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Long-Term Consumption of High-Fat-High-Fructose Diet Decreased Insulin Sensitivity and Damaged the Islets of *Langerhans* on *Sprague Dawley* Rats

Konsumsi Diet Tinggi Lemak Tinggi Fruktosa dalam Jangka Panjang Menurunkan Sensitivitas Insulin dan Merusak Pulau Langerhans pada Tikus Sprague Dawley

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ABSTRACT

Background: Obesity is linked to the rising incidence of type 2 diabetes, with excessive dietary fat intake being one of the primary causes. The development of fat animal models has been discovered to be a standard experimental strategy based on replicating human behaviour in food consumption.
Objectives: This study aims to investigate how long-term consumption of a high-fat, high-fructose (HFHF) diet affects the indicators of diabetes mellitus in *Sprague Dawley* (SD) rats, such as insulin sensitivity, by measuring the HOMA-IR, counting beta cells, and analyzing the histology of the pancreas.
Methods: This experiment was conducted with 36 male SD rats in normal and HFHF groups. The normal groups had a modified AIN-93 M, while the HFHF group received a high-fat diet with 30% fructose-based water. Feed and beverage intakes were monitored every 24 hours to calculate daily caloric consumption (energy intake) for 17 weeks.
Results: The results demonstrated a significant difference between the normal and HFHF groups in the HOMA-IR levels (insulin sensitivity) and number of pancreatic beta cells (p-value<0.05). This implied that following 17 weeks of HFHF intake, the HOMA-IR level of insulin sensitivity was reduced. However, the islet of *Langerhans* in pancreatic histopathology seemed damaged in the HFHF rats, as evidenced by the changes in their shape and lower beta cell number.
Conclusions: Consuming the HFHF diet over an extended period increased glucose level, decreased insulin sensitivity, and damaged pancreatic histopathology.

INTRODUCTION

Obesity is presently a chronic health problem, triggering metabolic syndrome due to its rapid global development. Although the exact causes of diabetes are not fully understood, obesity is still responsible for 80-85% of Type 2 Diabetes Mellitus (T2DM) risk development[1]. Based on the World Health Statistics in 2021, the WHO stated that the prevalence of obesity had increased by 50%, from 8.7-13.1% between 2000 and 2016[2]. The Global Burden of Disease (GBD) statistics update from 2017 also revealed that 462 million people worldwide, or 6.28% of the population, are estimated to have T2DM[3]. These were in line with the data obtained in Indonesia, where the prevalence of obesity increased from 15.4-21.8% between 2013 and 2018. Based on recent studies on the population of all ages, the prevalence of diabetes mellitus was 2% or approximately 1 million individuals[4].

Over the past few decades, fructose consumption has increased by approximately 30%, paralleling a significant rise in obesity and metabolic disorders[5]. Studies have shown that high fructose intake increases visceral fat accumulation. For instance, a 10-week research study reported an 8.6% increase in abdominal fat in participants consuming fructose-sweetened beverages, compared to a 4.8% increase in those consuming glucose-sweetened beverages[6]. These findings highlight the potential of fructose to more significantly promote fat deposition than other sugars, such as glucose. Consequently, investigating the effects of high fructose diets is crucial to understanding their impact on obesity and related metabolic disorders.

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Obesity reportedly impairs the ability of insulin to affect glucose uptake and metabolism in insulin-sensitive tissues. This is often referred to as Insulin Resistance (IR), as obesity is also found to increase plasma insulin secretion[7]. The Islets of *Langerhans* are clusters of cells in the pancreas that produce hormones, particularly the beta cells that produce insulin. The increased demand for insulin production can exhaust the beta cells, leading to a decrease in insulin production and eventually the onset of T2DM[8]. This indicates that obese people resist insulin, subsequently causing an increase in this hormone in the blood. Additionally, the presence of insulin reduces lipolysis or breakdown of fat and increases fat formation and uptake[7]. Fat intake is considered one of the leading causes of obesity, where more than 30% of the energy sources are found to affect the ailment significantly. A high-fat diet is also observed to influence the development of obesity in both humans and animals. Therefore, experimental animals, such as mice, are often used in several studies to deal with obesity[9]. A positive interaction was observed between high-fat-sucrose and weight gain in experimental rats.

