

# Zebra fish model of obesity: Relevance to metabolic syndrome

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## Abstract

**Aim:** Recently, the application of zebrafish as an experimental animal model has significantly increased, especially model for obesity. The aim of this research is to reveal the association between high-fat-diet-induced obesity and metabolic syndrome on the zebrafish model. **Materials and Methods:** Male zebra fish were used in this study. Acclimatization period was performed for 2 weeks when all fish received standard diet intake. Post acclimatization, zebra fish were divided into normal group and obese group. For 4 weeks, normal group received normal diet while the obese group received high-fat diet. Measurement parameters including body mass index (BMI) and blood biochemical parameters. Liver histopathological analysis was performed to observe nonalcoholic fatty liver disease (NAFLD). **Results and Discussion:** Zebra fish were divided into two groups such as normal group and obese group. Zebra fish were fed following the protocol for 4 weeks. Obese zebra fish showed increased of BMI and blood biochemical parameters compared to the normal group. In addition, excessive fat accumulation in obese zebrafish liver gave a contribution on the elevated NAFLD based on the histopathological study. **Conclusion:** It can be concluded that high-fat-diet-induced obesity on zebrafish has a strong association with metabolic syndrome.

**Key words:** High-fat diet, metabolic syndrome, model, obesity, zebrafish

## INTRODUCTION

Increase in obesity incidence has been observed and its becoming a major public health issue in the world.<sup>[1]</sup> Etiology of obesity was known as imbalance between energy intake and energy expenditure. Obesity has strong association against metabolic syndrome including Type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia.<sup>[2-4]</sup> The metabolic syndrome is described as metabolic abnormalities involving obesity, impaired glucose metabolism, dyslipidemia, and elevated blood pressure that contributed against cardiovascular disease. Other condition which associated with obesity is a nonalcoholic fatty liver disease (NAFLD). NAFLD is a condition which is characterized by excessive fat accumulation in the liver without a history of alcohol use. The incidence of NAFLD has increased in the world and is linear with the increase of obesity, T2DM, and dyslipidemia incidence.<sup>[5]</sup>

Various studies explained a relationship between excessive dietary intake and obesity

condition. In the experimental animal, especially rodents (rats or mice), the obese model was acquired by genetic mutations and the use of excessive calorie or hyperlipidemic diet intake methods. Although rodent models have given more contribution against on the understanding of human obesity, experiment using rodents need several supports including laboratory staff and good infrastructural system. Therefore, the options of simpler and more inexpensive animal models should be considered.<sup>[6]</sup>

Zebra fish (*Danio rerio*) are one of the simple and inexpensive animal models beside other animal models such as worms or fly which were used to observe metabolism process disorder. Zebra fish are a tropical fish which can be found in India

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and South Asia.<sup>[7]</sup> Recent studies, application of zebra fish as the experimental animal model significantly increased.<sup>[8]</sup> As a vertebrate, zebra fish have structural similarities with human involving the gastrointestinal system, cardiovascular system, visceral adipose tissues, musculoskeletal system, and neuroendocrine system.<sup>[9]</sup>

Consequently, the similarities of internal organs, neuroendocrine signaling system, and tissue of zebrafish allowed the use of this animal as a model of human disease, especially obesity. The results of this study have revealed that high-fat-diet-induced obesity in zebrafish has a strong association with elevated metabolic syndrome.

## MATERIALS AND METHODS

Animals adult zebra fish were obtained from Biopharmaca Research Center, Bogor Agricultural University Institut Pertanian Bogor (IPB), Bogor, Indonesia. All zebra fish in this study were maintained under a controlled environment involving 12:12-h light:dark cycle at 26°C, pH 7.5, and water quality conditions were maintained according to the zebrafish guidance.<sup>[10]</sup> The zebra fish which were used in this study were age 4 months post-fertilization, male fish, and with body weight is 0.1–0.2 g. All procedure in this study has been accepted by Animal Ethic Committee of Biopharmaca Research Center (IPB) (No.96-2018 IPB).

