig. 1A). Body weight variation was calculated as the difference between the first and last day of the experiment (BW_{16} - BW_0). On the 16th day, immediately after the last behavioral test, fish were euthanized and photographed laterally. The abdominal length and the standard length (SL) were quantified using the ImageJ 1.45 software. Body mass index (BMI; $g.cm^{-2}$) was calculated as weight (g) divided by SL squared (cm^2) (Oka et al., 2010).

2.4. Behavioral tests

Behavioral tests were performed between days 14–16 (Fig. 1A). Animals ($n = 15–18$ per group) were submitted to the behavioral tests at the same time period (9:00 am to 3:00 pm). The novel tank diving test (NTT), social preference test (SPT), and the mirror-induced aggression test (MIAT) were sequentially performed on the same rectangular tank (35 cm length × 25 cm height × 10 cm width) filled with 4 L of non-

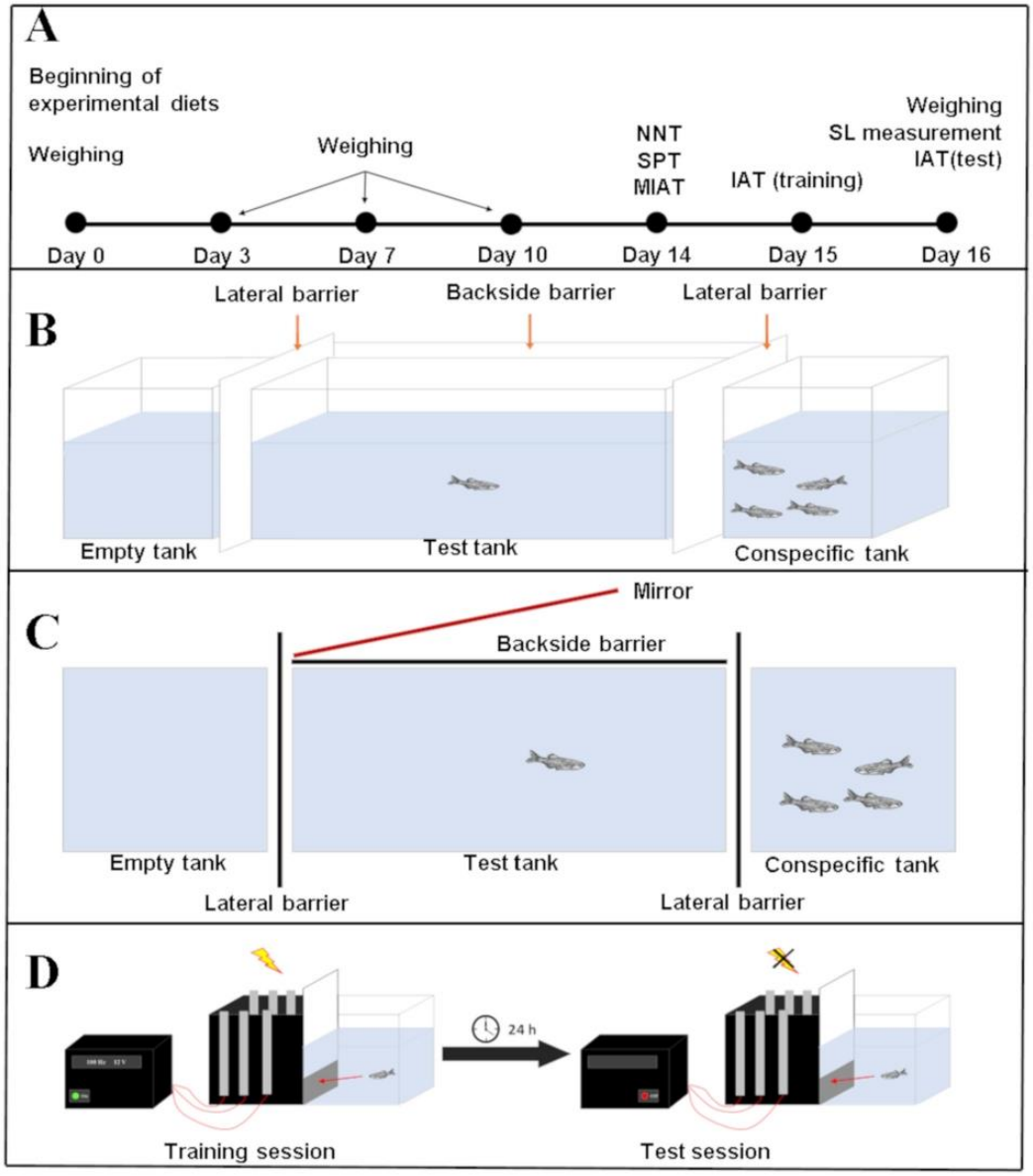

Fig. 1. Experimental design and schematic representation of the apparatus used in the behavioral tests. (A) Experimental design. The apparatus used on NNT, SPT, and MIAT is shown at (B) frontal- and (C) top- view perspectives. (D) Schematic representation of IAT experimental design and the apparatus used.

chlorinated water (27 ± 1 °C). Two tanks were positioned laterally and one of them received 4 conspecifics. A mirror was placed forming a 22.5° angle with the backside wall. Three removable opaque barriers were used to isolate the test tank from these objects (Fig. 1B and Fig. 1C). Upper- and frontal-view cameras were connected to a laptop to record the behavioral activities of fish using video-tracking software (ANY-maze™, Stoelting CO, USA) at 30 frames/s rate. The experimental strategy was adopted to minimize the handling stress and to assess all behavioral endpoints of the subject in the same trial/tank, fully adhering to the 3Rs principle of animal experimentation.

2.4.1. Novel tank diving test

Locomotor activity and anxiety-like behaviors were measured by the novel tank diving test (NTT) as described elsewhere (Fontana et al., 2019). The NTT is a well-characterized behavioral paradigm based on the natural tendency of zebrafish gradually explore the surface of the tank as novelty stress decreases across time, being highly sensitive to anxiolytic and anxiogenic manipulations (Bencan et al., 2009; Egan et al., 2009; Kalueff et al., 2013). Fish were individually placed on the apparatus with all the opaque barriers at the back and lateral walls of the tank closed and their behaviors were immediately recorded for 6 min. The aquarium was virtually divided into two equal areas (bottom and

top) on the lateral view. Locomotor activity was estimated by the distance traveled, absolute turn angle, and maximum speed, while vertical exploration was assessed by the latency to enter the top, number of transitions to top, and the time spent in the top area (Kalueff et al., 2013). All data were extracted offline and obtained by automated analysis using the ANY-maze™ software.

2.4.2. Social preference test

Zebrafish live in groups and display a natural preference to interact with conspecifics (Saverino and Gerlai, 2008). The social preference test is widely applied for measuring social behavior in zebrafish, which naturally form shoals in their natural environment. Notably, this behavioral task is also highly sensitive to various pharmacological treatments, which modulates the social preference as occurs in humans and rodent models (Fontana et al., 2018; Gerlai et al., 2000; Müller et al., 2020). Immediately after the NTT, both lateral barriers were removed exposing the test subject to an empty tank (one side of the tank) and a tank with four conspecifics (another side of the tank) simultaneously. Behavioral activities were recorded for 1 min. The experimental tank was virtually divided into four equal areas based on the distance to conspecifics. The time spent in the conspecific area and the number of entries to the conspecific area were quantified in an automated fashion using the ANY-maze™ software.

2.4.3. Mirror-induced aggression test

The mirror-induced aggression test (MIAT) was performed to assess the aggressive behavior of zebrafish. This behavioral task is based on the tendency of zebrafish to attack their reflection on the mirror when individually placed in a tank (Gerlai et al., 2000). Importantly, the MIAT is sensitive to drugs that positively influences aggression in rodents and humans (Gerlai et al., 2000; Gutiérrez et al., 2020; Norton and Bally-Cuif, 2010). After the SPT, both lateral opaque barriers were replaced, and the backside barrier was removed, allowing the test subject to interact with the inclined mirror. Behavioral activities were recorded in a top-view camera for 6 min and later analyzed offline by two analysts blinded to the experimental condition of fish (inter-rater reliability >0.85). The tank was virtually divided into three areas relatively with the proximity to the mirror. Both the number and duration of aggressive episodes were quantified. Aggression was defined by biting episodes, short bouts, or fast swimming directed to the tank wall closer to the mirror, aiming to attack the virtual conspecific (Kalueff et al., 2013; Zabegalov et al., 2019). We also measured the transitions to the area closer to the mirror and the time spent in the respective compartment as exploratory parameters.

2.4.4. Inhibitory avoidance test

Long-term memory was assessed by the inhibitory avoidance test (IAT) as reported and pharmacologically validated elsewhere (Bertoncello et al., 2019; Blank et al., 2009; Franscescon et al., 2020). The test is based on the avoidance of zebrafish to preferred places when a potentially dangerous stimulus (*e.g.,* mild electrick shock) is previously administered. The apparatus consisted of a tank (30 cm length × 10 cm height × 10 cm width) with two equally-sized compartments (black and white) divided by a manually operated guillotine-like door. Three pairs of steel bars attached to the black compartment connected to an electrical energy supplier of 12 V were used as an electrical shock source (100 Hz frequency; 5 ms duration of the pulse; 3 ± 0.2 V) for 5 s (Fig. 1D). Long-term memory formation was measured in tanks filled with 1.3 L of non-chlorinated water.

IAT comprises two sessions (training and test). In the training session, zebrafish were placed in the white compartment for 1 min. Then, the door was partially opened allowing the transition to the black compartment. The door was closed immediately after the fish completely entered the black compartment and the shock was applied. The animal was rapidly removed from the apparatus and individually placed in a perforated Plexiglass tank (50 cm length × 35 cm width × 6 cm height). This tank was equally divided in compartment (6 cm length × 6 cm width × 6 cm height) connected by small perforations on the compartment wall (0.5 cm diameter), which allows water flow and visual contact between individuals, minimizing isolation-evoked stress (Maximino et al., 2018; Ziani et al., 2018). Importantly, fishes that spent more than 300 s to cross the black side were removed from the test as an exclusion criterion.

The test session was performed 24 h after training. The same procedures were applied; however, the electrical supplier was turned off. After 1 min of acclimatization, the door was opened and the latency to enter the black compartment was measured. The retention index was calculated by the difference of the latencies obtained from the test session – to that of the training session. Higher values imply long-term memory consolidation capacity.

2.5. Statistics

Kolmogorov–Smirnov and Bartlett's tests were performed to determine the normality of data and homogeneity of variances, respectively. Data were presented as mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test. The body weight curve was analyzed by two-way ANOVA (time and treatment were set as factors) followed by Newman-Keuls post-hoc test. Due to the non-parametrical distribution of data, the latency to enter the black side in the IAT was expressed as median ± interquartile range and analyzed by Wilcoxon matched-pairs signed rank test. Statistical analysis was performed using the Graphpad Prism software (version 8.0.1). The significance level was set at $p < 0.05$ level.

3. Results

3.1. Short-term HFD induces obesity in zebrafish

To characterize obesity and evaluate the effects of the HFD, we measured morphological parameters. Two-way ANOVA revealed significant temporal effects of diet on the body weight (BW) (time x diet interaction: $F_{(8,\ 228)} = 6.601$; $p < 0.0001$) (Fig. 2A). Fig. 2B shows the data normalized by the BW of the control group on day 0. Both HFD groups showed increased BW after 7 days, which was sustained until the end of the experiments, while no weight change was observed in the SD group (diet as a factor on two-way ANOVA: $F_{(2,\ 46)} = 8.115$; $p = 0.001$). At day 16th, both HFD groups displayed a significant (+22%) increase on BW compared with SD group ($F_{(2,\ 46)} = 12.67$; $p < 0.0001$) (Fig. 2C). HFD also increased abdominal length ($F_{(2,\ 46)} = 3.819$; $p = 0.0292$) (Fig. 2D) and BMI ($F_{(2,\ 46)} = 6.893$; $p = 0.0024$) (Fig, 2E), which can be visualized in representative animals from each group (Fig. 2F).

3.2. HFD induces aggression, anxiety-like behavior, and memory formation impairment in zebrafish

The effects of HFD on behavioral functions were tested using the novel tank diving test (NTT), social preference test (SPT), mirror-induced aggression test (MIAT), and inhibitory avoidance test (IAT). HFD did not alter the total distance traveled ($F_{(2,\ 46)} = 0.09$; $p = 0.9141$) (Fig. 3A), the absolute turn angle ($F_{(2,\ 46)} = 0.05211$; $p = 0.9493$) (Fig. 3B), and the maximum speed ($F_{(2,\ 46)} = 0.2292$; $p = 0.7960$) (Fig. 3C) in the NTT. Although HFD did not change the number of transitions to the top area ($F_{(2,\ 46)} = 2.616$; $p = 0.0839$) (Fig. 3D), both HFD groups spent less time in the top ($F_{(2,\ 46)} = 11.18$; $p = 0.0001$) (Fig. 3E) and had a higher latency to enter the top area ($F_{(2,\ 46)} = 5.633$; $p = 0.0065$) (Fig. 3F). Temporal analyses of behavior (Figs. 3G–H) revealed that the SD group habituated faster to novelty when compared to both HFD groups, since significant effects of treatment x time interaction ($F_{(22,\ 506)} = 1.856$; $p = 0.0107$) and time ($F_{(11,\ 506)} = 4.378$; $p < 0.0001$) in the number of transitions to the top area were verified. Fig. 3H shows representative track plots of the exploratory behavior in

