



Abdominal Ultrasonography Imaging after High-Fat Diet Administration in Wistar Mice (*Rattus norvegicus*)

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ABSTRACT

According to World Health Organization (WHO) estimates, hypercholesterolemia contributes to over four million annual fatalities and more than half of coronary heart disease cases. Alterations in dietary patterns involving high-cholesterol and saturated fat foods lead to metabolic system abnormalities, impacting the digestive system, endocrine glands, reproductive system, immune system, and notably, the cardiovascular system. This study aimed to examine abdominal ultrasound imaging in Wistar mice (*Rattus norvegicus*) subjected to high-fat diets for 30 days to determine the concentration that can induce fat accumulation in visceral organs. The mice were categorized into five groups, each receiving a high-fat diet with varying concentrations (0.5, 1, 1.5, 2%) for 30 days. Abdominal ultrasonography was performed after the administration of the high-fat diet. The results showed that all control groups exhibited normal organ conditions. However, in the treatment groups, observations revealed no discernible boundaries of the atrial and ventricular cavities of the heart, indicating fat accumulation, visually identified as a hyperechoic appearance. In the liver, the entire treatment group displayed abnormal changes; the margins of the liver were hyperechoic in almost the entire liver, indicating fat buildup. Similarly, the spleen exhibited a hyperechoic image in almost the entire cavity. In conclusion, this study demonstrates that administering a high-fat diet comprising 0.5 to 2% of the total body weight of mice led to fat accumulation in the heart, liver, and spleen. This is characterized by a hyperechoic image, indicating fat presence and unclear boundaries between the cavities in these organs.

Keywords: High-fat diet, Hypercholesterolemia, Ultrasonography, Heart disease, Hyperechoic, Atherosclerosis

INTRODUCTION

Coronary heart disease (CHD) is currently the leading cause of death worldwide. The primary cause of this disease is coronary atherosclerosis, which develops slowly due to endothelial dysfunction, vascular inflammation and cholesterol accumulation in blood vessel walls (Antman and Braunwald 2007). CHD occurs as a result of narrowing and hardening of the coronary blood vessels, caused by the accumulation of fats and cholesterol in the walls of the blood vessels, a condition known as atherosclerosis. The primary factors contributing to the development of atherosclerosis and CHD include hypercholesterolemia, hyperlipidemia, and an unhealthy lifestyle, including the

consumption of high-fat foods, which can exacerbate the condition (Andrea et al. 2024, Zhuang et al. 2024).

Hypercholesterolemia is a major risk factor for CHD (Debra 2004). Hypercholesterolemia and hyperlipidemia, referring to elevated levels of total cholesterol and lipids in the blood, are major risk factors for atherosclerosis and the development of coronary heart disease. The World Health Organization (WHO) estimated that hypercholesterolemia is associated with more than half of all CHD cases and more than four million deaths each year (Andrea et al. 2024). Hypercholesterolemia is an increase in fasting low-density lipoprotein (LDL) cholesterol levels without a corresponding increase in triglyceride levels (Grundy 2006). Specifically, low-density lipoprotein (LDL)

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cholesterol is recognized as a key factor in the formation of atherosclerotic plaques that obstruct blood flow and worsen heart conditions. Recent research suggests that a high-fat diet, obesity, and lipid metabolism disorders play a significant role in increasing LDL levels in the blood and exacerbating cardiovascular conditions (Suowen and Jianping 2020).

Hypercholesterolemia is also associated with hypertriglyceridemia, which is defined as an increase in the level of triglycerides, the primary lipid in food, to ≥ 150 mg/dL (Webster-gandy et al. 2010). Elevated blood triglyceride levels can increase very low-density lipoprotein (VLDL) concentrations, which in turn can increase the risk of plaque formation in the arteries and, over time, lead to atherosclerosis (Miller et al. 2011). Increased triglyceride levels are influenced by several factors, including excessive fat and energy intake, obesity, and low physical activity (Krummel 2008). Causes of hypercholesterolemia include a diet high in cholesterol or saturated fatty acids, weight gain, aging and genetic factors (Grundy 2006).