### Experimental Design

Male adult zebra fish were used for this study. Acclimatization period was performed for 2 weeks when all fish received standard diet intake. Post acclimatization period, zebra fish were divided into normal group and obese group ( $n = 15$  in each group). For a period of 4 weeks, normal group received normal diet and obese group received high-fat diet. The obese group received 240 mg/group/fish of the experimental diet/daily. High-fat diet for this research was prepared with ingredient: Fat and oils (50%), protein (12%), fiber (1.4%), and other compounds (8.5%). Body weight of zebra fish was measured 2–3 times/weeks. Zebra fish length was measured from the head part to the end of the body. Obese criteria in zebra fish were determined based on the body mass index (BMI) calculation.<sup>[8]</sup> Blood collection for biochemical analysis was performed before and after the induction period. Technique for blood collection was based on Pedroso *et al.*<sup>[11]</sup> Histopathological analysis on the liver of obese zebrafish was performed to observe liver disorder. Histopathological analysis in this study was used hematoxylin-eosin staining.<sup>[12]</sup>

### Statistical Analysis

The data were expressed as means  $\pm$  standard error of the mean (SEM). Statistical comparisons were performed using

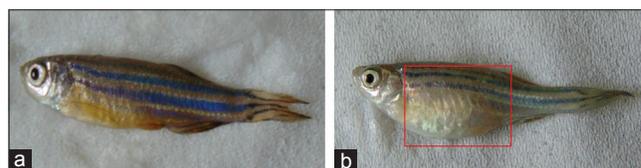
the *t*-test for unpaired data or one-way ANOVA followed by least significance difference test.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

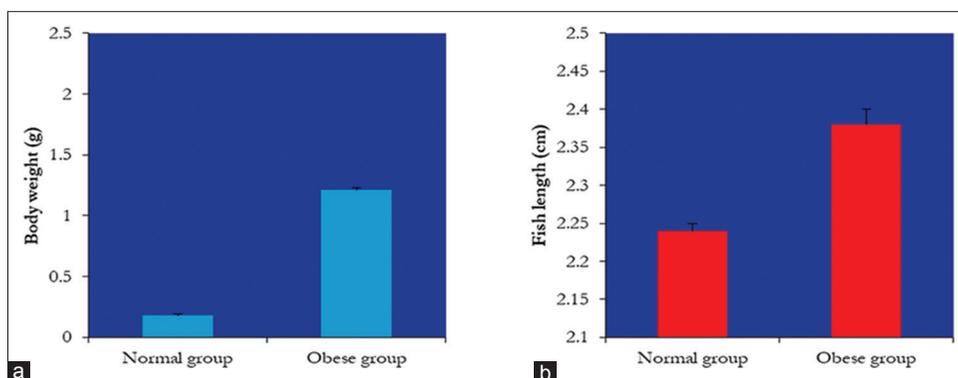
In this study, the obese group was developed by high-fat diet administration for 4 weeks (240 mg/group/fish of the experimental diet/daily). The obese group exhibited increased of body weight, BMI, fasting blood glucose, low-density lipoprotein (LDL), triglyceride (TG), and fat accumulation in the liver. Figure 1a and b showed size differences of normal and obese zebrafish group. Size differences have been seen between normal group and obese group, especially in an abdominal area [Figure 1b]. This condition indicated that abdominal area is one of the targets of fat accumulation. Increased of fat accumulation was followed by changes of BMI.

The body weight and fish length were measured 2–3 times/weeks during the study. Differences of body weight between normal group and obese group were seen on the week-2 (normal group:  $0.192 \pm 0.02$  g and obese group:  $0.354 \pm 0.02$  g). After week-4, obese group showed a significantly increased on body weight compared to normal group (normal group:  $0.255 \pm 0.01$  g and obese group:  $0.658 \pm 0.40$  g) ( $P < 0.05$ ). In addition, in the obese group, increase in the length of zebrafish compared to the normal group. Differences of body weight and fish length between normal and obese group showed in Figure 2a and b. Furthermore, body weight and fish length were used to measure BMI.

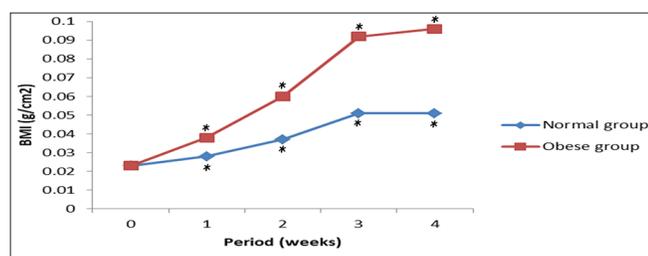
BMI was used to determine obese criteria. BMI of zebrafish was calculated using formula: Body weight (g) divided by the square of the fish body length (cm). Obesity criteria in male fish are when the BMI  $\geq 1.1$  fold and BMI  $\geq 1.3$  fold for female fish.<sup>[8]</sup> Figure 3 showed that obese group has significant difference in BMI compared to normal group ( $P < 0.05$ ). In the last of the induction period, BMI of normal group and obese group is  $0.051$  g/cm<sup>2</sup> and  $0.096$  g/cm<sup>2</sup>, respectively. High-fat-diet-induced obese zebra fish exhibited a hyperglycemia condition compared to normal group [Figure 4]. After 4-week induction with a high-fat diet, fasting blood glucose concentration of normal group was  $29.33 \pm 9.25$  mg/dl and the obese group was  $87.33 \pm 8.6$  mg/dl ( $P < 0.05$ ). Before induction with a high-fat diet, fasting blood glucose concentration of normal group and