Various sources are often used to induce obesity conditions in experimental animals, such as high-fat feeding through the addition of glucose/High-Fat Diet (HFD)[10,11] and fructose/High Fat Fructose Diet (HFFD)[12,13], respectively. Various applicable methods have also been found to create conditions for diabetic experimental animals, especially mice. Therefore, this study aims to evaluate the impact of long-term consumption of a high-fat, high-fructose (HFHF) diet on diabetes indicators in SD rats. This includes assessing glucose, insulin, and HOMA-IR levels, as well as the number of beta cells and the histopathology of the islets of *Langerhans*.

METHODS

This study was conducted from 2017 to 2018 across three laboratories. The animal treatments were carried out at the Biosains Laboratory of Universitas Brawijaya, where experimental protocols involving rats were performed. Subsequent analyses were conducted at two additional laboratories: plasma analysis was performed at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, while histopathological examination of pancreatic beta cells was carried out at the Anatomy-Histology Laboratory, Faculty of Medicine, Universitas Brawijaya.

Animals Model

Based on this study, 36 male SD rats (weighing 150 to 250 g) were purchased from the Animal Laboratory of Universitas Gajah Mada, Yogyakarta, Indonesia. These rats obtained veterinary care in the animal house of the Biosains Laboratory of Universitas Brawijaya, Indonesia. They were also acclimatized to their environment for four weeks before the start of the experiments. Furthermore, all animals were kept under similar environmental conditions, with daily free access to water and food. The rats were randomly divided into two classes using a randomized sampling method: the normal and HFHF groups. In this study, the standard AIN-93M was fed to the normal group, and the modified AIN-93M was fed to the HFHF group for 17 weeks[14]Each group contained 18 rats in a separate cage at room temperature (24°C) with day and night cycles. The institutional animal care and ethics committee of the Faculty of Medicine, Universitas Brawijaya (No. 368/EC/KEPK/10/2017) approved all protocols on October 24, 2017.

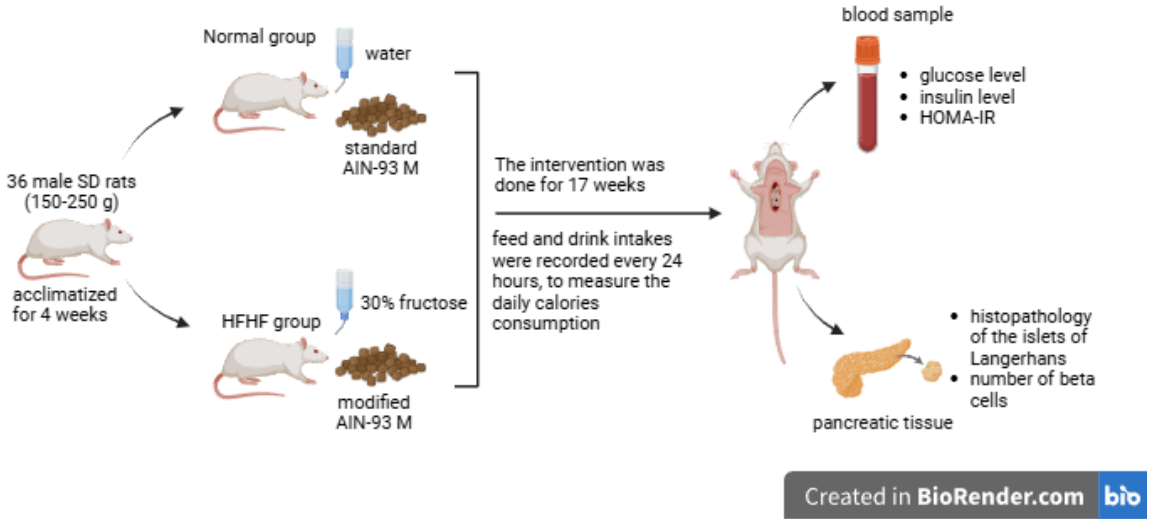

Figure 1. The outline of the research conducted

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Feeding Composition and Dietary Intake