Several recent animal studies have shown a strong relationship between a high-fat diet, obesity, and increased LDL levels, leading to the development of atherosclerosis (Saputri et al. 2023). In experimental animals, high-fat diets often result in elevated blood lipid levels, changes in the structure and function of blood vessels, and the development of atherosclerotic lesions similar to those observed in humans (Omachi et al. 2024). Furthermore, a reduction in LDL levels in animals has also shown improvements in lipid profiles and a decrease in the risk of atherosclerosis (Jorge et al. 2021). The fundamental cause of blood vessel congestion is the presence of cholesterol and lipoproteins, especially LDL, in the artery walls (Netala et al. 2024). Normally, LDL is able to exit and enter the artery walls through the endothelium. Oxidized LDL will trigger the accumulation of white blood cells (monocytes and T lymphocytes) in the walls of blood arteries, which will then trigger an inflammatory effect (Rastini et al. 2011).

The primary objective of this research is to ascertain the dosage required to elicit the effects of high cholesterol feeding on the liver, heart, and spleen. The findings of this research may offer insights into the implications of high cholesterol feed at varying proportions on CHD, which is caused by elevated cholesterol levels in the body. Moreover, this research aims to contribute to the prevention of escalating metabolic diseases and is deemed a crucial component in aiding the reduction of metabolic and cardiovascular disease incidences, thereby enhancing the overall quality of life. Additionally, this research will provide valuable information to assist veterinarians in managing cases related to diseases triggered by increased cholesterol levels in animals within Indonesia. Therefore, it is essential to investigate the impacts of high cholesterol feed.

MATERIALS AND METHODS

This experimental laboratory research was conducted at the Integrated Laboratory of the Veterinary Medicine Study Program, Faculty of Medicine, Hasanuddin University, Indonesia. The research employed a purposive

sampling method, a technique used when the researcher has a target individual with characteristics suitable for the research (Turner 2020). In this case, the experimental animal used was the white rat (*Rattus norvegicus*) with the ethical clearance number 0035/KKEH/RSHUH/EC/2023.

The study utilized 25 male white rats, distributed into five treatment groups. These rats, weighing between 150–250 grams, were obtained from rat breeders in Makassar, South Sulawesi. The control group (KK) was given a standard food composition *ad libitum* for 30 days. Treatment group 1 (KP1) received a high-fat diet composition of 2% of total body weight (BW), treatment group 2 (KP2) received 1.5% of total BW, treatment group 3 (KP3) received 1% of total BW, and treatment group 4 (KP4) received 0.5% of total BW, each administered for 30 days. The rats were housed in groups using plastic containers and covered with wire at the top of the cage. The rats were first weighed, acclimatized for seven days, and treated according to animal handling instructions. During adaptation, the rats were provided with food and drinking water *ad libitum*. Each group was maintained at the same location and time under appropriate conditions.

The study observed abdominal ultrasonography (USG) images of Wistar rats (*Rattus norvegicus*) that had been fed a high-fat diet to examine images of their heart, liver, and spleen organs. The aim was to determine whether fat accumulation occurs in the heart, liver, and spleen after being fed a high-fat diet.

Data analysis

Data were analyzed descriptively, focusing on the differences in organ interpretation across each treatment group that was administered a high-fat diet.

RESULTS & DISCUSSION

This research was undertaken to examine the differences in the organs of white rats that were fed a high-fat diet, which was clinically indicated to influence the accumulation of fatty tissue in the organs of white rats in each treatment group. A total of 25 male white rats were selected using purposive sampling. The ultrasound examination was conducted at the Veterinary Teaching Hospital of Hasanuddin University.

Generally, three types of reflections (echoes) can be observed on ultrasound: hyperechoic, hypoechoic and anechoic. Hyperechoic echoes are bright and appear white on the sonogram. Hypoechoic echoes are slightly lower than their surroundings and appear gray-black on the sonogram. Anechoic echoes produce no echo and appear black on sonograms. Fig. 1-5 present a comparison of changes in the organs of white mice from each treatment group found in this study.

Ultrasound results of heart in Fig. 1A after high-fat diet administration in control group (KK) show that no fatty heart was detected, boundaries between the atrial and ventricular cavities of the heart (the red circle) are clearly visible. The hypoechoic area represents the boundary between the heart cavities (the yellow arrow) and the anechoic area represents a cavity filled with fluid (the green arrow). The boundaries of the spleen organ are clearly visible (the red circle) in Fig. 1B. The spleen appears normal with a hypoechoic area in the spleen region (the yellow

arrow) and the boundaries of the liver are clearly visible (the red circle) in Fig. 1C. The liver appears normal with a hypoechoic area in the liver region (the yellow arrow).

