Zebra Fish Model - Can It be used to Unlock the Research Insights of Obesity in Humans? Let's Review

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Abstract

Genetic roots of human ailments were studied by using various laboratory methods. Animal models are required to define genetic mutations involved in manifestation of symptoms in patients. Obesity is a clinical condition characterized by subclinical inflammation due to the predominance of adipokines, often causing tissue damage and if not treated with adequate care can even lead to organ damage. The microarray investigation has demonstrated a specific gene pattern that was similar to human non-alcoholic fatty liver disease (NAFLD) in diet-induced obesity zebrafish model. It is now well documented that there is an eighty percent resemblance with human genome, with complete sequencing of zebrafish genome as indication ropes the theory of positive association amid increased lipid content in food items, obesogenic pathways, abnormal regulation and weight gain and the development of cardiovascular diseases and hence findings in zebrafish could be extrapolated to human beings. Zebrafish model is now being preferred over other commonly used animal models because of its cost effectiveness and good genetic compatibility when compared with human species as evident from success of various translational research with zebrafish on disorders of nervous system in humans.

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Introduction

Zebra fish model - An Overview in Scientific literature

Genetic causes of human diseases were investigated by using various laboratory methods. Animal models are required to define genetic mutations involved in occurrence of symptoms in patients. Zebrafish model is now being preferred over rats and mice because of its cost effectiveness and high fecundity with good genetic compatibility when compared with human species as evident from success of various translational research with zebrafish on disorders of nervous system in humans. For an inclusive analysis of gene function and signaling pathways zebrafish model is outstanding.[1] These researchers were possible because this model revealed diverse advantages when compared to other models. These zebrafish models are much easier to sustain in laboratory in their natural habitat than to mimic the conditions crucial for others vertebrae. Therefore it can be maintained in much feasible manner. The rate of study progress is faster since they have short lifespan of 3-5 months.[2] It facilitates the observational and experimental manipulation of embryos because the fertilization takes place externally, with large clutch size between 200 to 300 per fish which

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guarantees enough supply of animals for study purpose. The extraordinary optical clarity of embryos that shows fluorescently labelled individual genes during the developmental progress with non-invasive imaging technique is truly a unique advantage of these models.[3-8] Due to the small size of larvae and transparency of the embryo the genetic manipulations and screening of nueroactive compounds are effortlessly achieved. Scientific experimentations are usually reiterated manifold in order to substantiate that the outcomes are precise, so having an animal that can yield a hefty number of issues over and over is obliging.

Zebra fish model and obesity in humans - A survey

At present obesity is one of the major health issues among people worldwide as it may lead to health complications related to heart, kidney, endocrine system, GI system, musculoskeletal and diseases related to immune system.[9-12] Between 1975 and 2016, the occurrence of this health issue has augmented three times. As per the WHO, about two billion adult population were obese.[13,14] The present review is based on a bibliographic search carried out by the sciELO EMBASE, PubMed database Cand , Google Scholar search engine. In recent years, occurrence of obesity in children enhanced and it is now measured as a wide spread modifiable, non-communicable disease, across the globe.[15-18] In children obesity was about forty one million in the year 2016, with more than half the population residing in Asia whereas taking into consideration the adolescent age group, as compared to 4 percent in the year 1975, the number increased to 18 percent in 2016.[13,14] Childhood obesity which continues to excess weight-gain in adulthood indicates a risk factor for evolving long-lasting diseases that is challenging to accomplish in adults.[19,20] An additional intensification in adipose tissue in children is a known threat to advance into health issues, like intolerance to glucose, metabolic syndrome, diabetes mellitus 2, hypertension, heart failure, dyslipidemias, fatty liver disease and prostate, renal, endometrium, ovarian carcinomas, etc.,[21] The source of Diet induced obesity or DIO is found to be due to variations in the control of calorie intake. Weight gain and obesity is mostly due to buildup of triglycerides, lipids and adipocytes in the fatty tissues which is related to overeating leading to excess of calories.[22,23] A person's biotype in adulthood is determined by ones weight in childhood.[24-26]

Increase in adipose tissue can happen by two documented mechanisms: increase in cell size and/or number of cells of adipocytes. These mechanisms are under the control of factors like, aeging, food intake, hormones and genetics modifications.[27-29] which governs the expansion of fat tissues from two percent to seventy percent of one's own body weight in retort to a a positive energy balance.[26]