The AIN-93M standard feed consists of corn starch, dextrinized corn starch, sucrose, soybean oil, casein, egg-white flour, agar, minerals, and the AIN vitamin mix, along with L-cysteine, choline bitartrate, and tertiary butylhydroquinone (TBHQ). Meanwhile, the AIN-93M modified HFHF feed includes additional lard and fructose from the standard formulation of AIN-93M to increase fat and fructose content. This modification aims to simulate a high-fat, high-fructose diet. The HFHF group was also given high fructose drinks with 30% concentration, containing 0.3 kcal/mL energy and 1.2 g/mL carbohydrate. The energy density of the Normal and HFHF group feeds was 4.21 and 5.08 kcal/g, respectively[15,16]. The diet was given ad libitum and contained 20 g/day of rat feed and 250 mL/day of water. The actual food and drink intake was calculated by subtracting the remaining food (g) and drink (mL) per 24 hours from the initial weight.

Plasma Analysis

Euthanasia was carried out using a combination of ketamine (1 ml) and xylazine (0.5 ml), as blood samples were obtained at the end of the study. This was subsequently accompanied by the analysis of fasting blood glucose and insulin levels[13] and the HOMA-IR calculation[17]. These indicated that trunk blood samples were obtained from overnight fasted animals for 16 hours. This was subsequently accompanied by collecting blood samples from the heart, where the glucose levels were measured using a hand-held glucometer (ACCU check). However, the plasma blood samples were obtained and centrifuged at 2000 g and 4°C for 10 minutes to calculate insulin levels. This level was measured using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) (E-EL-R2466; Elabscience Biotechnology, China). This analysis was conducted by trained laboratory staff at The Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya.

Histopathology Procedures

The rats were then sacrificed for organ collection after 17 weeks of treatment, with the pancreas tissue utilized in this study. Subsequently, this tissue was fixed using the 10% formalin embedded in paraffin for approximately 7 hours, before being manually sectioned with a microtome to obtain 3-5 mm-thick sections. In addition, the dewaxed sections were heated using an oven at 70-80°C for 30 minutes. These were initially soaked in xylol and then stained with hematoxylin and eosin (H&E). The stained sections were rinsed with running water to remove excess stains. Subsequently, the sections were dehydrated through a series of graded ethanol solutions (70%, 80%, 90%, and absolute ethanol) for a few minutes at each concentration. After dehydration, the sections were cleared using xylene to enhance tissue transparency. Finally, the sections were mounted using a synthetic resin-based mounting medium and covered with glass coverslip. The prepared slides were then observed under a light microscope to analyze the histopathological characteristics[18]. This analysis was conducted by trained laboratory staff at the Anatomy-Histology Laboratory, Faculty of Medicine, Universitas Brawijaya.

Statistical Measurements

The statistical analysis was conducted using SPSS version 25. All data were presented as mean±standard deviation (SDev). An independent t-test was used to analyze the differences in energy intake and insulin levels. In contrast, glucose levels and the HOMA-IR index were analyzed using the Mann-Whitney U test. A p-value<0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

Obesity is one of the significant global issues influenced by energy intake and increased body weight. However, the weight level does not necessarily represent the ailment. The increasing body-fat accrual and triglyceride levels also supported the label of an obese status[19]. Several studies on obesity were found to be challenging due to its complex etiology involving genetic, metabolic, behavioral, and environmental factors. Using genetically modified animal obesity and leanness models, several studies have significantly expanded the knowledge of the physiological and molecular mechanisms affecting energy balance[9]. In this condition, the excessive consumption of fat and fructose played a vital role in increasing animal body weight[20]. Subsequently, weight gain increased the risk of heart disease and mortality in moderate and severe overweight.