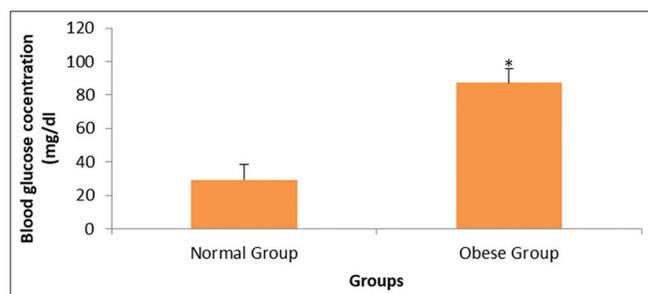
**Figure 1:** Normal (a) and obese zebrafish (b) Red box shows a change in the size of the abdomen of obese zebrafish



**Figure 2:** Difference in body weight (a) and length (b) between normal and obese group after 4 weeks ( $n=15$ ). Statistical analysis was done using unpaired  $t$ -test with a confidence interval of 95%. (a) Body weight (mean  $\pm$  standard error of the mean [SEM])  $0.255 \pm 0.01$  g from normal group and  $0.658 \pm 0.40$  g from obese group which showed a statistically significant difference ( $*P < 0.05$ ). (b) Statistically significant difference in the length of fish from normal (mean  $\pm$  SEM:  $2.24 \pm 0.01$  cm) and obese group ( $2.38 \pm 0.02$  cm;  $*P < 0.05$ )



**Figure 3:** Body mass index (BMI) of zebrafish. Statistical analysis was done using the least significant difference test with a 95% confidence interval. BMI of normal (◆) and obese (■) zebrafish over 4 weeks period



**Figure 4:** Fasting glucose concentration (mg/dl) difference between normal group and obese group after 4 weeks (\*): ( $P < 0.05$ )

obese group was  $29.28 \pm 8.20$  mg/dl and  $31.55 \pm 8.45$  mg/dl, respectively. Blood collection of zebrafish for hyperglycemia analysis was performed on before and after induction by high-fat diet using glucometer strip.

Based on the lipid parameter, high-fat diet-induced obese zebra fish showed increased of LDL and TG compared to the normal group. After 4-weeks induction with a high-fat diet, LDL concentration of normal group and obese group was  $28.20 \pm 5.60$  mg/dl and  $69.90 \pm 10.35$  mg/dl ( $P < 0.05$ ), respectively. TG concentration after induction by high-fat diet for normal group and obese group was  $151.20 \pm 9.60$  mg/dl and  $380.00 \pm 8.35$  mg/dl ( $P < 0.05$ ), respectively.

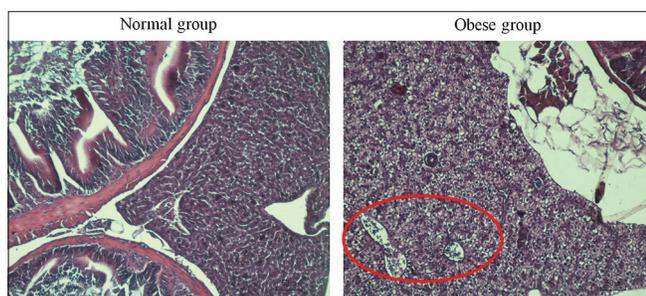
Blood collection of zebrafish for LDL and TG analysis was performed on before and after induction by high-fat diet.<sup>[11]</sup>

Figure 5 showed a liver histopathological of normal zebrafish and obese zebrafish. In the normal group, the histopathological study did not show an abnormality on liver tissues structural. Furthermore, in the obese group showed major enlargement on hepatocyte and elevated vacuolar degeneration compared to the normal zebrafish.