Association Amid Adipose Tissue And Obesity - An Insight Into

An overview on obesity studies

Studies have shown that in females, maternal weight gain, diabetes during pregnancy and alteration in nutritional intake leads to hyperplasia of adipose tissue cells, in one's initial part of life. So, a person's biotype in adulthood of becoming overweight or obese is determined by ones extra load in childhood due to hyperplasia of adipocytes in the fatty tissue.[16,24,26] Physiologically, adipose tissue is a part of endocrine system that is involved in the control of the cardiovascular and other organ function.[30] Obesity is a clinical condition characterized by subclinical inflammation due to the predominance of adipokines, often causing tissue damage and if not treated with adequate care can even lead to organ damage.[31] Obesity is a known cardiovascular risk factor as it causes endothelial dysfunction damaging the myocardial blood vessels and affecting the perfusion of multiple organs towards their end stages. However, studies still lack of information about the clarification of the underlying causes leading to these damages and hence basic biomedical research will play an important role to understand these topics.

Obesity research - use of rodents

Epidemiological and clinical studies have shown that fat intake in directly related to obesity as was evident in various animal studies but obesity research in various rodent models have also found that an abnormally great desire for food or excessive eating , also called as hyperphagia may not always be associated with weight gain as scientific literature reveals that

visceral adipose tissue accumulation occurs even with isocaloric diets as it has a direct proportion with the dietary fat content.[32,33] Studies in mice have concluded that in the subcutaneous tissue, adipocyte cell progenitors proliferate which is mainly induced by high fatty diet.[27,29]

Studies with rodent models - a known gold standard for research in obesity, have dual effects, i.e., its benefits of being easily correlated with human studies but cost effectiveness still remains a matter of discussion. Hence alternative research models is the need of the hour for the study of pathogenic contrivances of obesity and assessment of drug intercession, as obesity still remains one of the major modifiable causes of various co-morbidities which if not taken care or treated in correct time might lead to fatal conditions.

Zebra Fish - An Alternative Model For Research In Obesity

Zebra Fish - it's contribution to basic biomedical research

Our understanding of genetic and nutritional factors to regulate the expansion of AT (adipose tissue) in obesity was because of several studies conducted on animal models. To study on obesity, its metabolic disorders, to discern and examine the cause and development and to test novel drugs these zebrafish models have gained importance. Being an ideal model for biomedical research, including ease in maintenance and giving quick developmental process, this model reveals quite a lot of anatomic, genetic and functional features of mammals.[34] Additionally, there are a lot of similarity between adipocytes of zebrafish and that of mammals, and the incidence of all key viscera's required in fat metabolism makes this zebrafish model a perfect tool to study obesity, metabolic disorders and adipogenesis.[35] Microbiota in mammals have been exposed to have a role in obesity and obesity-related disorders, and this microbiota can be easily restrained in zebrafish. Hence, zebrafish models on obesity and diseases pertaining to various other metabolic disorders have been established which directed to finding out the genes and other medicinal constituents influencing metabolism in lipids and buildup of AT.[36]

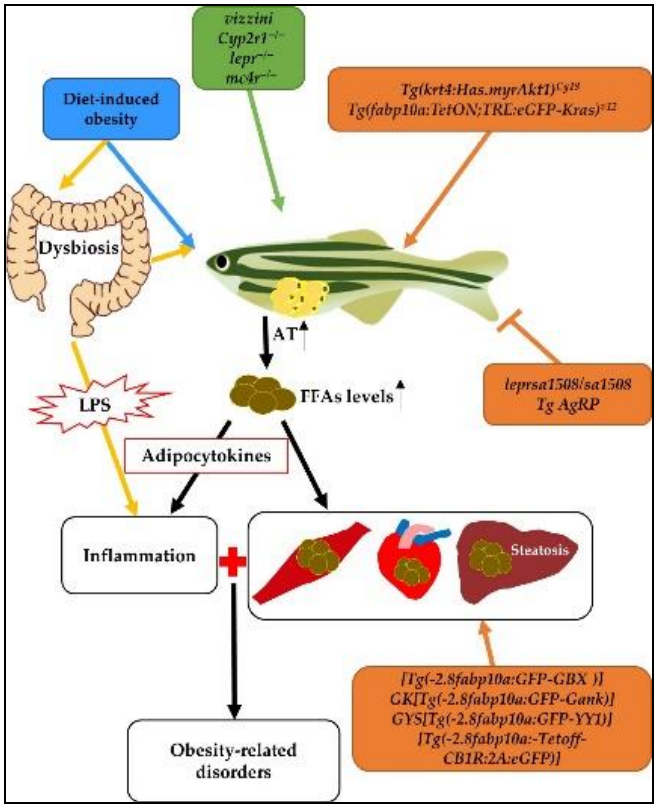

Figure 1. Genes involved and drugs having potential for altering metabolism of lipids and AT buildup.