Figure 2 shows that the rats' average energy intake calculated from diet and fructose consumption showed a significant difference between the two groups (p-value<0.05). However, fructose consumption increased energy intake than feed consumption in the HFHF group. The average feed intake in this group was also lower than that of the normal group. According to Ferreira in 2011, the AIN-hyper cholesterol diet 93M was used as the fat source to feed rats, where the average intake was 15.47 g, lower than that of the normal group[21]. This was caused by several factors regarding the rats' state and the feeding rhythmicity. For example, the variation in the total feed ingested, size, and frequency of meals played a role in the rats' physiological state with time[22,23]. Moving in and out of the cage daily increased the rats' stress during the feed intake measurement.

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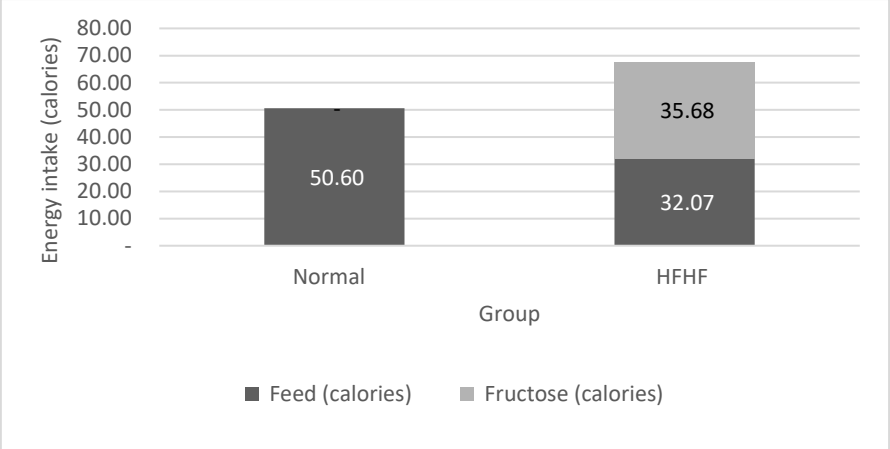

Figure 2. The average energy intake calculated from the diet and fructose consumption of the rats showed a significant difference between both groups (p-value<0.05).

This study is part of previous research[24] that investigated the effects of an HFHF diet on animal models' metabolic parameters. Previous findings demonstrated that body weight increased in the HFHF group while decreasing in the normal group. This body weight change (approximately 40%) was used to develop an obesity model in animals. The current study builds upon these findings by further examining the metabolic impact of an HFHF diet. Another study also observed the phenomenon of long-term fructose diet consumption, which indicated an interaction between a high-fructose diet and the regulation of food intake. The consumption of a fructose diet also induced metabolic disorders, although not accompanied by obesity or an increasingly significant body weight[25].

Bocarsly *et al.* in 2010 also examined both short- and long-term effects of a high fructose diet on body weight, fat, and circulating triglycerides. The result showed that the rats' long-term access to a high fructose diet led to obesity, while sucrose did not[19]. This indicated that the composition of the feed ingredients in the present experiment contained only vegetable fat (soybean oil), which made it more challenging to increase the weight of the experimental animals. According to Kylie Kavanagh in 2006, an animal-based diet provides higher fat than a plant-based diet. This indicated that the male monkeys fed with trans-fat diets, such as lamp, beef, and dairy products, had a 7.2% increase in body weight, compared to the 1.8% increment in those who ate monounsaturated fats, olive and soybean oil[21,26]. Feed composition and energy density are closely related to rat diet factors, which can influence their diet intake. Rat feed factors include texture, viscosity (for fructose drinks), and organoleptic conditions. In this study, the form of normal diet food had a denser texture, could be held by rats, and adapted to the shape of the rats' mouths as rodents. Meanwhile, the HFHF diet is coarser and breaks down easily, making it difficult for mice to consume. Based on observations from an organoleptic perspective, the aroma emitted by the HFHF feed is slightly sharp compared to a normal diet. The sharp aroma could be due to adding more significant amounts of lard to the HFHF diet. Lard contains saturated fatty acids, which can easily oxidize and cause a sharp aroma[27,28].