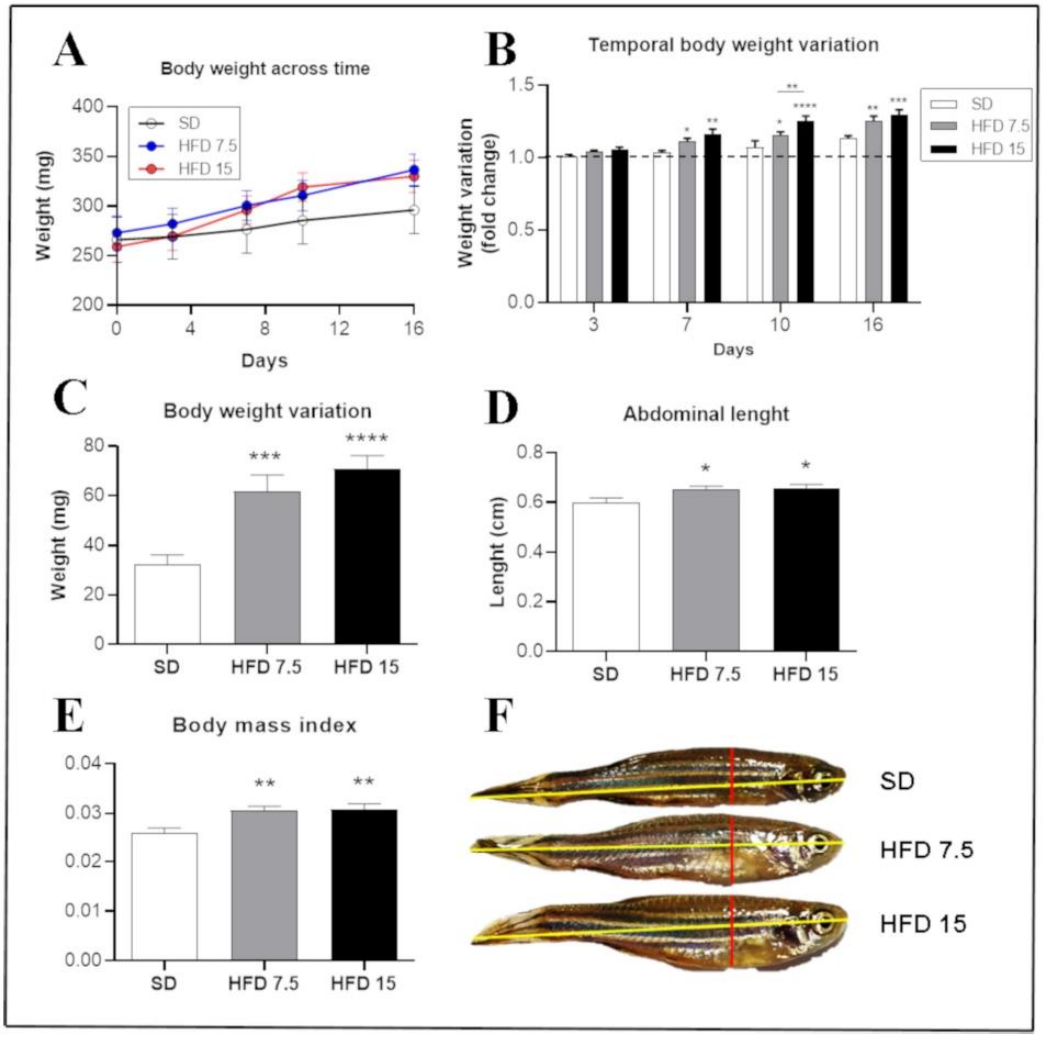

Fig. 2. Morphological analysis demonstrating the obesogenic effect of HFD in zebrafish. (A) Absolute body weight curve across the experiment. (B) Body weight (BW) normalized by the initial weight of the SD group. (C) BW variation calculated as the body weight difference between days 16 and 0. (D) Abdominal length and (E) BMI increased in both HFD groups. (F) Representative figure of the fish after the experimental period, showing increased abdominal length in both HFD groups. Data are expressed as mean ± standard error of the mean (S.E.M.) and analyzed by two-way ANOVA (graphs A and B; treatment and time were set as factors) or one-way ANOVA, followed by Student-Newman-Keuls post-hoc test. Asterisks indicate statistical differences compared with the SD group. $n = 15–18$ per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

the NTT, in which both HFD groups had similar anxiety-like responses.

Fig. 4 shows the behavioral parameters measured in the SPT. The short-term HFD did not affect the social preference, since no changes in time spent (Fig. 4A) and transition to the area closer to conspecific (Fig. 4B) ($F_{(2, 46)} = 0.7107$; $p = 0.4966$ and $F_{(2, 46)} = 0.7744$; $p = 0.4669$, respectively) were found. Moreover, the average duration per entry in the conspecific area was similar in all experimental groups ($F_{(2, 46)} = 0.9634$; $p = 0.3892$) (Fig. 4C).

In the MIAT, HFD modulated the aggression profile of zebrafish (Fig. 5). Although no differences were observed in the number of aggressive episodes ($F_{(2, 46)} = 0.2297$; $p = 0.7957$) (Fig. 5A), a higher duration of aggression episodes ($F_{(2, 46)} = 5.643$; $p = 0.0064$) was observed in HFD-7.5 and HFD-15 groups (Fig. 5B). Neither the number of entries ($F_{(2, 46)} = 0.3664$; $p = 0.6953$) or the time spent ($F_{(2, 46)} = 0.2333$; $p = 0.7928$) in the area closer to the mirror change in HFD groups.

Fig. 6 shows the effects of HFD on the aversive memory measured in the IAT. The latency to enter the dark compartment was higher in the test session in SD (W = 124.0; $p = 0.0005$) and HFD-7.5 (W = 103.0; $p = 0.0126$) groups, but not in HFD-15 (W = 22.0; $p = 0.5614$) (Fig. 6A). Accordingly, HFD-15 group had a significant lower retention index than the other experimental groups tested ($F_{(2, 46)} = 4.039$; $p = 0.0242$) (Fig. 6B).

4. Discussion

Although zebrafish is a relevant animal model of obesity and metabolic disarrangement (Gut et al., 2017; Zang et al., 2018), little is known

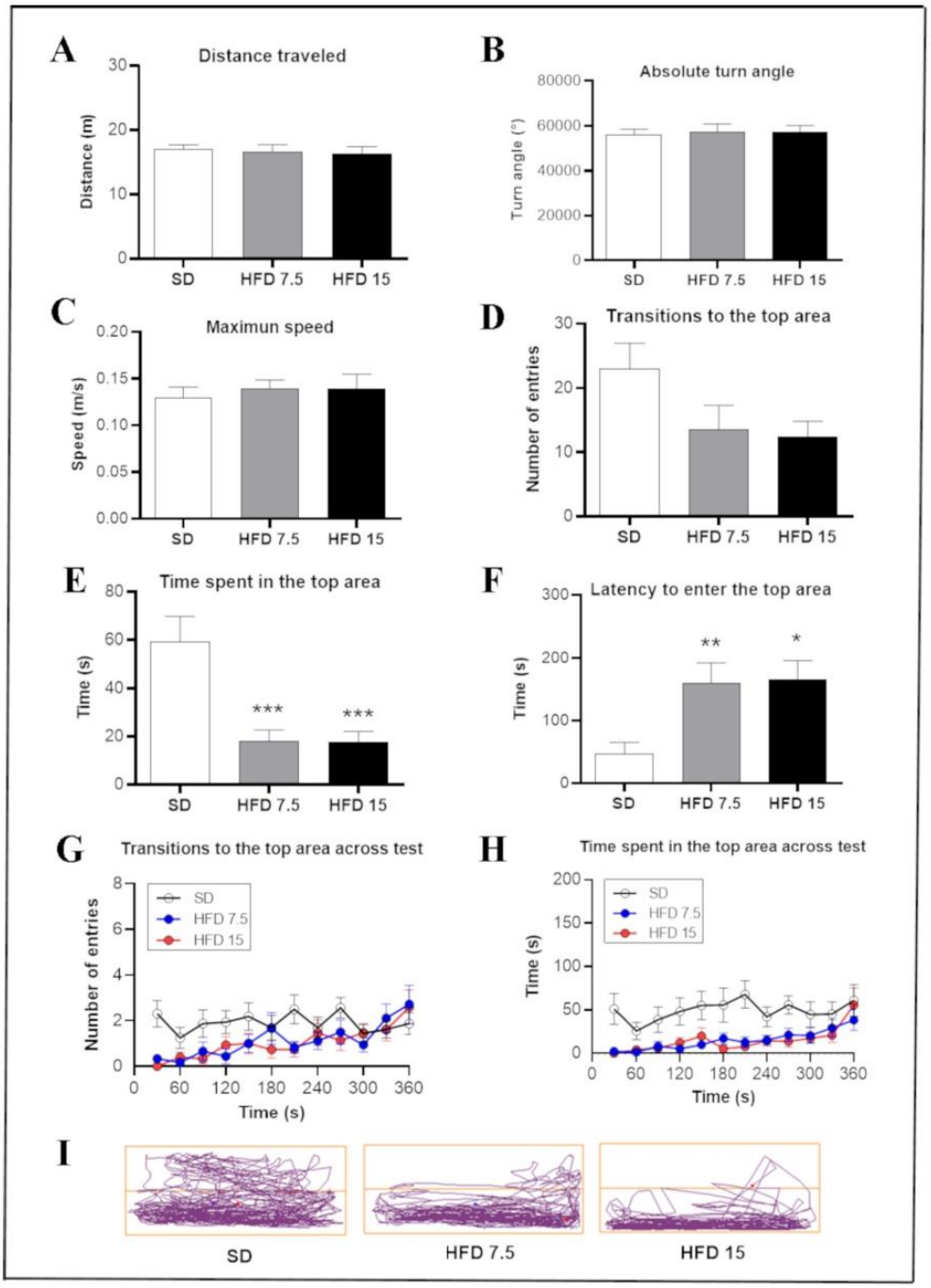

Fig. 3. HFD promotes anxiety-like behavior without influencing locomotion. Locomotion-related parameters were similar between all groups as determined by the (A) distance traveled, (B) absolute turn angle, and (C) maximum speed in the novel tank test (NTT). (D) Transitions to the top area, (E) time spent in the top area, and (F) latency to enter the top area were assessed to evaluate vertical exploration which reflects anxiety-like behaviors. (G-H) Transitions and time spent in the top area analyzed across time (data were grouped in 30 s intervals). (I) Representative images illustrate different behavioral profiles in HFD groups exploring less the top area compared with SD group. Data are expressed as means ± standard error of the mean (S.E.M.) and analyzed by two- way ANOVA (graphs G; treatment and time were set as factors) or one-way ANOVA, followed by Student-Newman-Keuls post-hoc test. Asterisks indicate statistical differences compared with the SD group. $n = 15–18$ per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

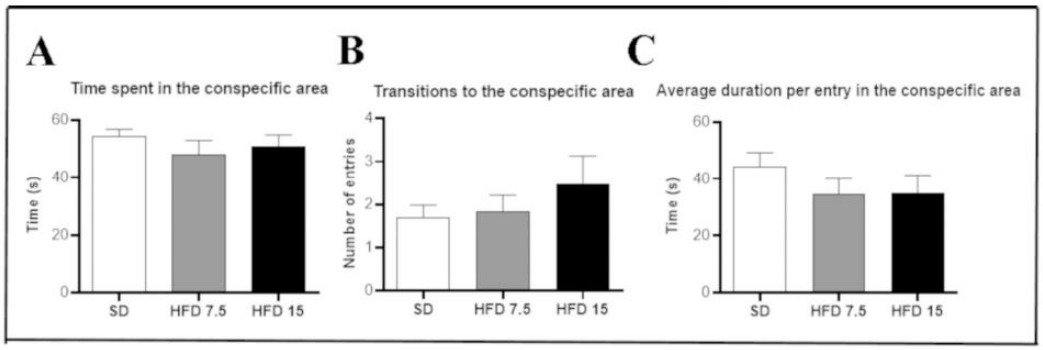

Fig. 4. HFD does not influence social preference in the social preference test (SPT). (A) time spent in the conspecific area, (B) transition to the conspecific area, and (C) average duration per entry in the conspecific area. Data are expressed as means ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA. n = 15–18 per group.

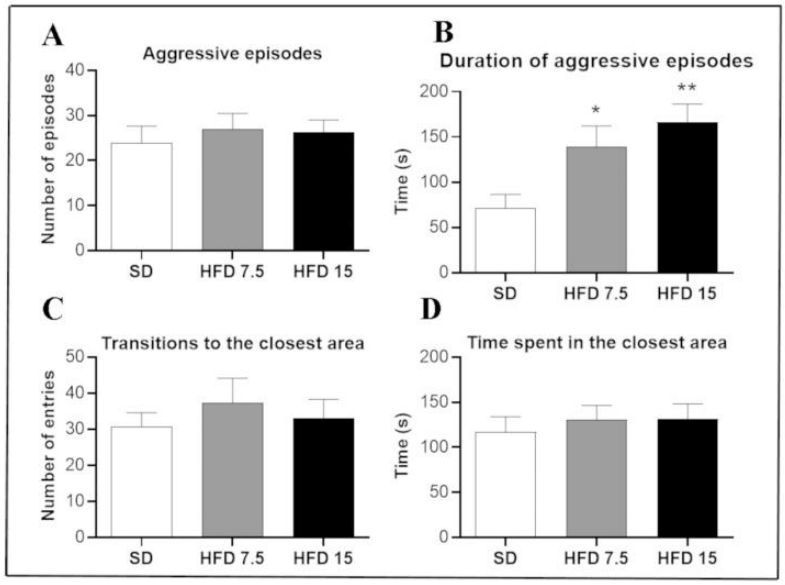

Fig. 5. HFD promotes aggressive behavior in zebrafish. Aggressive behavior was determined as (A) number of aggressive episodes, (B) duration of aggressive episodes, and (C—D) number of transitions and time spent in the area closer to the mirror on the mirror-induced aggression test (MIAT). Data are expressed as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test. * $p < 0.05$; ** $p < 0.01$. n = 15–18 per group.

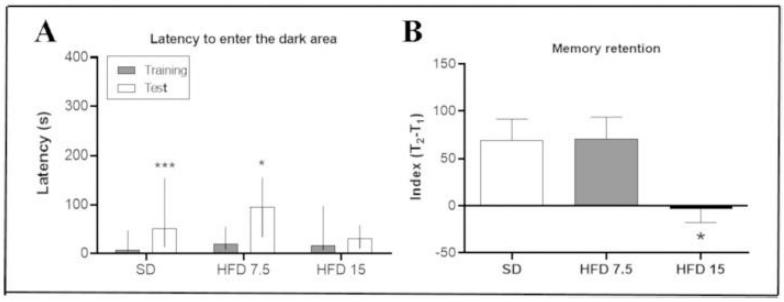

Fig. 6. HFD impairs memory formation in zebrafish. (A) Latency to enter the dark area in training and test sessions and (B) memory retention index evaluated by the inhibitory avoidance test (IAT). Latency to enter the dark area is expressed as median ± interquartile range and analyzed by Wilcoxon matched-pairs signed rank test. Memory retention index is expressed as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA followed by Newman-Keuls post-hoc test. n = 15–18 per group. * $p < 0.05$; *** $p < 0.001$.

about the behavior changes and CNS effects caused by these conditions. In this report, we assessed whether the HDF affect multiple behaviors of zebrafish after a short-term period, especially focusing on specific domains, such as anxiety, aggression, sociability, and memory. Our results showed that short-term HFD (2 weeks) induced an obesogenic effect and modulated a wide range of behaviors, supporting the use of zebrafish as a novel alternative model organism to assess the neurobehavioral effects of HFD, complementing the existing murine models. Since behaviors were measured using well-characterized tasks and parallel those observed in rodents and humans, these set of data support a high degree of face validity of the zebrafish model described.