Zebra Fish and mammals - effects observed in various obesity models

In recent times, zebrafish have been used in various basic biomedical research but literature lacks information about it being used extensively for studies in various obesity models, is spite of having multiple advantages, as compared to other animal models, as evident from various published articles.[37] Though, studies reveal that one of the first researches evident on zebrafish showing the relation between adipose tissue and obesity under normal conditions was evident in 2010.[38] experiments reported a direct relationship between the formation adipocytes and a fat rich diet, especially on live zebrafish larvae in the same year which was then followed in the corresponding year by various tests for evaluation of obesity in the larvae of Zebrafish.[39] Nile red, a dye which was injected into the adipose tissues, was found to be a key component, useful in the study of pathogenesis of obesity in this model.[40,41,42] A positive correlation exists between the metabolism of lipids in zebrafish and mammals as found in the studies. Researches have not only reported that

beta cell formation is stimulated by over nutrition but have also shown that, excess of insulin also called as hyperinsulinemia leads to changes in the adipose tissue as evident from changes in the metabolism due to accumulation of lipids.[43,44] since it is known that, acting as a growth factor, insulin can cause macrosomia most probably by inducing proliferation of precursors of adipocytes.[43] Also, like in mammals, the main source of lipid absorption is from dietary sources from where it is then delivered to the liver from the intestines and finally in the whole body, being transported by lipoprotein carriers like very low density lipoproteins or VLDL, low density lipoproteins or LDL and high density lipoproteins or HDL.[45,46] with leptin playing a key role in homeostatic mechanisms as it controls the melanocortical system response. With the confirmation of vital functions being played by adiponectin and adiponectin receptors, agouti-related protein, peroxisome proliferator-activated receptors or PPARs SCAP/SREBP in lipid metabolism in these species by various genetic studies along with the dietary effects on BMI and triglyceride levels, drugs with obesogenic and anti-obesogenic actions have also been documented successfully in this model.[47,48,49,50,51,52] It has been observed that in overfeeding with a high-fat diet over a long period, though, an initial phase of diet induced obesity or DIO resistance occurs in young zebrafish, long-term weight gain and BMI remain high in those overfed since an early age and hence defines the criteria of obesity in this model also.[53]

Studies on cardiac edifices starting from life in the embryo to full grown stage are well recognized in zebrafish models with the exception that these were all under physiological conditions only.[54,55] Looking into the cardiovascular changes like, globular-shaped heart, hypertrophy of visceral adipocytes, spongy myocardium, etc., ropes theories associated with dietetic inequities and the rapport amid obesity and cardiovascular ailments.

A positive correlation has been observed between overfeeding and obesity with respect to metabolically healthy obesity or MHO and metabolically unhealthy obesity or MUO. Studies on childhood obesity and zebrafish as a model for its correlation in adults with the use of inflammatory markers have been well documented in scientific literature.[56] as it is now known that in humans, children developing in obesogenic environment could be at a much higher risk of mortality and morbidity due to cardiovascular diseases in adulthood.[57,58]

Diet-persuaded obesity prototype in adult Zebrafish

The animal models *which simulate obesity* in humans *with revealing* metabolic pathways have gained high importance, of which diet-induced models are very common. The steady increase in weight that happens in human population as a result of energy balance for years is very well recapitulated by these models. The first model of diet-induced obesity or DIO in zebrafish was established by Oka et al in the year 2010.[39] He overfed these zebrafish with *Artemia nauplii* a usual live feed for short period that significantly increased the BMI that demonstrated hepatic steatosis and hypertriglyceridemia at the completion of treatment.[39] Another study with long-term exposure to hypercaloric diet demonstrated NAFLD, hepatic steatosis followed by fibrosis. When comparing overfed fertile female zebrafish with old females and all males the former (fertile females) developed less fatty liver and were free of fibrosis.[59]

The studies conducted on humans have validated that NAFLD is less in females than in males and the incidence of NAFLD was less common in females of reproductive phase.[59] This collective evidence was constant in relation to role of inflammation in conversion from NAFLD to NASH.[60] During the reproductive phase since the estrogen levels are higher there is less chances of inflammation.[59] These interpretations proposes that using zebrafish in estimation of fibrosis development in different reproductive phases, with an added benefit that ovarian senescence in zebrafish is spontaneous.[59,61] Furthermore, a consistent characteristic in these dual models was an increased penetrance of growth of liver steatosis and obesity, while in rodent models, only a few treated animals established these state of affairs.[62,63] These zebrafish models were fed in a meal feeding pattern that was same as that humans. The same method was intended to rodents but it gave an unreliable data that was different with each experimental animals. When the food was unceasingly available, it was then the obesity consistently developed in rodents.[64]