Rats have a body physiology that is almost the same as humans, as well as eating behavior, which environmental and psychological conditions can influence[29,30]. In this study, rats appeared to have relatively high levels of stress as evidenced by the presence of rats that had psychological disorders due to several factors, such as external disturbances during feeding, taking leftover feed, weighing, moving cages, and other disturbances. Stress is a non-specific response from the body to the demands it receives. Rats can also experience stress, especially from the environment, such as cage capacity, and external disturbances, such as sound, sight, and changes in cage position[31]. Stress can activate adrenocorticotropic hormone (ACTH) and cortisol secretion. Cortisol will stimulate an increase in central fat deposition, which will reduce the hormone leptin, a decrease in leptin, which will provide an adiposis signal, and an increase in ghrelin, which will provide an orexigenic signal, and the release of ghrelin, which contributes to the regulation of appetite[32].

Apart from the psychological condition, there is also a factor in the physiological condition of the mice, namely that the mice experience boredom while receiving the HFHF diet, which contains high fat and high fructose. Saturation is related to the feed content and energy density of the feed. The energy density of feed is influenced by the water and fat composition of the feed, feed with high energy density has low water and high fat content, while feed with low energy density has high water content and low fat. It is known that rats tend to prefer food with low energy density[29].

This was in line with Mayasari and Rahayuni's research in 2014, where the high-fat diet group had a lower feed intake (17.94 g) than the normal group (19.00 g)[33]. This signified that the high-fat feed content reduced intake and required more extended time in emptying the stomachs for rats to feel full quickly[34]. It also influenced the rats' appetite based on the feed's texture, smell, and organoleptic state. The rough texture and unscented feed (rancid) were among the factors causing a decrease in the appetite of rats[35].

Plasma Glucose, Insulin, and HOMA-IR

According to Table 1, both groups' glucose and HOMA-IR levels showed significant differences (p <0.05).

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This implied that the HFHF diet significantly increased blood glucose and insulin levels, with the decreased insulin sensitivity based on the increase of the HOMA-IR index (p-value<0.05). Meanwhile, the insulin levels between both groups did not show a significant difference, although they were higher in the HFHF group. These were analogous to several previous studies, which stated that high-fat and fructose diets (HFD and FRD) caused dyslipidemia in non-diabetic rodents[36]. This indicated that fructose and fat provided excess lipids from different sources, based on the increase in endogenous and exogenous lipids availability, respectively[20]. However, the feeding combination of the two diets in this study provided similar metabolic phenotypes in rats, as confirmed by the increased plasma glucose levels in the HFHF group. High fasting glucose was another frequently observed indicator of sugar intolerance. This was consistent with several earlier studies that reported glucose intolerance utilizing high-fat or high-fructose diets, with normal fasting sugar levels of 8.28. For instance, Han et al. fed Wistar rats diets containing 50% fat for 32 weeks. After 10 weeks, they saw enhanced weight gain, and starting at 16 weeks, their glucose levels were consistently high[37].

Table 1. Metabolic Parameters in SD Rats Maintained on Normal and HFHF Groups After 17 Weeks of Feeding

Parameter	Normal Group	HFHF Group	p-value
Energy Intake (calories)	54.02±7.59	72.41±6.30	0.000*
Insulin (mIU/L)	14.47±2.04	15.78±2.80	0.161*
Glucose (mmol/L)	7.58±1.60	8.89±1.69	0.023**
HOMA-IR index	4.78±0.76	6.30±1.97	0.016**

p-value was tested on the Independent T-Test (*) and Mann-Whitney (**), (p-value<0.05) indicates the parameters showed significant differences between the two groups. The Normal group received AIN-93M standard feed, while the HFHF group received AIN-93M modified with added lard and fructose, including 30% high-fructose drinking solution.