Ultrasound results of heart in Fig. 2A after high-fat diet administration in treatment group (KP1) show a fatty heart is visible as indicated by a hyperechoic appearance (the yellow arrow). Normally, an anechoic appearance of a blood-filled cardiac cavity is observed. The boundaries between the cavities in the heart (the red circle) are not clearly visible. The boundaries of the spleen (the red circle) in Fig. 2B are not clearly visible. A hyperechoic appearance is found in almost the entire cavity of the splenic area, indicating the presence of fatty deposits. The spleen appears abnormal with a hyperechoic area in the splenic area (the yellow arrow). The boundaries of the liver (the red circle) are not clearly visible. A hyperechoic image is found in almost the entire cavity of the liver area, indicating the presence of fat. The liver appears abnormal with a hyperechoic area in the liver area (the yellow arrow) in Fig. 2C.

Ultrasound results of heart in Fig. 3A after high-fat diet administration in treatment group (KP2) show that the boundaries of the atrial and ventricular cavities of the heart (the red circle) are not clearly visible. A hyperechoic image is found in almost all cavities in the heart area, indicating the presence of fat. A hypoechoic area is observed in the heart cavity filled with fluid (the yellow arrow). The boundaries of the spleen (the red circle) in Fig. 4B are not clearly visible. A hyperechoic appearance is found in almost the entire cavity of the splenic area, indicating the presence of fatty deposits. The spleen appears abnormal with a hyperechoic area in the splenic area (the yellow arrow). A hyperechoic appearance (the red circle) in Fig. 4C is visible in several places. Normally, a hypoechoic appearance of the liver is observed. The liver margins appear hyperechoic (the yellow arrows), indicating a fatty liver.

boundaries of the spleen (the red circle) in Fig. 3B are not clearly visible. A hyperechoic appearance is found in almost the entire cavity of the spleen area, indicating the presence of fatty deposits. The spleen appears abnormal with a hyperechoic area in the splenic area (the yellow arrow). A hyperechoic image is observed (the yellow arrow) in Fig. 3C. Normally, a hypoechoic appearance of the liver is seen. The boundaries between liver margins (the red circle) are unclear.

Ultrasound results of heart in Fig. 4A after high-fat diet administration in treatment group (KP3) show that the boundaries of the atrial and ventricular cavities of the heart (the red circle) are not clearly visible. A hyperechoic appearance is found in almost all cavities in the heart area, indicating the presence of fatty deposits. A hypoechoic area is observed in the heart cavity filled with fluid (the yellow arrow). The boundaries of the spleen (the red circle) in Fig. 4B are not clearly visible. A hyperechoic appearance is found in almost the entire cavity of the splenic area, indicating the presence of fatty deposits. The spleen appears abnormal with a hyperechoic area in the splenic area (the yellow arrow). A hyperechoic appearance (the red circle) in Fig. 4C is visible in several places. Normally, a hypoechoic appearance of the liver is observed. The liver margins appear hyperechoic (the yellow arrows), indicating a fatty liver.

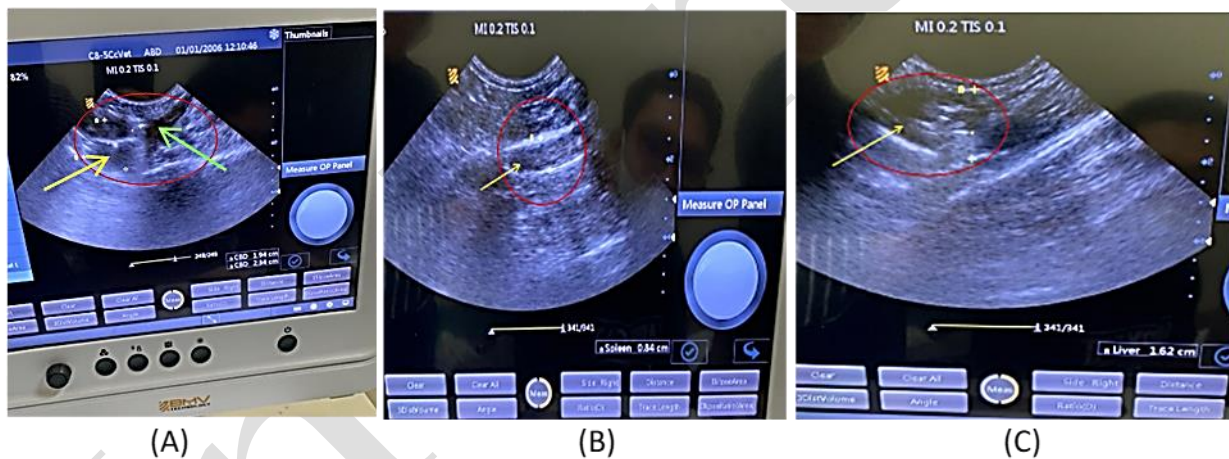


Fig. 1: Ultrasound Results of Heart (A), Spleen (B), and Liver (C) After High-Fat Diet Administration in Control Group (KK).