The microarray investigation has demonstrated a specific gene pattern that was similar to human NAFLD in diet-induced obesity zebrafish model.[65] There was significant deregulation in genes involved in fat digestion and absorption, clotting of blood and platelet instigation when associations between DNA microarray of zebrafish and mammalian VAT were carried out. Furthermore, zebrafish diet-induced models responded to caloric restriction and to treatments with natural compounds by decreasing BMI and adapting the manifestation of genes associated with obesity.[66,67,68]

In humans a high-fat diet is positively linked to obesity, which is true in the case of rats and mice that presented increase in body weight and fat when provided with high-fat diet.[69,70] Dietary lipids was also evaluated in zebrafish by giving a high cholesterol diet, that had gained weight and exhibited an increased accumulation of adipose tissue in the abdominal areas.[71] Another study by Meguro et al on zebrafish body fat volume was associated with effect of four various diets such as starch, gluten, corn oil and lard. Differences in feed efficacy amid the clusters were insignificant, but those which were treated with corn oil and lard revealed increased fat volumes, reliable with preceding trials on rodents. Certainly, in rodents the amount of nutritional fat, was displayed to be liable of increase in body fat.[70] In humans there was differences in fat accumulation and tendency to develop obesity associated metabolic disorders.[72] A few subjects presented with *'*metabolically healthy obesity' categorized by lack of metabolic irregularities, less VAT *with* reduced penetration of macrophages into adipocytes, with smaller cell size of adipocyte.[73] In contrasts the 'metabolically unhealthy obese' phenotypes exhibited a destructive distribution of adipose tissue, with larger fat cells, increased visceral fat and exposed inflammatory progression.[74] In a comparison done amongst zebrafish fed on high-fat diet (HFD) and normal-fat diet (NFD) confirmed that both dietary treatments significantly augmented the basal metabolic rate. MRI investigation of the same revealed larger visceral and smaller subcutaneous adipose tissue in HFD when compared to NFD animals. There was significantly increased blood glucose, triglyceride and cholesterol levels along with noticeable accrual of lipids in liver and muscles in HFD overfed zebrafish.[75] These facts propose that, zebrafish could be similar to both 'healthy' and 'unhealthy' obesity phenotypes depending on food regimen and type of diet that provide models for studies of regulatory mechanisms involved in these pathways and also metabolic conditions. Recently, a zebrafish model of T2DM was standardized.[76] To develop a model of overweight with high blood sugar levels, animals were fed with increased amount of commercial food. In relation to calories, each overfed animals was provided with 408 calories/per vs. 150 calories/day according to Oka et al.[39] This treatment method was able to rapidly induce an elevated BMI, increase adipose tissue volume and plasma triglycerides, it also diminished glucose tolerance, elevated insulin production and augmented beta cell mass, consistent with insulin resistance model of T2DM. Remarkably, this T2DM model was receptive to drugs having antagonistic actions to high blood sugar levels and has transcriptome pathways comparable to human disease.[77]

All the mentioned zebrafish models of DIO promote probability of these animals being used to find out progress of obesity with its associated disorders in an arrangement that bear a resemblance to the human pathological alterations. It is very unfortunate that a consistent food regime for collective solicitation in these models have not been expressed as yet.[76] Many diet regimens with varying dietary configuration have been implemented. When the diet varied in nutritional composition, there was alteration in the intake of energy, body conformation and adipose tissue accumulation. Difficulties in husbandry settings also disturb the energy outlay resulting in caloric imbalance.[64,76]

Conclusions

It is now well documented that there is an eighty percent resemblance with human genome, with complete sequencing of zebrafish[78] genome as indication ropes the theory of positive association amid increased lipid content in food items, obesogenic pathways, abnormal regulation and weight gain and the development of cardiovascular diseases[79] and hence findings in zebrafish could be extrapolated to human beings. Also, with studies providing information

about DIO in zebrafish model[80], it can be looked upon as a beneficial, cost effective experimental set up to introspect into the correlation existing between obesity and non-communicable chronic diseases, including obesity with its effects which in the long run might help to recommend prevention with pharmacological actions and hence could help in sustenance of public health-care agendas intended to the goal of plummeting the universal problem of ailments interrelated with obesity, at large.

Declaration of Interest

The authors report no conflict of interest.

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