According to this present study, the plasma insulin levels of the rats were measured by an Enzyme-linked Immunosorbent Assay (ELISA) at the end of the treatment period or week 17. This signified that the independent t-test did not show a significant difference between the insulin levels in the HFHF and normal groups. However, range values of the HFHF group were more significant than the normal group at 12.17 IU/ml (minimum) and 22.26 IU/ml (maximum), respectively. This was in line with D'Angelo *et al.* in 2005, where the fructose diet group had greater insulin levels at 1.3-2.5 than the control group[38]. However, the sensitivity of this hormone showed a different perspective, with several previous studies indicating that fructose-fed rats had metabolic abnormalities related to insulin resistance or the degradation of insulin sensitivity. Consequently, increased vasoconstrictor sensitivity, vascular dysfunction, decreased endothelium-dependent relaxation, potassium channel function, as well as elevated vascular superoxide production independent of hypertension, were some of the observed abnormalities[39–41].

Insulin sensitivity was measured by calculating the HOMA-IR (homeostasis model assessment score-insulin resistance), through the formulation described by Mathew *et al.* in 1985[17]. In this study, the values were calculated using the HOMA-IR blood code online calculator (https://www.thebloodcode.com/), marking the presence and extent of any expressed insulin sensitivity. This terrific method was used to show the dynamics between the baseline (fasting) blood sugar and the responsive insulin. According to this study, the HOMA-IR was used as a surrogate marker of insulin sensitivity and directly correlated with glucose level and energy intake in SD rats. As shown in Table 1, the values of the HOMA-IR index were significantly different between the normal and HFHF groups (p-value<0.05). However, the normal group showed increased metabolic abnormalities due to the high energy density in the feed formulation (4.2 calories/g). The increase in the HOMA-IR level caused by the high intake level also indicated the declining insulin sensitivity in the HFHF group[7].

Insulin sensitivity decreases with increasing insulin resistance. The pancreas produces more insulin when an individual is insulin-resistant because it takes more insulin to have the same effect on blood glucose regulation. Conversely, a high level of insulin sensitivity means that the pancreas does not need to work as hard to create large amounts of insulin because the body will respond well. This is typically a sign of excellent health and a decreased chance of type 2 diabetes or other metabolic issues.

Number of Pancreatic Beta Cells

Insulin is a hormone that reduces blood glucose levels. β-cells in the pancreas create, store, and secrete insulin. Each islet has many types of endocrine cells. Insulin-secreting β-cells comprise approximately 80% of the islets, followed by pancreatic α-cells (15%) that release glucagon and pancreatic δ-cells (5%) that secrete somatostatin[42]. The number of pancreatic beta cells was subsequently observed in this study after 17 weeks of treatment, as shown in Table 2. This indicated lower cell values in the HFHF group compared to the normal group. These results were in line with the consumption of a high-fat diet, triggering an increase in free fatty acids[43]. The accumulation of these acids also reduced insulin-mediated glucose utilization in peripheral tissues, commonly known as insulin resistance. Moreover, insulin resistance led to the failure of Insulin Receptor Substrate-1 (IRS-1) proliferation and decreased GLUT-4 translocation and glucose oxidation, resulting in the absence of sugar in the cells, subsequently causing hyperglycemia. This indicated that the pancreatic beta cells were compensated for responding to the phenomenon by producing large amounts of insulin, leading to hyperinsulinemia[44].

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Table 2. The number of Pancreatic Beta Cells on Normal and HFHF Groups After 17 Weeks of Feeding

Pancreatic Beta Cells	Normal Group	HFHF Group	p-value
Average Number (cells)	126.76±14.71	77.13±14.54	0.000*
Maximum (cells)	180	130	
Minimum (cells)	63	29	

p-value was tested by Independent T-Test (*), (p-value<0.05) indicates that the parameters showed significant differences between the two groups. The Normal group received AIN-93M standard feed, while the HFHF group received AIN-93M modified with added lard and fructose, including 30% high-fructose drinking solution.