Both HFD regimens used here increased BMI, the main indicator of obesity (Wang and Beydoun, 2007), as well as induced higher body weight and abdominal length, showing that obesity- and metabolic-related parameters in zebrafish were affected in a short-term period of HFD. We verified that HFD did not affect the locomotor capacity, as demonstrated by similar effects on total distance traveled, absolute turn angle, and maximum velocity in the NTT compared to control group. Importantly, the vertical exploration was also unaffected, as demonstrated by the number of entries in the top measured in the NTT. These set of data corroborate the applicability of various behavioral tests with obese zebrafish.

Mounting evidence shows the effects of fat-rich diet in obesity-related parameters and metabolic status (Landgraf et al., 2017; Meguro et al., 2015; Oka et al., 2010). In line with this, an HFD study using chicken egg yolk, increased blood glucose, cholesterol, and triglycerides levels, as well as visceral and subcutaneous fat content (associated with increased abdominal length) and BMI in zebrafish, while body weight increased only after 4, but not 2 weeks of such diet (Landgraf et al., 2017). Feeding zebrafish with corn oil or lard also affected metabolic health and obesity-related parameters (Meguro et al., 2015). Similarly, overfeeding zebrafish with *Artemia*, a live food rich in fat, leads to increased BMI, and triglyceride levels, leading to hepatic steatosis and higher lipid storage (Oka et al., 2010). Although the precise mechanisms underlying the effects of HFD on zebrafish behaviors still merit future scrutiny, our findings reinforce the susceptibility of zebrafish models to HFD, which can further serve as a valuable tool to explore both biochemical and morphological parameters associated with obesity.

Obesity and/or fat intake is associated with neurobehavioral changes, including cognitive decline, in humans (Freeman et al., 2014) and rodents (Karimi et al., 2013). Using the IAT, we verified that the HFD impaired memory acquisition in HFD-15, but not in HFD-7.5 group. HFD-induced cognitive impairment was recently described in zebrafish fed with lard for 8 weeks subjected to the active avoidance learning test (Meguro et al., 2019). Here, we showed a similar behavioral response in the passive avoidance learning, corroborating the deleterious influence of HFD on memory. Active or passive avoidance tests access different neurophysiological process of memory formation (Hauser et al., 2016; Krypotos et al., 2015) and are differentially affected by modulation of the cholinergic system (Dimitrova and Getova-Spassova, 2006). In mice, active avoidance learning is serotonin-dependent, while the passive avoidance learning is more dependent of dopaminergic activity (Allen et al., 1974). Although the mechanisms involved in such effects still require future studies in zebrafish models, HDF may impair memory formation in zebrafish by modulating different neurotransmitter systems. Notably, HFD-induced cognitive decline in mammals is usually associated with hippocampal vulnerability to insults arising from fat consumption (Beilharz et al., 2015). The effects of HFD on the CNS are associated with morphological changes (Valladolid-Acebes et al., 2013), impaired synaptic plasticity (Stranahan et al., 2008), insulin resistance (McNay et al., 2010), reduced expression of memory-related genes (*e.g.*, *sirt1* and *pp1*) (Heyward et al., 2012), and reduced neurogenesis (Grayson et al., 2014). Here, we demonstrated that HDF-induced memory acquisition impairment occurs within 2 weeks of supplementation. Despite the anatomical differences between teleost and mammals, zebrafish have evolutionarily conserved genome and physiology of neurotransmitter systems (Horzmann and Freeman, 2016; Howe et al., 2013), and the dorsal lateral pallium area is homologous to the mammalian hippocampus (Cheng et al., 2014; Salas et al., 2006). Interestingly, the memory impairment reported in zebrafish fed with a lard-based HFD was associated with the modulation of genes known as regulator of neuronal function, oxidative response, and blood-brain barrier integrity, supporting a conserved basis of HFD-induced pathogenesis (Meguro et al., 2019). Importantly, future studies aiming to elucidate how each brain area is affected by both short- and long-term HDF in zebrafish models of obesity are warranted in order to improve pharmacological and construct validity.

Obesity and overweight are also correlated with aggressive behavior and anxiety/depression in humans (Cerniglia et al., 2018; Lindberg et al., 2020). Although it is difficult to establish a causal relationship (*i.e.*, socioeconomic and self-esteem factors are involved), animal models allow a proper evaluation of the link between obesity/overweight and behavioral changes (Baker and Reichelt, 2016; Buchenauer et al., 2009). Here, the short-term HFD markedly changed the aggressive profile and anxiety-like responses in zebrafish, as represented by a higher duration of aggressive episodes in the MIAT and increased bottom dwelling in the NTT. Similar results were shown with rats and mice fed with HFD (Baker and Reichelt, 2016; Buchenauer et al., 2009; de Noronha et al., 2017) demonstrating a conserved biological response in zebrafish, reinforcing the translatability of zebrafish models for obesity research (Gut et al., 2017).

Obese individuals are more vulnerable to neurological (Cadenas-Sanchez et al., 2020) and psychiatric disorders (Hamer et al., 2012) when metabolic alterations are present. Here we observed an increase in abdominal length after the HFD intake, which is one of the diagnostic criteria of the metabolic syndrome, representing higher visceral adiposity (Bigaard et al., 2005). Accordingly, zebrafish fed with chicken egg yolk showed higher propensity to develop metabolic changes compared with animals overfed with standard diet (Landgraf et al., 2017), displaying increased levels of glucose, cholesterol, triglycerides, and visceral adiposity (Alberti et al., 2009). Triglycerides can cross the BBB promoting central leptin resistance (Banks et al., 2018), which in turn could promote anxiety-like behavior, through modulation of the neuropeptide Y levels on the brain of obese individuals (Karl et al., 2008; Widdowson et al., 1999). Leptin is highly conserved between fish and humans (Prokop et al., 2012), promoting anxiolytic-like effects in rodents (Wang et al., 2015), while prominent anxiety-like behavior is observed in zebrafish knockouts (Audira et al., 2018). Hypertriglyceridemia also plays a role on hippocampal impairment and cognitive decline, since lower triglycerides levels facilitate the recovery of memory functioning (Banks et al., 2018; Farr et al., 2008). Similarly, hyperglycemic zebrafish and rats showed anxiety-like behavior and the glycemic control was associated with an anxiolytic effect (dos Santos et al., 2018; Gambeta et al., 2016). Although further experiments are necessary to understand the role of metabolic dysfunction on CNS disorders in zebrafish, we verified that animals displayed cognitive decline, anxiety-like and aggressive behavior following a short-term HFD. These set of data corroborate the use of zebrafish models to investigate the molecular basis underling obesity and neuropsychiatry conditions in a medium-to-high throughput manner.

5. Conclusion

Our findings show that HFD modulates a wide range of behavioral domains in zebrafish, since heightened aggression, anxiety-like behaviors, and impaired memory formation were verified. Importantly, HFD did not change locomotion and vertical exploratory capacity, supporting the results observed and the use of obese zebrafish in various behavioral tasks. We hypothesize that metabolic alterations may play an important role on the behavioral changes observed here, corroborating data from other animal models and humans. Further investigation is necessary to

elucidate this hypothesis and the underlying mechanisms involved in the behavioral phenotypes observed following a short-term HFD. Collectively, our study fosters a cross-species analyses in a translational perspective and highlights the use of zebrafish as a versatile and promising tool for assessing the neurobehavioral effects of HFD-induced obesity with high degree of face validity.

Author contributions

1. Conceived and designed the experiments: VLP, CKG, JTG, CP, AFB, DBR.
2. Performed the experiments: VLP, VAQ, JC.
3. Analyzed the data: VLP, AFB, DBR.
4. Contributed reagents/materials/analysis tools DBR, CKG.
5. Wrote the paper: VLP, AFB, DBR.

Declaration of Competing Interest

The authors declare that no competing interests exists.

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References

Alberti, K.G.M.M., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P.T., Loria, C.M., Smith, S.C., 2009. Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; world heart federation, International. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.109.192644.

Allen, C., Allen, B.S., Rake, A.V., 1974. Pharmacological distinctions between "active" and "passive" avoidance memory formation as shown by manipulation of biogenic amine active compounds. Psychopharmacologia. https://doi.org/10.1007/BF00421215.

Anstey, K.J., Cherbuin, N., Budge, M., Young, J., 2011. Body mass index in midlife and late-life as a risk factor for dementia: a meta-analysis of prospective studies. Obes. Rev. https://doi.org/10.1111/j.1467-789X.2010.00825.x.

Attuquayefio, T., Stevenson, R.J., Oaten, M.J., Francis, H.M., 2017. A four-day Western-style dietary intervention causAttuquayefio, T., Stevenson, R. J., Oaten, M. J., & Francis, H. M. (2017). A four-day Western-style dietary intervention causes reductions in hippocampal-dependent learning and memory and interoceptive sen. PLoS One 12, e0172645. doi:https://doi.org/10.1371/journal.pone.0172645.

Audira, G., Sarasamma, S., Chen, J.R., Juniardi, S., Sampurna, B.P., Liang, S.T., Lai, Y.H., Lin, G.M., Hsieh, M.C., Der Hsiao, C., 2018. Zebrafish mutants carrying leptin a (Lepa) gene deficiency display obesity, anxiety, less aggression and fear, and circadian rhythm and color preference dysregulation. Int. J. Mol. Sci. 19 https://doi.org/10.3390/ijms19124038.

Baker, K.D., Reichelt, A.C., 2016. Impaired fear extinction retention and increased anxiety-like behaviours induced by limited daily access to a high-fat/high-sugar diet in male rats: implications for diet-induced prefrontal cortex dysregulation. Neurobiol. Learn. Mem. https://doi.org/10.1016/j.nlm.2016.10.002.

Banks, W.A., Farr, S.A., Salameh, T.S., Niehoff, M.L., Rhea, E.M., Morley, J.E., Hanson, A. J., Hansen, K.M., Craft, S., 2018. Triglycerides cross the blood-brain barrier and induce central leptin and insulin receptor resistance. Int. J. Obes. https://doi.org/10.1038/ijo.2017.231.

Beilharz, J.E., Maniam, J., Morris, M.J., 2014. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. Brain Behav. Immun. 37, 134–141. https://doi.org/10.1016/j.bbi.2013.11.016.

Beilharz, J.E., Maniam, J., Morris, M.J., 2015. Diet-induced cognitive deficits: the role of fat and sugar, potential mechanisms and nutritional interventions. Nutrients. https://doi.org/10.3390/nu7085307.

Bencan, Z., Sledge, D., Levin, E.D., 2009. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol. Biochem. Behav. https://doi.org/10.1016/j.pbb.2009.07.009.

Bertoncello, K.T., Müller, T.E., Fontana, B.D., Franscescon, F., Filho, G.L.B., Rosemberg, D.B., 2019. Taurine prevents memory consolidation deficits in a novel alcohol-induced blackout model in zebrafish. Prog. Neuro-Psychopharmacology Biol. Psychiatry 93, 39–45. https://doi.org/10.1016/j.pnpbp.2019.03.006.

Bigaard, J., Frederiksen, K., Tjønneland, A., Thomsen, B.L., Overvad, K., Heitmann, B.L., Sørensen, T., 2005. Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. Int. J. Obes. https://doi.org/10.1038/sj.ijo.0802976.

Blank, M., Guerim, L.D., Cordeiro, R.F., Vianna, M.R.M., 2009. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol. Learn. Mem. 92, 529–534. https://doi.org/10.1016/j.nlm.2009.07.001.

Blüher, M., 2020. Metabolically healthy obesity. Endocr. Rev. https://doi.org/10.1210/endrev/bnaa004.

Buchenauer, T., Behrendt, P., Bode, F.J., Horn, R., Brabant, G., Stephan, M., Nave, H., 2009. Diet-induced obesity alters behavior as well as serum levels of corticosterone in F344 rats. Physiol. Behav. 98, 563–569. https://doi.org/10.1016/j.physbeh.2009.09.003.

Cadenas-Sanchez, C., Esteban-Cornejo, I., Migueles, J.H., Labayen, I., Verdejo-Román, J., Mora-Gonzalez, J., Henriksson, P., Maldonado, J., Gómez-Vida, J., Hillman, C.H., Erickson, K.I., Kramer, A.F., Catena, A., Ortega, F.B., 2020. Differences in brain volume between metabolically healthy and unhealthy overweight and obese children: the role of fitness. J. Clin. Med. https://doi.org/10.3390/jcm9041059.

Cai, D., 2013. Neuroinflammation and neurodegeneration in overnutrition-induced diseases. Trends Endocrinol. Metab. https://doi.org/10.1016/j.tem.2012.11.003.

Cerniglia, L., Cimino, S., Erriu, M., Jezek, S., Almenara, C.A., Tambelli, R., 2018. Trajectories of aggressive and depressive symptoms in male and female overweight children: do they share a common path or do they follow different routes? PLoS One 13, e0190731. https://doi.org/10.1371/journal.pone.0190731.

Cheng, R.K., Jesuthasan, S.J., Penney, T.B., 2014. Zebrafish forebrain and temporal conditioning. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2012.0462.

Cordain, L., Eaton, S.B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B.A., O'Keefe, J. H., Brand-Miller, J., 2005. Origins and evolution of the Western diet: health implications for the 21st century. Am. J. Clin. Nutr. https://doi.org/10.1093/ajcn.81.2.341.

de Noronha, S.R., Campos, G.V., Abreu, A.R., de Souza, A.A., Chianca, D.A., de Menezes, R.C., 2017. High fat diet induced-obesity facilitates anxiety-like behaviors due to GABAergic impairment within the dorsomedial hypothalamus in rats. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2016.08.042.

Dimitrova, D.S., Getova-Spassova, D.P., 2006. Effects of galantamine and donepezil on active and passive avoidance tests in rats with induced hypoxia. J. Pharmacol. Sci. https://doi.org/10.1254/jphs.FPE05006X.

dos Santos, M.M., de Macedo, G.T., Prestes, A.S., Loro, V.L., Heidrich, G.M., Picoloto, R. S., Rosemberg, D.B., Barbosa, N.V., 2018. Hyperglycemia elicits anxiety-like behaviors in zebrafish: protective role of dietary diphenyl diselenide. Prog. Neuro-Psychopharmacology Biol. Psychiatry 85, 128–135. https://doi.org/10.1016/j.pnpbp.2018.04.017.

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205, 38–44. https://doi.org/10.1016/j.bbr.2009.06.022.