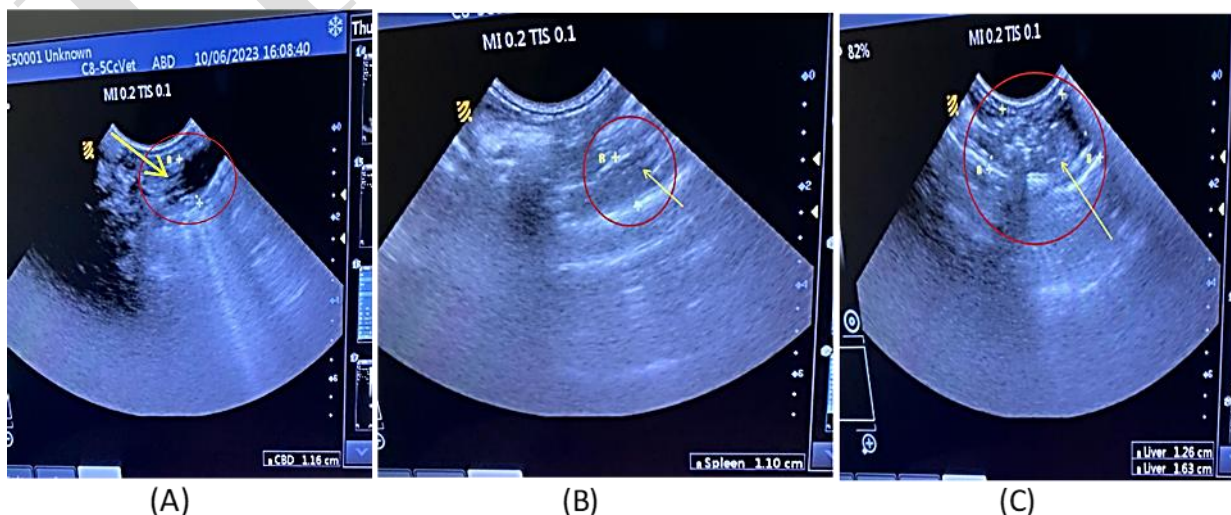


Fig. 2: Ultrasound Results of Heart (A), Spleen (B), and Liver (C) After High-Fat Diet Administration in Treatment Group 1 (KP1).

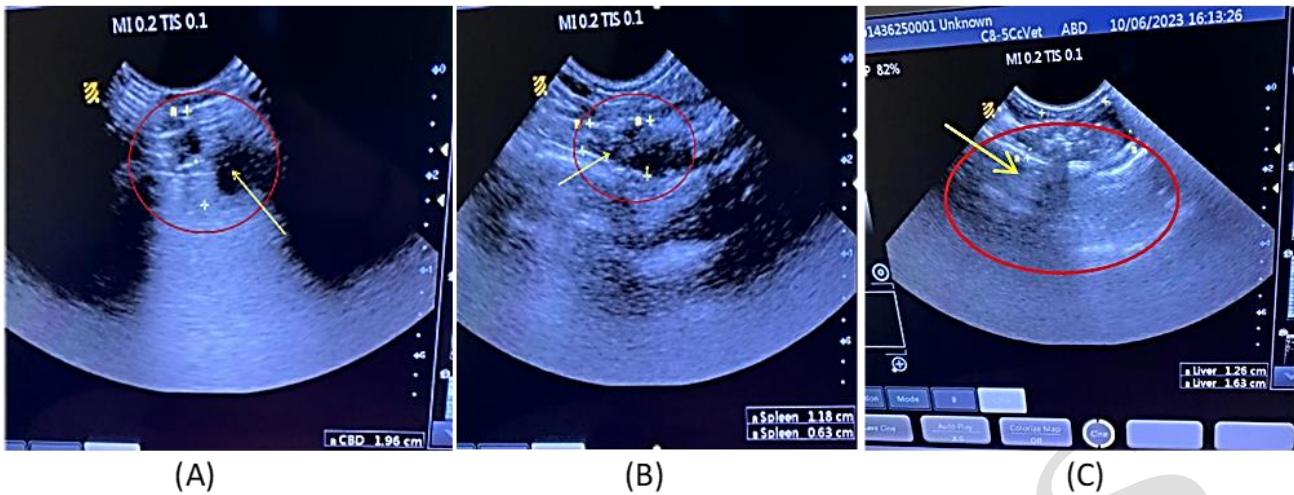


Fig. 3: Ultrasound Results of Heart (A), Spleen (B), and Liver (C) After High-Fat Diet Administration in Treatment Group 2 (KP2).