In this study, the continuous occurrence of hyperinsulinemia caused the failure of beta cells to respond to high blood glucose levels, subsequently leading to insulin resistance and abnormalities in the insulin signal transduction pathways[5,45]. The insulin resistance in the pancreatic beta cells further led to activation of the caspase pathway and increased ceramide levels, which induced apoptosis. Hyperinsulinemia conditions also cause an increase in the production of free radicals, especially Reactive Oxygen Species (ROS), whose excessive accumulation over a long period causes chronic oxidative stress and cell death[45,46]. The level of insulin resistance is inversely proportional to insulin sensitivity, which means that the reduced number of pancreatic beta cells was in line with the decreasing insulin sensitivity.

Subsequently, the combination of the HFHF diet triggered the release of pro-inflammatory cytokines, which induced apoptosis in the pancreatic beta cells. The release of these cytokines, such as IL-1, TNF, IFN, IFN gamma, and nuclear factor, was also triggered by an excessive increase in MCP-1, caused by high consumption of fat and fructose[13]. These compounds induced apoptosis in beta c through a series of gene transcriptions. The activation of NF-kappa-β resulted in the generation of NO, chemokines, and calcium depletion in the endoplasmic reticulum (reticulum stress). This then triggered and released Mitogen-Activated Protein Kinase (MAPK) and apoptotic signals through the mitochondria, resulting in beta-cell death[47].

Chronic exposure to high glucose or fructose concentrations can reduce β-cell viability through oxidative stress. Hyperglycemia-induced oxidative stress has been found to cause mitochondrial permeability transition and cell death in human endothelial cells[48]. In the HFHF group, the decrease in the number of beta cells was caused by high-fat and fructose consumption. This was in line with a previous study, which stated that the provision of a High-Fructose Diet (HFR) caused an increase in body weight and lipolysis, continuously leading to an elevation in apoptotic free fatty acids[47,49,50]. The results also revealed that the apoptotic elevation was due to fructose being very efficient in inducing De Novo Lipogenesis (DNL) by providing carbon atoms for glycerol and acyl-CoA. Furthermore, a synthesis of triglycerides and increased liver fat accumulation was observed in this study, subsequently causing a decrease in insulin sensitivity[50]. This was in line with several previous studies, where rats were fed a diet containing 30% fructose, which induced metabolic syndrome. Adding 60% fructose to the rat diet for eight weeks also caused hyperuricemia, hypertriglyceridemia, and VLDL. These conditions consequently led to hyperinsulinemia, which triggered apoptosis in beta cells[10].

Pancreatic Histopathology

The staining of Hematoxylin and Eosin on pancreatic organs was used to determine the level of damage to the islets of *Langerhans* due to the HFHF diet. These islets are clusters of endocrine cells scattered in the exocrine glands of the pancreas, where each *Langerhans* islet contains three main cell types: alpha, beta, and delta[51]. Damages to the islets of *Langerhans'* structure and morphology are often caused by systemic metabolic changes, due to insulin insensitivity and loss of glucose control. Additionally, these islets' size increase (hypertrophy) and decrease (atrophy) are stimulated by insulin hypersecretion-induced IR[35].