Farr, S.A., Yamada, K.A., Butterfield, D.A., Abdul, H.M., Xu, L., Miller, N.E., Banks, W.A., Morley, J.E., 2008. Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology. https://doi.org/10.1210/en.2007-1722.

Flegal, K.M., Carroll, M.D., Ogden, C.L., Curtin, L.R., 2010. Prevalence and trends in obesity among US adults, 1999-2008. JAMA- J. Am. Med. Assoc. https://doi.org/10.1001/jama.2009.2014.

Fontana, B.D., Stefanello, F.V., Mezzomo, N.J., Müller, T.E., Quadros, V.A., Parker, M.O., Rico, E.P., Rosemberg, D.B., 2018. Taurine modulates acute ethanol-induced social behavioral deficits and fear responses in adult zebrafish. J. Psychiatr. Res. 104, 176–182. https://doi.org/10.1016/j.jpsychires.2018.08.008.

Fontana, B.D., Cleal, M., Clay, J.M., Parker, M.O., 2019. Zebrafish (Danio rerio) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses. Anim. Cogn. 22, 1051–1061. https://doi.org/10.1007/s10071-019-01296-9.

Francis, H.M., Stevenson, R.J., 2011. Higher reported saturated fat and refined sugar intake is associated with reduced hippocampal-dependent memory and sensitivity to interoceptive signals. Behav. Neurosci. https://doi.org/10.1037/a0025998.

Franscescon, F., Müller, T.E., Bertoncello, K.T., Rosemberg, D.B., 2020. Neuroprotective role of taurine on MK-801-induced memory impairment and hyperlocomotion in zebrafish. Neurochem. Int. 104710 https://doi.org/10.1016/j.neuint.2020.104710.

Freeman, L.R., Granholm, A.C.E., 2012. Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. J. Cereb. Blood Flow Metab. https://doi.org/10.1038/jcbfm.2011.168.

Freeman, L.R., Haley-Zitlin, V., Rosenberger, D.S., Granholm, A.-C., 2014. Damaging effects of a high-fat diet to the brain and cognition: a review of proposed mechanisms NIH public access. Nutr. Neurosci. 17, 241–251. https://doi.org/10.1179/1476830513Y.0000000092.

Gambeta, E., de Souza, C.P., de Morais, H., Zanoveli, J.M., 2016. Reestablishment of the hyperglycemia to the normal levels seems not to be essential to the anxiolytic-like effect induced by insulin. Metab. Brain Dis. https://doi.org/10.1007/s11011-015-9770-1.

Gerlai, R., Lahav, M., Guo, S., Rosenthal, A., 2000. Drinks like a fish: Zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacol. Biochem. Behav. 67, 773–782. https://doi.org/10.1016/S0091-3057(00)00422-6.

Grayson, B.E., Fitzgerald, M.F., Hakala-Finch, A.P., Ferris, V.M., Begg, D.P., Tong, J., Woods, S.C., Seeley, R.J., Davidson, T.L., Benoit, S.C., 2014. Improvements in hippocampal-dependent memory and microglial infiltration with calorie restriction and gastric bypass surgery, but not with vertical sleeve gastrectomy. Int. J. Obes. https://doi.org/10.1038/ijo.2013.100.

Greenwood, C.E., Winocur, G., 1990. Learning and memory impairment in rats fed a high saturated fat diet. Behav. Neural Biol. https://doi.org/10.1016/0163-1047(90)90831-P.

Gut, P., Reischauer, S., Stainier, D.Y.R., Arnaout, R., 2017. Little fish, big data: zebrafish as a model for cardiovascular and metabolic disease. Physiol. Rev. 97, 889–938. https://doi.org/10.1152/physrev.00038.2016.

Gutiérrez, H.C., Vacca, I., Schoenmacker, G., Cleal, M., Tochwin, A., O'Connor, B., Young, A.M.J., Vasquez, A.A., Winter, M.J., Parker, M.O., Norton, W.H.J., 2020. Screening for drugs to reduce zebrafish aggression identifies caffeine and sildenafil. Eur. Neuropsychopharmacol. 30, 17–29. https://doi.org/10.1016/j.euroneuro.2019.10.005.

Hamer, M., Batty, G.D., Kivimaki, M., 2012. Risk of future depression in people who are obese but metabolically healthy: the English longitudinal study of ageing. Mol. Psychiatry. https://doi.org/10.1038/mp.2012.30.

Hauser, T.U., Eldar, E., Dolan, R.J., 2016. Neural mechanisms of harm-avoidance learning a model for obsessive-compulsive disorder? JAMA Psychiatry. https://doi.org/10.1001/jamapsychiatry.2016.1938.

Heyward, F.D., Walton, R.G., Carle, M.S., Coleman, M.A., Garvey, W.T., Sweatt, J.D., 2012. Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. Neurobiol. Learn. Mem. https://doi.org/10.1016/j.nlm.2012.04.005.

Horzmann, K.A., Freeman, J.L., 2016. Zebrafish get connected: investigating neurotransmission targets and alterations in chemical toxicity. Toxics. https://doi.org/10.3390/toxics4030019.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assunção, J.A., Zhou, Y., Gu, Y., Yen, J., Vogel, J.H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird, G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Eliott, D., Threadgold, G., Harden, G., Ware, D., Mortimer, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C. M., Ersan-Ürün, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberländer, M., Rudolph-Geiger, S., Teucke, M., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M.J., Enright, A., Geisler, R., Plasterk, R.H.A., Lee, C., Westerfield, M., De Jong, P.J., Zon, L.I., Postlethwait, J.H., Nüsslein-Volhard, C., Hubbard, T.J.P., Crollius, H.R., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496, 498–503. https://doi.org/10.1038/nature12111.

Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., Gilman, C.P., Pittman, J., Rosemberg, D.B., Gerlai, R., Echevarria, D., Lamb, E., Neuhauss, S.C.F., Weng, W., Bally-Cuif, L., Schneider, H., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish. https://doi.org/10.1089/zeb.2012.0861.

Karimi, S.A., Salehi, I., Komaki, A., Sarihi, A., Zarei, M., Shahidi, S., 2013. Effect of high-fat diet and antioxidants on hippocampal long-term potentiation in rats: an in vivo study. Brain Res. https://doi.org/10.1016/j.brainres.2013.09.029.

Karl, T., Duffy, L., Herzog, H., 2008. Behavioural profile of a new mouse model for NPY deficiency. Eur. J. Neurosci. https://doi.org/10.1111/j.1460-9568.2008.06306.x.

Krypotos, A.M., Effting, M., Kindt, M., Beckers, T., 2015. Avoidance learning: a review of theoretical models and recent developments. Front. Behav. Neurosci. https://doi.org/10.3389/fnbeh.2015.00189.

Landgraf, K., Schuster, S., Meusel, A., Garten, A., Riemer, T., Schleinitz, D., Kiess, W., Körner, A., 2017. Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development o1. Landgraf, K. et al. short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealt. BMC Physiol. 17, 4. https://doi.org/10.1186/s12899-017-0031-x.

Lindberg, L., Hagman, E., Danielsson, P., Marcus, C., Persson, M., 2020. Anxiety and depression in children and adolescents with obesity: a nationwide study in Sweden. BMC Med. 18 https://doi.org/10.1186/s12916-020-1498-z.

Malnick, S.D.H., Knobler, H., 2006. The medical complications of obesity. QJM. https://doi.org/10.1093/qjmed/hcl085.

Maximino, C., Meinerz, D.L., Fontana, B.D., Mezzomo, N.J., Stefanello, F.V., Prestes, De S., Batista, C.B., Rubin, M.A., Barbosa, N.V., Rocha, J.B.T., Lima, M.G., Rosemberg, D.B., 2018. Extending the analysis of zebrafish behavioral endophenotypes for modeling psychiatric disorders: fear conditioning to conspecific alarm response. Behav. Process. 149, 35–42. https://doi.org/10.1016/j.beproc.2018.01.020.

Mazon, J.N., de Mello, A.H., Ferreira, G.K., Rezin, G.T., 2017. The impact of obesity on neurodegenerative diseases. Life Sci. https://doi.org/10.1016/j.lfs.2017.06.002.

McNay, E.C., Ong, C.T., McCrimmon, R.J., Cresswell, J., Bogan, J.S., Sherwin, R.S., 2010. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiol. Learn. Mem. https://doi.org/10.1016/j.nlm.2010.02.002.

Meguro, S., Hasumura, T., Hase, T., 2015. Body fat accumulation in zebrafish is induced by a diet rich in fat and reduced by supplementation with green tea extract. PLoS One 10, e0120142. https://doi.org/10.1371/journal.pone.0120142.

Meguro, S., Hosoi, S., Hasumura, T., 2019. High-fat diet impairs cognitive function of zebrafish. Sci. Rep. 9 https://doi.org/10.1038/s41598-019-53634-z.

Müller, T.E., Fontana, B.D., Bertoncello, K.T., Franscescon, F., Mezzomo, N.J., Canzian, J., Stefanello, F.V., Parker, M.O., Gerlai, R., Rosemberg, D.B., 2020. Understanding the neurobiological effects of drug abuse: lessons from zebrafish models. Prog. Neuro-Psychopharmacology Biol. Psychiatry. https://doi.org/10.1016/j.pnpbp.2020.109873.

Norton, W., Bally-Cuif, L., 2010. Adult zebrafish as a model organism for behavioural genetics. BMC Neurosci. https://doi.org/10.1186/1471-2202-11-90.

O'Brien, P.D., Hinder, L.M., Callaghan, B.C., Feldman, E.L., 2017. Neurological consequences of obesity. Lancet Neurol. https://doi.org/10.1016/S1474-4422(17)30084-4.

Oka, T., Nishimura, Y., Zang, L., Hirano, M., Shimada, Y., Wang, Z., Umemoto, N., Kuroyanagi, J., Nishimura, N., Tanaka, T., 2010. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. BMC Physiol. 10, 21. https://doi.org/10.1186/1472-6793-10-21.

Pannevis, M.C., Earle, K.E., 1994. Maintenance energy requirement of five popular species of ornamental fish. J. Nutr. 124, 2616S–2618S. https://doi.org/10.1093/jn/124.suppl_12.2616s.

Parichy, D.M., Elizondo, M.R., Mills, M.G., Gordon, T.N., Engeszer, R.E., 2009. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. Dev. Dyn. 238, 2975–3015. https://doi.org/10.1002/dvdy.22113.

Pedditizi, E., Peters, R., Beckett, N., 2016. The risk of overweight/obesity in mid-life and late life for the development of dementia: a systematic review and meta-analysis of longitudinal studies. Age Ageing. https://doi.org/10.1093/ageing/afv151.

Procaccini, C., Santopaolo, M., Faicchia, D., Colamatteo, A., Formisano, L., De Candia, P., Galgani, M., De Rosa, V., Matarese, G., 2016. Role of metabolism in neurodegenerative disorders. Metabolism. https://doi.org/10.1016/j.metabol.2016.05.018.

Prokop, J.W., Duff, R.J., Ball, H.C., Copeland, D.L., Londraville, R.L., 2012. Leptin and leptin receptor: analysis of a structure to function relationship in interaction and evolution from humans to fish. Peptides. https://doi.org/10.1016/j.peptides.2012.10.002.

Salas, C., Broglio, C., Durán, E., Gómez, A., Ocaña, F.M., Jiménez-Moya, F., Rodríguez, F., 2006. Neuropsychology of learning and memory in teleost fish. Zebrafish. https://doi.org/10.1089/zeb.2006.3.157.

Saverino, C., Gerlai, R., 2008. The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish. Behav. Brain Res. 191, 77–87. https://doi.org/10.1016/j.bbr.2008.03.013.

Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (Danio rerio). Behav. Brain Res. https://doi.org/10.1016/j.bbr.2007.10.031.

Stranahan, A.M., Norman, E.D., Lee, K., Cutler, R.G., Telljohann, R.S., Egan, J.M., Mattson, M.P., 2008. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus. https://doi.org/10.1002/hipo.20470.

Tamashiro, K.L.K., 2015. Developmental and environmental influences on physiology and behavior - 2014 Alan N, Epstein Research Award. Physiol. Behav. https://doi.org/10.1016/j.physbeh.2015.08.019.

Tan, B.L., Norhaizan, M.E., 2019. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients. https://doi.org/10.3390/nu11112579.

Valladolid-Acebes, I., Fole, A., Martín, M., Morales, L., Victoria Cano, M., Ruiz-Gayo, M., Del Olmo, N., 2013. Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol. Learn. Mem. https://doi.org/10.1016/j.nlm.2013.06.012.

Vandevijvere, S., Chow, C.C., Hall, K.D., Umali, E., Swinburn, B.A., 2015. Increased food energy supply as a major driver of the obesity epidemic: a global analysis. Bull. World Health Organ. https://doi.org/10.2471/blt.14.150565.

Wang, Y., Beydoun, M.A., 2007. The obesity epidemic in the United States - gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. Epidemiol. Rev. https://doi.org/10.1093/epirev/mxm007.

Wang, W., Liu, S.L., Li, K., Chen, Y., Jiang, B., Li, Y.K., Xiao, J.L., Yang, S., Chen, T., Chen, J.G., Li, J.G., Wang, F., 2015. Leptin: a potential anxiolytic by facilitation of fear extinction. CNS Neurosci. Ther. https://doi.org/10.1111/cns.12375.

5. DISCUSSION

Although zebrafish is a relevant animal model of obesity and metabolic disarrangement (GUT *et al.*, 2017; ZANG; MADDISON; CHEN, 2018), little is known about the behavior changes and CNS effects caused by these conditions. In this study, we assessed whether the high-fat diet (HFD) affect multiple behaviors of zebrafish after a short-term period, especially focusing on specific domains, such as anxiety, aggression, sociability, and memory to perform a face validation of zebrafish model. Our results showed that short-term HFD (2 weeks) induced an obesogenic effect and modulated a wide range of behaviors, supporting the use of zebrafish as a novel alternative model organism to assess the neurobehavioral effects of HFD, complementing the existing murine models. Since behaviors were measured using well-characterized tasks and resemble those observed in rodents and humans, these set of data support a high degree of face validity of the zebrafish model described.

Both HFD regimens used here increased BMI, the main indicator of obesity (WANG, Y.; BEYDOUN, 2007), as well as induced higher body weight and abdominal length, showing that obesity-related and metabolic parameters in zebrafish were affected in a short-term period of HFD. We verified that HFD did not affect the locomotor capacity, as demonstrated by similar effects on total distance traveled, absolute turn angle, and maximum velocity in the NTT compared to control group. Importantly, the vertical exploration was also unaffected, as demonstrated by the number of entries in the top measured in the NTT. These set of data corroborate the applicability of various behavioral tests with obese zebrafish.