## DISCUSSION

Increased of fat consumption has been associated to obesity condition. On the next stage, this condition followed by elevated metabolic syndrome and the development of NAFLD.<sup>[13]</sup> Several researches explained that high-fat diet not only induces obesity in human but also influencing obesity in animals. Therefore, fat accumulation in the animal as a response to high-fat diet administration is most commonly used in obesity research application.<sup>[14,15]</sup> Different with carbohydrate and protein, fat has ability to stimulate excess energy intake by its high luscious and influencing the satiating power. Long-term exposure of high-fat diet, especially in hungry condition, might be sufficient to cause overconsumption of energy with obesity as a result.<sup>[16]</sup> Some studies have demonstrated the ability of hypercaloric and hyperlipidemic diets administration to induce obesity and its metabolic disorders, especially in rodents.<sup>[17]</sup> Another animal model, such as zebra fish have been observed as an obesity model and this model is very appropriate to be developed as an obesity model through high-fat diet administration.<sup>[8-18]</sup>

In the recent study, we use zebra fish (*D. rerio*) to explore the association between high-fat diet consumption, obesity, and metabolic syndrome. The results of this study found that high-fat-diet-induced obesity in zebrafish has an impact on elevated metabolic syndrome and the development of NAFLD. Excess fat accumulation on white adipose tissue



**Figure 5:** Liver histopathology of normal (left) and obese zebrafish (right). Red circle shows enlargement on hepatocyte and elevated vacuolar degeneration

is influencing the release of pro-inflammatory cytokines, providing the pathophysiologic basis of comorbid conditions associated with obesity such as T2DM, dyslipidemia, and the development of NAFLD. Adipose tissue is known to release several proteins which modulate metabolism, energy intake, and fat depot. Adipose tissue not only has a function as energy reservoir but also an immune organ in the body. More than 50 adipokines have been identified and have various functions including leptin, adiponectin, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- $\alpha$ ). These adipokines have important roles in metabolic syndrome-associated obesity.<sup>[19,20]</sup>

The pathogenesis of obesity and T2DM is complex and multifactorial, associated with various tissues and organs involving pancreas, liver, skeletal muscle, and adipose tissue.<sup>[21]</sup> T2DM is a condition characterized by hyperglycemia and abnormalities of carbohydrate, lipid, and protein metabolism. T2DM also produce essential changes in the intracellular process in various tissues. Fat accumulation in visceral adipose tissue has a contribution to the release of pro-inflammatory cytokines and elevated T2DM. Pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, TNF- $\alpha$ , interferon- $\gamma$ , and pancreatic-derived factor influence apoptosis mechanism in pancreatic beta cells. Cytokines in beta cells activate several metabolic pathways to stimulate the cell death which followed by pancreatic beta cells damage.<sup>[22]</sup> It can be explained by the occurrence of hyperglycemia in the high-fat-diet-induced obese zebrafish compared to the normal group. This is in line with other studies which described obese zebra fish have developed by high-fat diet administration showed results of hyperglycemia.

Increased of LDL and TG was found in obese zebrafish compared to the normal group. Increased of LDL and TG can influence the development of insulin resistance, atherosclerosis, and NAFLD.<sup>[23,24]</sup> Lipid abnormalities as a typical characteristic of the metabolic syndrome might be associated with pro-inflammatory cytokines which released from adipose tissue. In addition, the important association between obesity, the metabolic syndrome, and dyslipidemia appears to be the development of insulin resistance in peripheral tissues stimulating to an increased hepatic flux fatty acids from dietary sources, intravascular lipolysis,

and adipose tissue resistant to the antilipolytic effects of insulin.<sup>[25,26]</sup>

The next consequence of metabolic syndrome is NAFLD development. Excess high-fat diet consumption may affect the liver. NAFLD is condition with excess fat accumulation in liver without a history of alcohol abuse. NAFLD is associated with obesity, insulin resistance, and DM.<sup>[27,28]</sup> NAFLD was classified into simple steatosis and nonalcoholic steatohepatitis (NASH). In NASH, not only steatosis occur but also inflammation in intralobular and hepatocellular ballooning. Long-term NASH might developed to cirrhosis and hepatocellular carcinoma. Animal model of NAFLD/ NASH should be reflected by its liver histopathology.<sup>[29]</sup> Furthermore, the animal model should exhibit metabolic abnormalities such as obesity, fasting hyperglycemia, dyslipidemia, and adipokine profile. In this study, NAFLD condition on obese zebra fish might be reflected. Obese group showed abnormality in liver tissues structural by histopathology study compared to the normal group. Major enlargement on hepatocyte and elevated vacuolar degeneration has seen on the obese group. This result consistent with several researches which described obese zebra fish have developed by high-fat diet administration showed results of NAFLD.<sup>[13-30]</sup> Recent scientific reports showed that a high-fat diet is an ideal model to develop the obese condition and metabolic syndrome on zebrafish.

## CONCLUSION

High-fat diet administration on zebrafish could result in the obese condition. This condition has an impact on metabolic processes such as elevated hyperglycemia, dyslipidemia, and liver disorder in zebrafish. It can be concluded that obesity has strong with association metabolic syndrome.

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