According to Figure 3, the pancreatic histopathology showed that the *Langerhans'* islets were damaged. In the HFHF group, these organs became irregular in shape, with some parts also observed to be empty. The *Langerhans'* cell density and nucleus were also discovered to decrease and disappear. Meanwhile, the histopathology of the control group showed that the morphology and structure of these organs were normal. It is as if the cells were homogeneously distributed throughout the *Langerhans*. Damages were not observed in these organs' cells and structures. The density and shape were found to be good and almost spherical. This indicated that the high-fat and fructose diet caused the pancreatic histopathological changes in the HFHF group. The damage observed in this group was also caused by the apoptotic performance of the cells, which affected the density of pancreatic beta cells. Furthermore, an increase in free fatty acids caused beta-cell apoptosis, MCP-1, ROS elevation, and hyperinsulinemia, which occurred due to the consumption of the HFHF diet[22]. Previous research conducted by Mutiyani *et al*. in 2014 by providing an HFD to experimental animals showed that a high-fat diet can cause a decrease in pancreatic beta cell density. Mice given an HFD had a lower density, namely 57.98 mm^2, while those on a normal diet had a density of 86.95 mm^2. The number of beta cells influences beta cell density; the more beta cells there are, the greater the beta cell density value[52].

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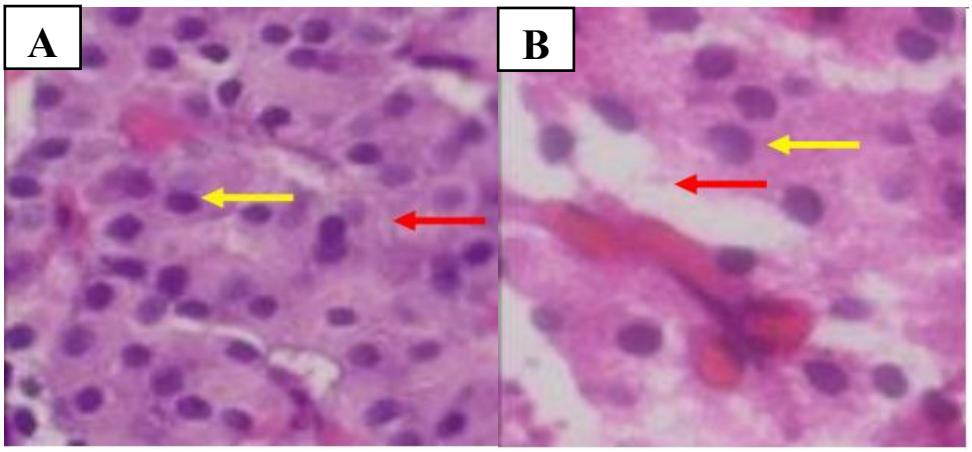

Figure 3. The histopathological description of the pancreas in the normal group (A) and HFHF group (B), using the Hematoxylin Eosin (HE) staining method with 400x magnification. The yellow and red arrows indicate the pancreatic beta cells and empty spaces on the *Langerhans*, respectively. The normal histopathological tissue showed higher cell density than the HFHF group.

This study provides valuable insights into the metabolic impacts of an HFHF diet, particularly its role in impairing insulin sensitivity and damaging pancreatic beta cells. Using animal models allows controlled conditions and direct measurement of key metabolic markers. However, the findings may not fully translate to human physiology, and further research involving clinical trials is necessary to validate these results.

CONCLUSIONS

The study of metabolic abnormalities in animal models has significantly contributed to obesity and insulin sensitivity. In these models, several aspects of metabolic function were available for direct measurements, such as glucose and insulin levels, and calculating the HOMA-IR index to measure insulin sensitivity levels. The results showed that the long-term consumption of an HFHF diet contributed to glucose elevation and reduced insulin sensitivity. Meanwhile, the insulin level showed an insignificant difference between groups. The histopathological observation also showed that the HFHF diet damaged *Langerhans'* islets and reduced the number of pancreatic beta cells. Notably, the normal group exhibited better metabolic markers than the HFHF group, emphasizing the adverse effects of the HFHF diet.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors have no conflict of interest in this article. This study was funded by the Faculty of Medicine, Universitas Brawijaya, with contract number 28/SK/UN10.7/PN/BPPM/2017.

AUTHOR CONTRIBUTIONS

ADK: conceptualization, investigation, methodology, writing–original draft and editing; LSK and AH: investigation and data analysis; ES: methodology, formal analysis, writing–review; IK and DH: conceptualization, methodology, supervision, writing review.

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