Mounting evidence shows the effects of fat-rich diet in obesity-related parameters and metabolic status (LANDGRAF *et al.*, 2017; MEGURO; HASUMURA; HASE, 2015; OKA *et al.*, 2010). In line with this, a HFD study using chicken egg yolk, increased blood glucose, cholesterol, and triglycerides levels, as well as visceral and subcutaneous fat content (associated with increased abdominal length) and BMI in zebrafish, while body weight increased only after 4, but not 2 weeks of such diet (LANDGRAF *et al.*, 2017). Feeding zebrafish with corn oil or lard also affected metabolic health and obesity-related parameters (MEGURO; HASUMURA; HASE, 2015). Similarly, overfeeding zebrafish with Artemia, a live food rich in fat, leads to increased BMI, and triglyceride levels, leading to hepatic steatosis and higher lipid storage (OKA *et al.*, 2010). Although the precise mechanisms underlying the effects of HFD on zebrafish behaviors still merit future scrutiny, our findings reinforce the susceptibility of

zebrafish models to HFD, which can further serve as a valuable tool to explore both biochemical and morphological parameters associated with obesity.

Obesity and/or fat intake is associated with neurobehavioral changes, including cognitive decline, in humans (FREEMAN *et al.*, 2014) and rodents (KARIMI *et al.*, 2013). Using the IAT, we verified that the HFD impaired memory acquisition in HFD-15, but not in HFD-7.5 group. HFD-induced cognitive impairment was recently described in zebrafish fed with lard for 8 weeks subjected to the active avoidance learning test (MEGURO; HOSOI; HASUMURA, 2019). Here, we showed a similar behavioral response in the passive avoidance learning, corroborating the deleterious influence of HFD on memory. Active or passive avoidance tests access different neurophysiological process of memory formation (HAUSER; ELDAR; DOLAN, 2016; KRYPOTOS *et al.*, 2015) and are differentially affected by modulation of the cholinergic system (DIMITROVA; GETOVA-SPASSOVA, 2006). In mice, active avoidance learning is serotonin-dependent, while the passive avoidance learning is more dependent of dopaminergic activity (ALLEN; ALLEN; RAKE, 1974). Although the mechanisms involved in such effects still require future studies in zebrafish models, HDF may impair memory formation in zebrafish by modulating different neurotransmitter systems. Notably, HFD-induced cognitive decline in mammals is usually associated with hippocampal vulnerability to insults arising from fat consumption (BEILHARZ; MANIAM; MORRIS, 2015). The effects of HFD on the CNS are associated with morphological changes (VALLADOLID-ACEBES *et al.*, 2013), impaired synaptic plasticity (STRANAHAN *et al.*, 2008), insulin resistance (MCNAY *et al.*, 2010), reduced expression of memory-related genes (e.g., sirt1 and pp1) (HEYWARD *et al.*, 2012), and reduced neurogenesis (GRAYSON *et al.*, 2014). Similarly, zebrafish overfeed with artemia for 5 weeks display altered expression of leptin, ghrelin and orexin on the brain (MONTALBANO *et al.*, 2018). Here, we demonstrated that HFD-induced memory acquisition impairment occurs within 2 weeks of supplementation. Despite the anatomical differences between teleost and mammals, zebrafish have evolutionarily conserved genome and physiology of neurotransmitter systems (HORZMANN; FREEMAN, 2016; HOWE *et al.*, 2013), and the lateral pallium area is homologous to the mammalian hippocampus (CHENG; JESUTHASAN; PENNEY, 2014; SALAS *et al.*, 2006). Interestingly, the memory impairment reported in zebrafish fed with a lard-based HFD was associated with the modulation of genes known as regulator of neuronal function, oxidative response, and blood-brain barrier integrity, supporting a conserved basis of HFD-induced pathogenesis (MEGURO; HOSOI; HASUMURA, 2019). Importantly, future studies aiming to elucidate how each brain area is affected by both short-

and long-term HFD in zebrafish models of obesity are warranted in order to improve pharmacological and construct validity.

Obesity and overweight are also correlated with aggressive behavior and anxiety/depression in humans (CERNIGLIA *et al.*, 2018; LINDBERG *et al.*, 2020). Although it is difficult to establish a causal relationship (i.e., socioeconomic and self-esteem factors are involved), animal models allow a proper evaluation of the link between obesity/overweight and behavioral changes (BAKER; REICHELT, 2016; BUCHENAUER *et al.*, 2009). Here, the short-term HFD markedly changed the aggressive profile and anxiety-like responses in zebrafish, as represented by a higher duration of aggressive episodes in the MIAT and increased bottom dwelling in the NTT. Similar results were shown with rats and mice fed with HFD (BAKER; REICHELT, 2016; BUCHENAUER *et al.*, 2009; DE NORONHA *et al.*, 2017) demonstrating a conserved biological response in zebrafish, reinforcing the translatability of zebrafish models for obesity research (GUT et al., 2017).

Obese individuals are more vulnerable to neurological (CADENAS-SANCHEZ *et al.*, 2020) and psychiatric disorders (HAMER; BATTY; KIVIMAKI, 2012) when metabolic alterations are present. Similarly, zebrafish models of metabolic disarrangements also display neurobehavioral alteration (AUDIRA *et al.*, 2018; DOS SANTOS *et al.*, 2018). Here we observed an increase in abdominal length after the HFD intake, which is one of the diagnostic criteria of the metabolic syndrome, representing higher visceral adiposity (BIGAARD *et al.*, 2005). Accordingly, zebrafish fed with chicken egg yolk showed higher propensity to develop metabolic changes compared with animals overfed with an isocaloric standard diet (LANDGRAF *et al.*, 2017), displaying increased levels of glucose, cholesterol, triglycerides, and visceral adiposity (ALBERTI *et al.*, 2009). Triglycerides can cross the BBB promoting central leptin resistance (BANKS *et al.*, 2018), which in turn could promote anxiety-like behavior, through modulation of the neuropeptide Y levels on the brain of obese individuals (KARL; DUFFY; HERZOG, 2008; WIDDOWSON *et al.*, 1999). Leptin is highly conserved between fish and humans (PROKOP *et al.*, 2012), promoting anxiolytic-like effects in rodents (WANG, W. *et al.*, 2015), while prominent anxiety-like behavior is observed in zebrafish knockouts (AUDIRA *et al.*, 2018). Hypertriglyceridemia also plays a role on hippocampal impairment and cognitive decline, since lower triglycerides levels facilitate the recovery of memory functioning (BANKS *et al.*, 2018; FARR *et al.*, 2008). Similarly, hyperglycemic zebrafish and rats showed anxiety-like behavior and the glycemic control was associated with an anxiolytic effect (DOS SANTOS *et al.*, 2018; GAMBETA *et al.*, 2016). Although further

experiments are necessary to understand the role of metabolic dysfunction on CNS disorders in zebrafish, we verified that animals displayed cognitive decline, anxiety-like and aggressive behavior following a short-term HFD. These set of data corroborate the use of zebrafish models to investigate the molecular basis underling obesity and neuropsychiatry conditions in a medium-to-high throughput manner.

6. CONCLUSION

Our results demonstrated the following effects of HFD on obesity-related and metabolic parameters in adult zebrafish:

- Increase of BW,
- Increase of BMI and
- Increase of abdominal length.

Plus, behavioral analysis showed effect of HFD on the following parameters:

- Heightened aggressiveness as evaluated by the MIAT,
- Heightened anxiety-like behavior as evaluated by the NNT,
- Impairment of memory formation induced by the highest concentration of chicken egg yolk as evaluated by the IAT,
- The locomotion and vertical exploratory capacity were not affected in obese zebrafish, supporting the results of behavioral tasks used here.

Taken together, our findings show that HFD modulates metabolic parameters and a wide range of behavioral domains in zebrafish, since heightened aggression, anxiety-like behaviors, and impaired memory formation were verified. We hypothesize that metabolic alterations may play an important role on the behavioral changes observed here, corroborating data from other animal models and humans. Further investigation is necessary to elucidate this hypothesis and the underlying mechanisms involved in the behavioral phenotypes observed following a short-term HFD. Collectively, our study fosters a cross-species analyses in a translational perspective and highlights the use of zebrafish as a versatile and promising tool for assessing the neurobehavioral effects of HFD-induced obesity with high degree of face validity.

7. BIBLIOGHAPIC REFERENCES

AFSHIN, Ashkan *et al.* Health effects of overweight and obesity in 195 countries over 25 years. **New England Journal of Medicine**, [*s. l.*], 2017. Disponível em: https://doi.org/10.1056/NEJMoa1614362

ALBERTI, K. G.M.M. *et al.* **Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International** . [*S. l.: s. n.*], 2009. Disponível em: https://doi.org/10.1161/CIRCULATIONAHA.109.192644

ALLEN, C.; ALLEN, Blair S.; RAKE, A. V. Pharmacological distinctions between "active" and "passive" avoidance memory formation as shown by manipulation of biogenic amine active compounds. **Psychopharmacologia**, [*s. l.*], 1974. Disponível em: https://doi.org/10.1007/BF00421215

ALSOP, Derek; VIJAYAN, Mathilakath. The zebrafish stress axis: Molecular fallout from the teleost-specific genome duplication event. **General and Comparative Endocrinology**, [*s. l.*], v. 161, n. 1, p. 62–66, 2009. Disponível em: https://doi.org/10.1016/j.ygcen.2008.09.011

ANICHTCHIK, Oleg V. *et al*. Neurochemical and behavioural changes in zebrafish Danio rerio after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. **Journal of Neurochemistry**, [*s. l.*], v. 88, n. 2, p. 443–53, 2004. Disponível em: https://doi.org/10.1111/j.1471-4159.2004.02190.x

ANSTEY, K. J. *et al.* Body mass index in midlife and late-life as a risk factor for dementia: A meta-analysis of prospective studies. **Obesity Reviews**, [*s. l.*], 2011. Disponível em: https://doi.org/10.1111/j.1467-789X.2010.00825.x

ATTUQUAYEFIO, Tuki *et al.* A four-day Western-style dietary intervention causes reductions in hippocampal-dependent learning and memory and interoceptive sensitivity. **PLoS ONE**, [*s. l.*], v. 12, n. 2, 2017. Disponível em: https://doi.org/10.1371/journal.pone.0172645. Acesso em: 7 jul. 2020.

AUDIRA, Gilbert *et al*. Zebrafish mutants carrying leptin a (Lepa) gene deficiency display

obesity, anxiety, less aggression and fear, and circadian rhythm and color preference dysregulation. **International Journal of Molecular Sciences**, [*s. l.*], v. 19, n. 12, 2018. Disponível em: https://doi.org/10.3390/ijms19124038

BAKER, Kathryn D.; REICHELT, Amy C. Impaired fear extinction retention and increased anxiety-like behaviours induced by limited daily access to a high-fat/high-sugar diet in male rats: Implications for diet-induced prefrontal cortex dysregulation. **Neurobiology of Learning and Memory**, [*s. l.*], 2016. Disponível em: https://doi.org/10.1016/j.nlm.2016.10.002

BANKS, W. A. *et al.* Triglycerides cross the blood-brain barrier and induce central leptin and insulin receptor resistance. **International Journal of Obesity**, [*s. l.*], 2018. Disponível em: https://doi.org/10.1038/ijo.2017.231

BANTING, F. G. *et al.* Pancreatic extracts in the treatment of diabetes mellitus. **The Indian journal of medical research**, [*s. l.*], v. 12, p. 141–146, 1922. Disponível em: https://doi.org/10.2337/diab.5.1.69

BARABAN, S. C. *et al.* Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. **Neuroscience**, [*s. l.*], v. 131, n. 3, p. 759–68, 2005. Disponível em: https://doi.org/10.1016/j.neuroscience.2004.11.031

BEILHARZ, Jessica E.; MANIAM, Jayanthi; MORRIS, Margaret J. **Diet-induced cognitive deficits: The role of fat and sugar, potential mechanisms and nutritional interventions**. [*S. l.: s. n.*], 2015. Disponível em: https://doi.org/10.3390/nu7085307

BEILHARZ, Jessica E.; MANIAM, Jayanthi; MORRIS, Margaret J. Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats. **Brain, Behavior, and Immunity**, [*s. l.*], v. 37, p. 134–141, 2014. Disponível em: https://doi.org/10.1016/j.bbi.2013.11.016. Acesso em: 7 jul. 2020.

BENCAN, Zachary; SLEDGE, Damiyon; LEVIN, Edward D. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. **Pharmacology Biochemistry and Behavior**, [*s. l.*], v. 94, n. 1, p. 75–80, 2009. Disponível em: https://doi.org/10.1016/j.pbb.2009.07.009

BERRINGTON DE GONZALEZ, Amy *et al.* Body-Mass Index and Mortality among 1.46 Million White Adults. **New England Journal of Medicine**, [*s. l.*], v. 363, p. 2211–2219, 2010. Disponível em: https://doi.org/10.1056/nejmoa1000367

BERTONCELLO, Kanandra T. *et al.* Taurine prevents memory consolidation deficits in a novel alcohol-induced blackout model in zebrafish. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [*s. l.*], v. 93, n. November 2018, p. 39–45, 2019. Disponível em: https://doi.org/10.1016/j.pnpbp.2019.03.006

BIGAARD, J. *et al.* Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. **International Journal of Obesity**, [*s. l.*], 2005. Disponível em: https://doi.org/10.1038/sj.ijo.0802976

BLANK, Martina *et al.* A one-trial inhibitory avoidance task to zebrafish: Rapid acquisition of an NMDA-dependent long-term memory. **Neurobiology of Learning and Memory**, [*s. l.*], v. 92, n. 4, p. 529–534, 2009. Disponível em: https://doi.org/10.1016/j.nlm.2009.07.001

BLÜHER, Matthias. Metabolically Healthy Obesity. **Endocrine reviews**, [*s. l.*], 2020. Disponível em: https://doi.org/10.1210/endrev/bnaa004

BOITARD, Chloé *et al.* Juvenile obesity enhances emotional memory and amygdala plasticity through glucocorticoids. **Journal of Neuroscience**, [*s. l.*], v. 35, p. 4092–4103, 2015. Disponível em: https://doi.org/10.1523/JNEUROSCI.3122-14.2015

BRADFORD, Yvonne M. *et al.* Zebrafish models of human disease: Gaining insight into human disease at ZFIN. **ILAR Journal**, [*s. l.*], v. 58, n. 1, p. 4–16, 2017. Disponível em: https://doi.org/10.1093/ilar/ilw040. Acesso em: 22 dez. 2020.