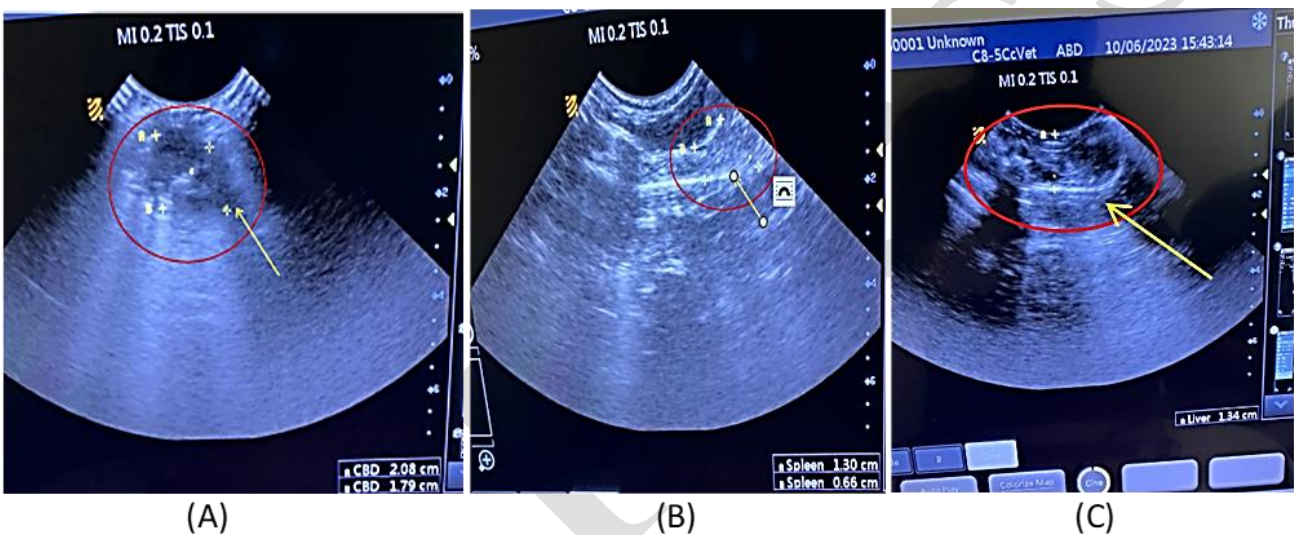


Fig. 4: Ultrasound Results of Heart (A), Spleen (B), and Liver (C) After High-Fat Diet Administration in Treatment Group 3 (KP3).

Ultrasound results of heart in Fig. 5A after high-fat diet administration in treatment group (KP4) show that the boundaries of the atrial and ventricular cavities of the heart (the red circle) are not clearly visible. A hyperechoic image is found in almost all cavities in the heart area, indicating the presence of fat. A hypoechoic area is observed in the heart cavity filled with fluid (the yellow arrow). The boundaries of the spleen (the red circle) in Fig. 5B are not clearly visible. A hyperechoic appearance is found in almost the entire cavity of the spleen area, indicating the presence of fat. The spleen appears abnormal with a hyperechoic area in the splenic area (the yellow arrow). A hyperechoic image (the red circle) in Fig. 5C can be seen in several places. Normally, a hypoechoic appearance of the liver is observed. The margin of the liver appears hyperechoic (the yellow arrow), indicating a fatty liver.

Fig. 1-5 show the differences in each treatment group after being administered a high-fat diet. The heart plays a central role in maintaining physiological homeostasis by acting as the primary pump for blood circulation, ensuring the delivery of oxygen to tissues and the removal of metabolic waste products. These vital functions are indispensable for the survival and health of animals, as they support cellular respiration, metabolic processes, and organ

function. The cardiovascular system is responsible for the continuous flow of oxygenated blood throughout the body, while simultaneously collecting carbon dioxide and other waste metabolites to be excreted via the lungs and kidneys. Recent studies have illuminated the interaction between the heart's pumping capacity, lipid metabolism, and the potential impact of low-density lipoprotein (LDL) levels on these processes (Suowen and Jianping 2020). In the control group (KK), there were no changes or accumulation of tissue in the heart and the boundaries between the ventricles and atria of the heart were clearly visible. However, in the treatment groups (KP1, KP2, KP3, and KP4), the boundaries of the atrial and ventricular cavities were not visible, accompanied by fat accumulation interpreted as a hyperechoic appearance.