BRAFORD, M. R. Comparative aspects of forebrain organization in the ray-finned fishes: touchstones or not? **Brain, behavior and evolution**, [*s. l.*], v. 46, p. 259–274, 1995. Disponível em: https://doi.org/10.1159/000113278

BRUSTEIN, Edna *et al.* Steps during the development of the zebrafish locomotor network. **Journal of Physiology Paris**, [*s. l.*], v. 97, n. 1, p. 77–86, 2003. Disponível em: https://doi.org/10.1016/j.jphysparis.2003.10.009

BUCHENAUER, T. *et al.* Diet-induced obesity alters behavior as well as serum levels of corticosterone in F344 rats. **Physiology and Behavior**, [*s. l.*], v. 98, n. 5, p. 563–569, 2009. Disponível em: https://doi.org/10.1016/j.physbeh.2009.09.003

CADENAS-SANCHEZ, Cristina *et al.* Differences in Brain Volume between Metabolically Healthy and Unhealthy Overweight and Obese Children: The Role of Fitness. **Journal of Clinical Medicine**, [*s. l.*], 2020. Disponível em: https://doi.org/10.3390/jcm9041059

CAI, Dongsheng. **Neuroinflammation and neurodegeneration in overnutrition-induced diseases**. [*S. l.: s. n.*], 2013. Disponível em: https://doi.org/10.1016/j.tem.2012.11.003

CAI, Huan *et al.* Metabolic Dysfunction in Alzheimers Disease and Related Neurodegenerative Disorders. **Current Alzheimer Research**, [*s. l.*], 2012. Disponível em: https://doi.org/10.2174/156720512799015064

CAO, Jingli *et al.* Single epicardial cell transcriptome sequencing identifies caveolin 1 as an essential factor in zebrafish heart regeneration. **Development (Cambridge)**, [*s. l.*], v. 143, p. 232–243, 2016. Disponível em: https://doi.org/10.1242/dev.130534

CAPIOTTI, Katiucia Marques *et al.* Hyperglycemia induces memory impairment linked to increased acetylcholinesterase activity in zebrafish (Danio rerio). **Behavioural Brain Research**, [*s. l.*], v. 274, p. 319–325, 2014. Disponível em: https://doi.org/10.1016/j.bbr.2014.08.033

CERNIGLIA, Luca *et al.* Trajectories of aggressive and depressive symptoms in male and female overweight children: Do they share a common path or do they follow different routes? **PLOS ONE**, [*s. l.*], v. 13, n. 1, p. e0190731, 2018. Disponível em: https://doi.org/10.1371/journal.pone.0190731. Acesso em: 18 jun. 2020.

CHEN, Hong *et al.* Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in db/db mice. **Cell**, [*s. l.*], v. 84, p. 491–495, 1996. Disponível em: https://doi.org/10.1016/S0092-8674(00)81294-5

CHENG, Ruey Kuang; JESUTHASAN, Suresh J.; PENNEY, Trevor B. **Zebrafish forebrain and temporal conditioning**. [*S. l.*]: The Royal Society, 2014. Disponível em: https://doi.org/10.1098/rstb.2012.0462

CHU, Che Yu *et al.* Overexpression of Akt1 enhances adipogenesis and leads to lipoma formation in zebrafish. **PLoS ONE**, [*s. l.*], 2012. Disponível em: https://doi.org/10.1371/journal.pone.0036474

CLEMENTE, Diego *et al.* Cholinergic elements in the zebrafish central nervous system: Histochemical and immunohistochemical analysis. **Journal of Comparative Neurology**, [*s. l.*], v. 474, n. 1, p. 75–107, 2004. Disponível em: https://doi.org/10.1002/cne.20111

CRUZ, Shelly A. *et al.* Ghrelin affects carbohydrate-glycogen metabolism via insulin inhibition and glucagon stimulation in the zebrafish (Danio rerio) brain. **Comparative**

Biochemistry and Physiology - A Molecular and Integrative Physiology, [*s. l.*], v. 156, p. 190–200, 2010. Disponível em: https://doi.org/10.1016/j.cbpa.2010.01.019

D'ALESSIO, David A. *et al.* Activation of the parasympathetic nervous system is necessary for normal meal-induced insulin secretion in rhesus macaques. **Journal of Clinical Endocrinology and Metabolism**, [*s. l.*], v. 86, p. 1253–1259, 2001. Disponível em: https://doi.org/10.1210/jc.86.3.1253

DE NORONHA, Sylvana Rendeiro *et al.* High fat diet induced-obesity facilitates anxiety-like behaviors due to GABAergic impairment within the dorsomedial hypothalamus in rats. **Behavioural Brain Research**, [*s. l.*], 2017. Disponível em: https://doi.org/10.1016/j.bbr.2016.08.042

DELGADO, Luz; SCHMACHTENBERG, Oliver. Immunohistochemical localization of GABA, GAD65, and the receptor subunits GABAAα1 and GABAB1 in the zebrafish cerebellum. **Cerebellum**, [*s. l.*], v. 7, n. 3, p. 444–50, 2008. Disponível em: https://doi.org/10.1007/s12311-008-0047-7

DEN BROEDER, Marjo *et al.* Altered Adipogenesis in Zebrafish Larvae Following High Fat Diet and Chemical Exposure Is Visualised by Stimulated Raman Scattering Microscopy. **International Journal of Molecular Sciences**, [*s. l.*], v. 18, n. 4, p. 894, 2017. Disponível em: https://doi.org/10.3390/ijms18040894. Acesso em: 20 jun. 2019.

DEN BROEDER, Marjo J. *et al.* Zebrafish as a Model to Study the Role of Peroxisome Proliferating-Activated Receptors in Adipogenesis and Obesity. **PPAR Research**, [*s. l.*], 2015. Disponível em: https://doi.org/10.1155/2015/358029

DIMITROVA, Darinka Slavcheva; GETOVA-SPASSOVA, Damianka Peteva. Effects of galantamine and donepezil on active and passive avoidance tests in rats with induced hypoxia. **Journal of Pharmacological Sciences**, [*s. l.*], 2006. Disponível em: https://doi.org/10.1254/jphs.FPE05006X

DOS SANTOS, Matheus M. *et al.* Hyperglycemia elicits anxiety-like behaviors in zebrafish: Protective role of dietary diphenyl diselenide. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [*s. l.*], v. 85, p. 128–135, 2018. Disponível em: https://doi.org/10.1016/j.pnpbp.2018.04.017. Acesso em: 10 jul. 2020.

EAMES, Stefani C. *et al.* Blood sugar measurement in zebrafish reveals dynamics of glucose homeostasis. **Zebrafish**, [*s. l.*], v. 7, p. 205–213, 2010. Disponível em:

https://doi.org/10.1089/zeb.2009.0640

EGAN, Rupert J. *et al.* Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. **Behavioural Brain Research**, [*s. l.*], v. 205, n. 1, p. 38–44, 2009. Disponível em: https://doi.org/10.1016/j.bbr.2009.06.022

ELO, B. *et al.* Larval zebrafish as a model for glucose metabolism: Expression of phosphoenolpyruvate carboxykinase as a marker for exposure to anti-diabetic compounds. **Journal of Molecular Endocrinology**, [*s. l.*], v. 38, p. 433–440, 2007. Disponível em: https://doi.org/10.1677/JME-06-0037

ERIKSSON, Krister S. *et al.* Development of the histaminergic neurons and expression of histidine decarboxylase mRNA in the zebrafish brain in the absence of all peripheral histaminergic systems. **European Journal of Neuroscience**, [*s. l.*], v. 10, p. 3799–812, 1998. Disponível em: https://doi.org/10.1046/j.1460-9568.1998.00394.x

FARR, Susan A. *et al.* Obesity and hypertriglyceridemia produce cognitive impairment. **Endocrinology**, [*s. l.*], 2008. Disponível em: https://doi.org/10.1210/en.2007-1722

FLINN, Laura *et al.* Zebrafish as a new animal model for movement disorders. **Journal of Neurochemistry**, [*s. l.*], v. 106, p. 7, 2008. Disponível em: https://doi.org/10.1111/j.1471-4159.2008.05463.x

FLYNN, Edward J *et al.* Ontogeny and nutritional control of adipogenesis in zebrafish (Danio rerio). **Journal of lipid research**, [*s. l.*], v. 50, n. 8, p. 1641–1652, 2009. Disponível em: https://doi.org/10.1194/jlr.M800590-JLR200. Acesso em: 18 jun. 2019.

FOLCH, J.; LEES, M.; SLOANE STANLEY, G. H. A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of biological chemistry**, [*s. l.*], v. 226, p. 497 – 509, 1957. Disponível em: https://doi.org/10.1016/s0021-9258(18)64849-5

FONTAINE, Kevin R. *et al.* Years of life lost due to obesity. **Journal of the American Medical Association**, [*s. l.*], v. 289, p. 187–193, 2003. Disponível em: https://doi.org/10.1001/jama.289.2.187

FONTANA, Barbara D. *et al.* Taurine modulates acute ethanol-induced social behavioral deficits and fear responses in adult zebrafish. **Journal of Psychiatric Research**, [*s. l.*], v. 104, p. 176–182, 2018. Disponível em: https://doi.org/10.1016/j.jpsychires.2018.08.008. Acesso em: 13 mar. 2020.

FONTANA, Barbara D. *et al.* Zebrafish (Danio rerio) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses. **Animal Cognition**, [*s. l.*], v. 22, n. 6, p. 1051–1061, 2019. Disponível em: https://doi.org/10.1007/s10071-019-01296-9

FRANSCESCON, Francini *et al.* Neuroprotective role of taurine on MK-801-induced memory impairment and hyperlocomotion in zebrafish. **Neurochemistry International**, [*s. l.*], p. 104710, 2020. Disponível em: https://doi.org/10.1016/j.neuint.2020.104710

FREEMAN, Linnea R *et al.* Damaging effects of a high-fat diet to the brain and cognition: A review of proposed mechanisms NIH Public Access. **Nutr Neurosci**, [*s. l.*], v. 17, n. 6, p. 241–251, 2014. Disponível em: https://doi.org/10.1179/1476830513Y.0000000092

FREEMAN, Linnea R.; GRANHOLM, Ann Charlotte E. Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet. **Journal of Cerebral Blood Flow and Metabolism**, [*s. l.*], 2012. Disponível em: https://doi.org/10.1038/jcbfm.2011.168

GABRIEL, Jens Peter *et al.* Serotonergic modulation of locomotion in zebrafish - Endogenous release and synaptic mechanisms. **Journal of Neuroscience**, [*s. l.*], v. 29, n. 33, p. 10387–10395, 2009. Disponível em: https://doi.org/10.1523/JNEUROSCI.1978-09.2009

GAMBETA, Eder *et al.* Reestablishment of the hyperglycemia to the normal levels seems not to be essential to the anxiolytic-like effect induced by insulin. **Metabolic Brain Disease**, [*s. l.*], 2016. Disponível em: https://doi.org/10.1007/s11011-015-9770-1

GERLAI, R. *et al.* Drinks like a fish: Zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. **Pharmacology Biochemistry and Behavior**, [*s. l.*], v. 67, n. 4, p. 773–782, 2000. Disponível em: https://doi.org/10.1016/S0091-3057(00)00422-6

GORISSEN, Marnix *et al.* Two different leptin paralogues in zebrafish (Danio rerio) that originate early in teleostean evolution. **Journal of Endocrinology**, [*s. l.*], v. 201, p. 329–339, 2009. Disponível em: https://doi.org/10.1677/JOE-09-0034

GRAYSON, B. E. *et al.* Improvements in hippocampal-dependent memory and microglial infiltration with calorie restriction and gastric bypass surgery, but not with vertical sleeve gastrectomy. **International Journal of Obesity**, [*s. l.*], 2014. Disponível em: https://doi.org/10.1038/ijo.2013.100

GUT, Philipp *et al.* Little fish, big data: Zebrafish as a model for cardiovascular and metabolic disease. **Physiological Reviews**, [*s. l.*], v. 97, n. 3, p. 889–938, 2017. Disponível

em: https://doi.org/10.1152/physrev.00038.2016. Acesso em: 8 jul. 2020.

GUTIÉRREZ, Héctor Carreño *et al.* Screening for drugs to reduce zebrafish aggression identifies caffeine and sildenafil. **European Neuropsychopharmacology**, [*s. l.*], v. 30, p. 17–29, 2020. Disponível em: https://doi.org/10.1016/j.euroneuro.2019.10.005

HAMER, M.; BATTY, G. D.; KIVIMAKI, M. Risk of future depression in people who are obese but metabolically healthy: The English longitudinal study of ageing. **Molecular Psychiatry**, [*s. l.*], 2012. Disponível em: https://doi.org/10.1038/mp.2012.30

HARIRI, Niloofar; THIBAULT, Louise. High-fat diet-induced obesity in animal models. **Nutrition Research Reviews**, [*s. l.*], v. 23, n. 2, p. 270–299, 2010. Disponível em: https://doi.org/10.1017/S0954422410000168. Acesso em: 22 dez. 2020.

HASUMURA, Takahiro *et al*. Green tea extract suppresses adiposity and affects the expression of lipid metabolism genes in diet-induced obese zebrafish. **Nutrition & Metabolism**, [*s. l.*], v. 9, n. 1, p. 73, 2012. Disponível em: https://doi.org/10.1186/1743-7075-9-73. Acesso em: 13 jun. 2019.

HAUSER, Tobias U.; ELDAR, Eran; DOLAN, Raymond J. **Neural mechanisms of harm-Avoidance learning a model for obsessive-compulsive disorder?**. [*S. l.: s. n.*], 2016. Disponível em: https://doi.org/10.1001/jamapsychiatry.2016.1938

HAVEL, P. J.; VALVERDE, C. Autonomic mediation of glucagon secretion during insulin-induced hypoglycemia in rhesus monkeys. **Diabetes**, [*s. l.*], v. 45, p. 960–966, 1996. Disponível em: https://doi.org/10.2337/diab.45.7.960

HEYWARD, Frankie D. *et al.* Adult mice maintained on a high-fat diet exhibit object location memory deficits and reduced hippocampal SIRT1 gene expression. **Neurobiology of Learning and Memory**, [*s. l.*], 2012. Disponível em: https://doi.org/10.1016/j.nlm.2012.04.005

HILAKIVI-CLARKE, Leena; CHO, Elizabeth; ONOJAFE, Ighovie. High-fat diet induces aggressive behavior in male mice and rats. **Life Sciences**, [*s. l.*], v. 58, n. 19, p. 1653–1660, 1996. Disponível em: https://doi.org/10.1016/0024-3205(96)00140-3. Acesso em: 8 jul. 2020.

HILL, James O.; WYATT, Holly R.; PETERS, John C. Energy balance and obesity. **Circulation**, [*s. l.*], p. 126–132, 2012. Disponível em: https://doi.org/10.1161/CIRCULATIONAHA.111.087213

HORZMANN, Katharine A.; FREEMAN, Jennifer L. **Zebrafish get connected: Investigating neurotransmission targets and alterations in chemical toxicity**. [*S. l.: s. n.*], 2016. Disponível em: https://doi.org/10.3390/toxics4030019

HOWE, Kerstin *et al*. The zebrafish reference genome sequence and its relationship to the human genome. **Nature**, [*s. l.*], v. 496, p. 498–503, 2013. Disponível em: https://doi.org/10.1038/nature12111

HSU, Chia Chun *et al.* MicroRNA-27b depletion enhances endotrophic and intravascular lipid accumulation and induces adipocyte hyperplasia in zebrafish. **International Journal of Molecular Sciences**, [*s. l.*], v. 19, n. E93, 2018. Disponível em: https://doi.org/10.3390/ijms19010093

IBRAHIM, M. Mohsen. Subcutaneous and visceral adipose tissue: Structural and functional differences. **Obesity Reviews**, [*s. l.*], v. 11, p. 11–18, 2010. Disponível em: https://doi.org/10.1111/j.1467-789X.2009.00623.x

INGALLS, Ann M.; DICKIE, Margaret M.; SNELL, G. D. Obese, a new mutation in the house mouse. **Journal of Heredity**, [*s. l.*], v. 41(12), p. 317–8, 1950. Disponível em: https://doi.org/10.1093/oxfordjournals.jhered.a106072

JACKA, Felice N. *et al.* Association of western and traditional diets with depression and anxiety in women. **American Journal of Psychiatry**, [*s. l.*], v. 73, p. 483–490, 2010. Disponível em: https://doi.org/10.1176/appi.ajp.2009.09060881

JI, Juling *et al.* Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation. **FEBS Letters**, [*s. l.*], v. 583, p. 759–766, 2009. Disponível em: https://doi.org/10.1016/j.febslet.2009.01.034

JOHNSON, Paul M.; KENNY, Paul J. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. **Nature Neuroscience**, [*s. l.*], v. 13, p. 635–641, 2010. Disponível em: https://doi.org/10.1038/nn.2519

JONES, Lauren J.; NORTON, William H.J. Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders. **Behavioural Brain Research**, [*s. l.*], v. 276, n. ;HY:^**I98;171-180, p. 171–180, 2015. Disponível em: https://doi.org/10.1016/j.bbr.2014.05.055

KALUEFF, Allan V. *et al.* **Towards a comprehensive catalog of zebrafish behavior 1.0**

and beyond. [*S. l.*]: Mary Ann Liebert, Inc., 2013. Disponível em: https://doi.org/10.1089/zeb.2012.0861

KALUEFF, Allan V.; STEWART, Adam Michael; GERLAI, Robert. **Zebrafish as an emerging model for studying complex brain disorders**. [*S. l.*]: NIH Public Access, 2014. Disponível em: https://doi.org/10.1016/j.tips.2013.12.002. Acesso em: 24 abr. 2021.

KANOSKI, Scott E.; DAVIDSON, Terry L. Different Patterns of Memory Impairments Accompany Short- and Longer-Term Maintenance on a High-Energy Diet. **Journal of Experimental Psychology: Animal Behavior Processes**, [*s. l.*], v. 36, n. 2, p. 313–319, 2010. Disponível em: https://doi.org/10.1037/a0017228. Acesso em: 7 jul. 2020.

KARIMI, Seyed Asaad *et al.* Effect of high-fat diet and antioxidants on hippocampal long-term potentiation in rats: An in vivo study. **Brain Research**, [*s. l.*], 2013. Disponível em: https://doi.org/10.1016/j.brainres.2013.09.029

KARL, Tim; DUFFY, Liesl; HERZOG, Herbert. Behavioural profile of a new mouse model for NPY deficiency. **European Journal of Neuroscience**, [*s. l.*], v. 28, p. 173–80, 2008. Disponível em: https://doi.org/10.1111/j.1460-9568.2008.06306.x

KASLIN, Jan *et al.* The Orexin/Hypocretin System in Zebrafish Is Connected to the Aminergic and Cholinergic Systems. **Journal of Neuroscience**, [*s. l.*], v. 24, n. 11, p. 2678–89, 2004. Disponível em: https://doi.org/10.1523/JNEUROSCI.4908-03.2004

KAWAI, Hitomi; ARATA, Noriko; NAKAYASU, Hiroshi. Three-dimensional distribution of astrocytes in zebrafish spinal cord. **GLIA**, [*s. l.*], v. 36, p. 406–413, 2001. Disponível em: https://doi.org/10.1002/glia.1126

KIM, Yong Jung *et al.* Identification and functional evidence of GABAergic neurons in parts of the brain of adult zebrafish (Danio rerio). **Neuroscience Letters**, [*s. l.*], v. 355, n. 1–2, p. 29–32, 2004. Disponível em: https://doi.org/10.1016/j.neulet.2003.10.024

KIMMEL, Charles B *et al.* Stages of Embryonic Development of the Zebrafish. [*s. l.*], v. 203, p. 253–310, 1995. Disponível em: https://onlinelibrary.wiley.com/doi/pdf/10.1002/aja.1002030302. Acesso em: 19 jun. 2019.

KLEINERT, Maximilian *et al.* **Animal models of obesity and diabetes mellitus**. [*S. l.: s. n.*], 2018. Disponível em: https://doi.org/10.1038/nrendo.2017.161

KOJIMA, Masayasu *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from

stomach. **Nature**, [*s. l.*], v. 402, p. 656–660, 1999. Disponível em: https://doi.org/10.1038/45230

KOOPMAN, René; SCHAART, Gert; HESSELINK, Matthijs K. Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. **Histochemistry and Cell Biology**, [*s. l.*], v. 116, n. 1, p. 63–68, 2001. Disponível em: https://doi.org/10.1007/s004180100297

KOPP, R. *et al.* Bringing obesity to light: Rev-erbα, a central player in light-induced adipogenesis in the zebrafish? **International Journal of Obesity**, [*s. l.*], v. 40, p. 824–832, 2016. Disponível em: https://doi.org/10.1038/ijo.2015.240

KRYPOTOS, Angelos Miltiadis *et al.* **Avoidance learning: A review of theoretical models and recent developments**. [*S. l.: s. n.*], 2015. Disponível em: https://doi.org/10.3389/fnbeh.2015.00189

KYZAR, Evan *et al.* Behavioral effects of bidirectional modulators of brain monoamines reserpine and d-amphetamine in zebrafish. **Brain Research**, [*s. l.*], v. 1527, p. 108–116, 2013. Disponível em: https://doi.org/10.1016/j.brainres.2013.06.033

LANDGRAF, Kathrin *et al.* Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealt. **BMC physiology**, [*s. l.*], v. 17, n. 1, p. 4, 2017. Disponível em: https://doi.org/10.1186/s12899-017-0031-x. Acesso em: 24 jun. 2019.

LEIBOLD, Sandra; HAMMERSCHMIDT, Matthias. Long-term hyperphagia and caloric restriction caused by low- or high-density husbandry have differential effects on zebrafish postembryonic development, somatic growth, fat accumulation and reproduction. **PLoS ONE**, [*s. l.*], v. 10, n. 3, 2015. Disponível em: https://doi.org/10.1371/journal.pone.0120776

LEVIN, Edward D.; BENCAN, Zachary; CERUTTI, Daniel T. Anxiolytic effects of nicotine in zebrafish. **Physiology and Behavior**, [*s. l.*], v. 90, n. 1, p. 54–8, 2007. Disponível em: https://doi.org/10.1016/j.physbeh.2006.08.026

LEVIN, Edward D.; CHEN, Elaine. Nicotinic involvement in memory function in zebrafish. **Neurotoxicology and Teratology**, [*s. l.*], v. 26, n. 6, p. 731–5, 2004. Disponível em: https://doi.org/10.1016/j.ntt.2004.06.010

LIESCHKE, Graham J.; CURRIE, Peter D. **Animal models of human disease: Zebrafish**

swim into view. [*S. l.: s. n.*], 2007. Disponível em: https://doi.org/10.1038/nrg2091

LILLESAAR, Christina. The serotonergic system in fish. **Journal of Chemical Neuroanatomy**, [*s. l.*], v. 41, n. 4, p. 294–308, 2011. Disponível em: https://doi.org/10.1016/j.jchemneu.2011.05.009

LINDBERG, Louise *et al.* Anxiety and depression in children and adolescents with obesity: A nationwide study in Sweden. **BMC Medicine**, [*s. l.*], v. 18, n. 1, 2020. Disponível em: https://doi.org/10.1186/s12916-020-1498-z

MA, Ji *et al.* A Comprehensive Study of High Cholesterol Diet-Induced Larval Zebrafish Model: A Short-Time In Vivo Screening Method for Non-Alcoholic Fatty Liver Disease Drugs. **International journal of biological sciences**, [*s. l.*], v. 15, n. 5, p. 973–983, 2019. Disponível em: https://doi.org/10.7150/ijbs.30013. Acesso em: 21 jul. 2020.

MACMAHON, S. *et al.* Body-mass index and cause-specific mortality in 900 000 adults: Collaborative analyses of 57 prospective studies. **The Lancet**, [*s. l.*], n. 373, p. 1083–1096, 2009. Disponível em: https://doi.org/10.1016/S0140-6736(09)60318-4

MARTÍNEZ, Rubén *et al.* Changes in lipid profiles induced by bisphenol A (BPA) in zebrafish eleutheroembryos during the yolk sac absorption stage. **Chemosphere**, [*s. l.*], v. 246, n. 125704, 2020. Disponível em: https://doi.org/10.1016/j.chemosphere.2019.125704

MAXIMINO, Caio *et al.* **Measuring anxiety in zebrafish: A critical review**. [*S. l.: s. n.*], 2010. Disponível em: https://doi.org/10.1016/j.bbr.2010.05.031

MAZON, Janaína Niero *et al.* **The impact of obesity on neurodegenerative diseases**. [*S. l.: s. n.*], 2017. Disponível em: https://doi.org/10.1016/j.lfs.2017.06.002

MCMENAMIN, Sarah K. *et al.* Dwarfism and increased adiposity in the gh1 mutant zebrafish vizzini. **Endocrinology**, [*s. l.*], v. 154, p. 1476–1487, 2013. Disponível em: https://doi.org/10.1210/en.2012-1734

MCNAY, Ewan C. *et al.* Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. **Neurobiology of Learning and Memory**, [*s. l.*], 2010. Disponível em: https://doi.org/10.1016/j.nlm.2010.02.002

MEGURO, Shinichi; HASUMURA, Takahiro; HASE, Tadashi. Body Fat Accumulation in Zebrafish Is Induced by a Diet Rich in Fat and Reduced by Supplementation with Green Tea Extract. **PLOS ONE**, [*s. l.*], v. 10, n. 3, p. e0120142, 2015. Disponível em:

https://doi.org/10.1371/journal.pone.0120142. Acesso em: 24 nov. 2019.

MEGURO, Shinichi; HOSOI, Sayaka; HASUMURA, Takahiro. High-fat diet impairs cognitive function of zebrafish. **Scientific Reports**, [*s. l.*], v. 9, n. 1, 2019. Disponível em: https://doi.org/10.1038/s41598-019-53634-z

MICHAEL STEWART, Adam; V. KALUEFF, Allan. The Developing Utility of Zebrafish Models for Cognitive Enhancers Research. **Current Neuropharmacology**, [*s. l.*], v. 10, p. 263–271, 2012. Disponível em: https://doi.org/10.2174/157015912803217323

MILLER, Noam; GERLAI, Robert. From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (Danio rerio). **PLoS ONE**, [*s. l.*], v. 7, n. e48865, 2012. Disponível em: https://doi.org/10.1371/journal.pone.0048865

MILLER, Noam Y.; GERLAI, Robert. Shoaling in zebrafish: What we don't know. **Reviews in the Neurosciences**, [*s. l.*], v. 22, p. 17–25, 2011. Disponível em: https://doi.org/10.1515/RNS.2011.004

MINCHIN, James E.N.; RAWLS, John F. In vivo Analysis of White Adipose Tissue in Zebrafish. **Methods in Cell Biology**, [*s. l.*], v. 105, p. 63–86, 2011. Disponível em: https://doi.org/10.1016/B978-0-12-381320-6.00003-5

MONTALBANO, G. *et al.* Melatonin treatment suppresses appetite genes and improves adipose tissue plasticity in diet-induced obese zebrafish. **Endocrine**, [*s. l.*], v. 62, n. 2, p. 381–393, 2018. Disponível em: https://doi.org/10.1007/s12020-018-1653-x

MUELLER, Thomas *et al.* The dorsal pallium in zebrafish, Danio rerio (Cyprinidae, Teleostei). **Brain Research**, [*s. l.*], v. 1381, p. 95–105, 2011. Disponível em: https://doi.org/10.1016/j.brainres.2010.12.089

MUELLER, Thomas; VERNIER, Philippe; WULLIMANN, Mario F. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish Danio rerio. **Brain Research**, [*s. l.*], v. 1011, n. 2, p. 156–69, 2004. Disponível em: https://doi.org/10.1016/j.brainres.2004.02.073

MÜLLER, Talise E. *et al.* **Understanding the neurobiological effects of drug abuse: Lessons from zebrafish models**. [*S. l.: s. n.*], 2020. Disponível em: https://doi.org/10.1016/j.pnpbp.2020.109873

NISHIO, Shin Ichi *et al.* Fasting induces CART down-regulation in the zebrafish nervous

system in a cannabinoid receptor 1-dependent manner. **Molecular Endocrinology**, [*s. l.*], v. 26, p. 1316–1326, 2012. Disponível em: https://doi.org/10.1210/me.2011-1180

NOLTE, Hendrik *et al.* Dynamics of zebrafish fin regeneration using a pulsed SILAC approach. **Proteomics**, [*s. l.*], v. 15, p. 739–751, 2015. Disponível em: https://doi.org/10.1002/pmic.201400316

NORTHCUTT, R. Glenn. Connections of the lateral and medial divisions of the goldfish telencephalic pallium. **Journal of Comparative Neurology**, [*s. l.*], v. 494, p. 903–943, 2006. Disponível em: https://doi.org/10.1002/cne.20853

NORTON, William; BALLY-CUIF, Laure. **Adult zebrafish as a model organism for behavioural genetics**. [*S. l.*]: BioMed Central, 2010. Disponível em: https://doi.org/10.1186/1471-2202-11-90

O'BRIEN, Phillipe D. *et al.* **Neurological consequences of obesity**. [*S. l.: s. n.*], 2017. Disponível em: https://doi.org/10.1016/S1474-4422(17)30084-4

OKA, Takehiko *et al*. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. **BMC Physiology**, [*s. l.*], v. 10, n. 1, p. 21, 2010. Disponível em: https://doi.org/10.1186/1472-6793-10-21. Acesso em: 13 jun. 2019.

PANNACCIULLI, Nicola *et al*. Brain abnormalities in human obesity: A voxel-based morphometric study. **NeuroImage**, [*s. l.*], v. 31, p. 1419–1425, 2006. Disponível em: https://doi.org/10.1016/j.neuroimage.2006.01.047

PANULA, Pertti *et al.* Modulatory neurotransmitter systems and behavior: Towards zebrafish models of neurodegenerative diseases. **Zebrafish**, [*s. l.*], v. 3, n. 2, p. 235–247, 2006. Disponível em: https://doi.org/10.1089/zeb.2006.3.235

PEITSARO, Nina *et al*. Identification of zebrafish histamine H1, H2 and H3 receptors and effects of histaminergic ligands on behavior. **Biochemical Pharmacology**, [*s. l.*], v. 73, n. 8, p. 1205–14, 2007. Disponível em: https://doi.org/10.1016/j.bcp.2007.01.014

PEITSARO, Nina *et al*. Modulation of the histaminergic system and behaviour by α-fluoromethylhistidine in zebrafish. **Journal of Neurochemistry**, [*s. l.*], v. 86, p. 432–41, 2003. Disponível em: https://doi.org/10.1046/j.1471-4159.2003.01850.x

PEITSARO, Nina; ANICHTCHIK, Oleg V.; PANULA, Pertti. Identification of a histamine H3-like receptor in the zebrafish (Danio rerio) brain. **Journal of Neurochemistry**, [*s. l.*], v.

75, p. 718–24, 2000. Disponível em: https://doi.org/10.1046/j.1471-4159.2000.0750718.x

PERI, Francesca; NÜSSLEIN-VOLHARD, Christiane. Live Imaging of Neuronal Degradation by Microglia Reveals a Role for v0-ATPase a1 in Phagosomal Fusion In Vivo. **Cell**, [*s. l.*], v. 33, p. 916–92, 2008. Disponível em: https://doi.org/10.1016/j.cell.2008.04.037

PHILLIPS, Jennifer B.; WESTERFIELD, Monte. Zebrafish models in translational research: Tipping the scales toward advancements in human health. **DMM Disease Models and Mechanisms**, [*s. l.*], v. 7, n. 7, p. 739–743, 2014. Disponível em: https://doi.org/10.1242/dmm.015545. Acesso em: 23 dez. 2020.

PICOLO, Victor L. *et al.* Short-term high-fat diet induces cognitive decline, aggression, and anxiety-like behavior in adult zebrafish. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [*s. l.*], v. 110, p. 110288, 2021. Disponível em: https://doi.org/10.1016/j.pnpbp.2021.110288

PORTAVELLA, M. *et al.* The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. **Brain Research Bulletin**, [*s. l.*], v. 57, p. 397–399, 2002. Disponível em: https://doi.org/10.1016/S0361-9230(01)00699-2

PROCACCINI, Claudio *et al.* **Role of metabolism in neurodegenerative disorders**. [*S. l.: s. n.*], 2016. Disponível em: https://doi.org/10.1016/j.metabol.2016.05.018

PROKOP, J. W. *et al*. Leptin and leptin receptor: Analysis of a structure to function relationship in interaction and evolution from humans to fish. **Peptides**, [*s. l.*], 2012. Disponível em: https://doi.org/10.1016/j.peptides.2012.10.002

PUDER, J. J.; MUNSCH, S. Psychological correlates of childhood obesity. **International Journal of Obesity**, [*s. l.*], v. 34, p. S37—S43, 2010. Disponível em: https://doi.org/10.1038/ijo.2010.238

REES, D. A.; ALCOLADO, J. C. **Animal models of diabetes mellitus**. [*S. l.: s. n.*], 2005. Disponível em: https://doi.org/10.1111/j.1464-5491.2005.01499.x

RICO, Eduardo Pacheco *et al*. Expression and functional analysis of Na+-dependent glutamate transporters from zebrafish brain. **Brain Research Bulletin**, [*s. l.*], v. 81, n. 4–5, p. 517–23, 2010. Disponível em: https://doi.org/10.1016/j.brainresbull.2009.11.011

RINGHOLM, Aneta *et al.* One melanocortin 4 and two melanocortin 5 receptors from zebrafish show remarkable conservation in structure and pharmacology. **Journal of**

Neurochemistry, [*s. l.*], v. 82, p. 6 –18, 2002. Disponível em: https://doi.org/10.1046/j.1471-4159.2002.00934.x

SACKERMAN, James *et al.* Zebrafish Behavior in Novel Environments: Effects of Acute Exposure to Anxiolytic Compounds and Choice of Danio rerio Line. **International journal of comparative psychology**, [*s. l.*], v. 23, n. 1, p. 43–61, 2010.

SALAS, Cosme *et al.* **Neuropsychology of learning and memory in teleost fish**. [*S. l.: s. n.*], 2006. Disponível em: https://doi.org/10.1089/zeb.2006.3.157

SAVERINO, Cristina; GERLAI, Robert. The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. **Behavioural Brain Research**, [*s. l.*], v. 191, n. 1, p. 77–87, 2008. Disponível em: https://doi.org/10.1016/j.bbr.2008.03.013

SCHLEGEL, Amnon; STAINIER, Didier Y.R. **Lessons from "lower" organisms: What worms, flies, and zebrafish can teach US about human energy metabolism**. [*S. l.: s. n.*], 2007. Disponível em: https://doi.org/10.1371/journal.pgen.0030199

SHEN, Peiyi; YUE, Yiren; PARK, Yeonhwa. **A living model for obesity and aging research: Caenorhabditis elegans**. [*S. l.: s. n.*], 2018. Disponível em: https://doi.org/10.1080/10408398.2016.1220914

SICCARDI, Anthony J. *et al.* Growth and survival of Zebrafish (Danio rerio) fed different commercial and laboratory diets. **Zebrafish**, [*s. l.*], v. 6, p. 275–280, 2009. Disponível em: https://doi.org/10.1089/zeb.2008.0553

SMITH, Daniel L. *et al.* Dietary protein source influence on body size and composition in growing zebrafish. **Zebrafish**, [*s. l.*], v. 10, p. 439–446, 2013. Disponível em: https://doi.org/10.1089/zeb.2012.0864

SMITH, Wanli W. *et al.* **From fat fruit fly to human obesity**. [*S. l.: s. n.*], 2014. Disponível em: https://doi.org/10.1016/j.physbeh.2014.01.017

SONG, Youngsup *et al.* Agouti-Related Protein (AGRP) Is Conserved and Regulated by Metabolic State in the Zebrafish, Danio rerio. **Endocrine**, [*s. l.*], v. 22, p. 257–265, 2003. Disponível em: https://doi.org/10.1385/ENDO:22:3:257

SONG, Youngsup; CONE, Roger D. Creation of a genetic model of obesity in a teleost. **The FASEB Journal**, [*s. l.*], v. 21, p. 2042–2049, 2007. Disponível em: https://doi.org/10.1096/fj.06-7503com

STEWART, Adam Michael *et al.* Developing zebrafish models of autism spectrum disorder (ASD). **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, [*s. l.*], v. 3, n. 50, p. 27–36, 2014. Disponível em: https://doi.org/10.1016/j.pnpbp.2013.11.014

STEWART, Adam Michael *et al.* Perspectives of zebrafish models of epilepsy: What, how and where next? **Brain Research Bulletin**, [*s. l.*], v. 87, p. 135–143, 2012. Disponível em: https://doi.org/10.1016/j.brainresbull.2011.11.020

STRANAHAN, Alexis M. *et al.* Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. **Hippocampus**, [*s. l.*], 2008. Disponível em: https://doi.org/10.1002/hipo.20470

STREISINGER, George *et al*. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). **Nature**, [*s. l.*], v. 291, n. 5813, p. 293–296, 1981. Disponível em: https://doi.org/10.1038/291293a0

TAINAKA, Toshiyuki *et al*. Transcriptome analysis of anti-fatty liver action by Campari tomato using a zebrafish diet-induced obesity model. **Nutrition and Metabolism**, [*s. l.*], v. 8, 2011. Disponível em: https://doi.org/10.1186/1743-7075-8-88

TAN, Bee Ling; NORHAIZAN, Mohd Esa. **Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function**. [*S. l.: s. n.*], 2019. Disponível em: https://doi.org/10.3390/nu11112579

TELES, Magda C. *et al.* Social modulation of brain monoamine levels in zebrafish. **Behavioural Brain Research**, [*s. l.*], v. 253, p. 17–24, 2013. Disponível em: https://doi.org/10.1016/j.bbr.2013.07.012

TINGAUD-SEQUEIRA, Angèle; OUADAH, Nafia; BABIN, Patrick J. Zebrafish obesogenic test: a tool for screening molecules that target adiposity. **Journal of lipid research**, [*s. l.*], v. 52, n. 9, p. 1765–1772, 2011. Disponível em: https://doi.org/10.1194/jlr.D017012. Acesso em: 20 jun. 2019.

VALDEARCOS, Martin *et al.* Microglia Dictate the Impact of Saturated Fat Consumption on Hypothalamic Inflammation and Neuronal Function. **Cell Reports**, [*s. l.*], 2014. Disponível em: https://doi.org/10.1016/j.celrep.2014.11.018

VALLADOLID-ACEBES, Ismael *et al.* Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of

leptin? **Neurobiology of Learning and Memory**, [*s. l.*], 2013. Disponível em: https://doi.org/10.1016/j.nlm.2013.06.012

VANDEVIJVERE, Stefanie *et al*. Increased food energy supply as a major driver of the obesity epidemic: a global analysis. **Bulletin of the World Health Organization**, [*s. l.*], 2015. Disponível em: https://doi.org/10.2471/blt.14.150565

VERGAUWEN, Lucia *et al.* Assessing the impact of thermal acclimation on physiological condition in the zebrafish model. **Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology**, [*s. l.*], v. 183, p. 109–121, 2013. Disponível em: https://doi.org/10.1007/s00360-012-0691-6

VICKERS, Kasey C. *et al.* MicroRNA-27b is a regulatory hub in lipid metabolism and is altered in dyslipidemia. **Hepatology**, [*s. l.*], v. 57, p. 533–542, 2013. Disponível em: https://doi.org/10.1002/hep.25846

WANG, Wei *et al.* Leptin: A Potential Anxiolytic by Facilitation of Fear Extinction. **CNS Neuroscience and Therapeutics**, [*s. l.*], 2015. Disponível em: https://doi.org/10.1111/cns.12375

WANG, Youfa; BEYDOUN, May A. The Obesity Epidemic in the United States-Gender, Age, Socioeconomic, Racial/Ethnic, and Geographic Characteristics: A Systematic Review and Meta-Regression Analysis. **Epidemiologic Reviews**, [*s. l.*], 2007. Disponível em: https://doi.org/10.1093/epirev/mxm007. Acesso em: 25 jun. 2020.

WARD, Michael A. *et al.* The effect of body mass index on global brain volume in middle-aged adults: A cross sectional study. **BMC Neurology**, [*s. l.*], 2005. Disponível em: https://doi.org/10.1186/1471-2377-5-23

WATSON, G. Stennis *et al.* Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. **The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry**, [*s. l.*], v. 13, p. 950–958, 2005. Disponível em: https://doi.org/10.1176/appi.ajgp.13.11.950

WIDDOWSON, Peter S. *et al.* Hypothalamic NPY status during positive energy balance and the effects of the NPY antagonist, BW1229U91, on the consumption of highly palatable energy-rich diet. **Peptides**, [*s. l.*], v. 20, p. 367–72, 1999. Disponível em: https://doi.org/10.1016/S0196-9781(99)00044-3

WORLD HEALTH ORGANIZATION. **Obesity and overweight**. [*S. l.*], 2016. Disponível em: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Acesso em: 17 jun. 2020.

WULLIMANN, M. F.; RUPP, B.; REICHERT, H. Introduction: neuroanatomy for a neurogenetic model system. **Neuroanatomy of the Zebrafish Brain**, [*s. l.*], v. 1, p. 1–5, 1996. Disponível em: https://doi.org/10.1007/978-3-0348-8979-7_1

YOGANANTHARJAH, Prusothman; GIBERT, Yann. The Use of the Zebrafish Model to Aid in Drug Discovery and Target Validation. **Current Topics in Medicinal Chemistry**, [*s. l.*], v. 17, n. 18, 2017. Disponível em: https://doi.org/10.2174/1568026617666170130112109. Acesso em: 23 dez. 2020.

YOSHIDA, Mika; MACKLIN, Wendy B. Oligodendrocyte development and myelination in GFP-transgenic zebrafish. **Journal of Neuroscience Research**, [*s. l.*], v. 81, p. 1–8, 2005. Disponível em: https://doi.org/10.1002/jnr.20516

YU, Lili *et al.* Cognitive aging in zebrafish. **PLoS ONE**, [*s. l.*], v. e14, n. 1, 2006. Disponível em: https://doi.org/10.1371/journal.pone.0000014

ZABEGALOV, Konstantin N. *et al.* **Understanding zebrafish aggressive behavior**. [*S. l.*]: Elsevier B.V., 2019. Disponível em: https://doi.org/10.1016/j.beproc.2018.11.010

ZANG, Liqing; MADDISON, Lisette A.; CHEN, Wenbiao. **Zebrafish as a model for obesity and diabetes**. [*S. l.*]: Frontiers Media S.A., 2018. Disponível em: https://doi.org/10.3389/fcell.2018.00091

ZHANG, Yiying *et al.* Positional cloning of the mouse obese gene and its human homologue. **Nature**, [*s. l.*], v. 372, n. 6505, p. 425–432, 1994. Disponível em: https://doi.org/10.1038/372425a0

ZHOU, Juan *et al.* Rapid analysis of hypolipidemic drugs in a live zebrafish assay. **Journal of Pharmacological and Toxicological Methods**, [*s. l.*], v. 72, p. 47–52, 2015. Disponível em: https://doi.org/10.1016/j.vascn.2014.12.002

ZIV, L. *et al.* An affective disorder in zebrafish with mutation of the glucocorticoid receptor. **Molecular Psychiatry**, [*s. l.*], v. 18, n. 6, p. 681–691, 2013. Disponível em: https://doi.org/10.1038/mp.2012.64