Increased thickness of epicardial fat is associated with insulin resistance (Shi et al. 2023). This condition disrupts the active and efficient transfer of glucose into myocardial cells. Consequently, myocardial cells predominantly rely on breaking down fatty acids for energy utilization. This heightened energy demand is predominantly met through the use of fatty acids from triglycerides stored in epicardial fat cells. Within adipose tissue, triglycerides—which serve as the energy reservoir in the form of fat—are broken down

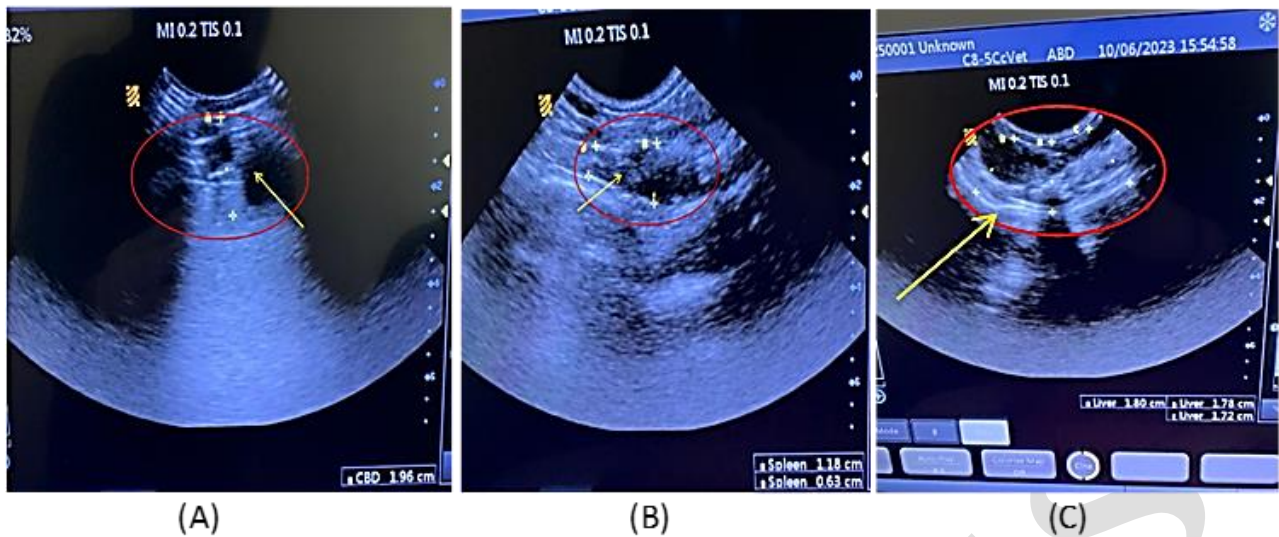


Fig. 5: Ultrasound Results of Heart (A), Spleen (B), and Liver (C) After High-Fat Diet Administration in Treatment Group 4 (KP4).

into glycerol and three fatty acids. These fatty acids are then transported into cells through blood vessels by binding to lipoproteins, forming LDL. An increase in LDL levels within visceral fat tissue, including epicardial fat, contributes to increased lipotoxicity in both the myocardium and coronary arteries. The LDL can directly infiltrate the tunica intima and tunica media of coronary vessels and initiate the process of atherosclerosis. The end product of fatty acid breakdown is ketone bodies, which can induce endothelial injury. Furthermore, the buildup of ketones decreases pH, exacerbating endothelial injury progression (Gaborit et al. 2017).

The role of the heart as the organ responsible for delivering oxygen to tissues is well-established (Zhao et al. 2023). Oxygenated blood is carried from the lungs via the heart to tissues, where it is utilized in cellular respiration to produce energy. As noted by Suowen and Jianping (2020), the heart's ability to pump blood efficiently is crucial for maintaining adequate oxygen delivery, particularly in tissues with high metabolic demand such as the brain, muscles, and kidneys. However, lipid metabolism, specifically elevated LDL levels, has been shown to negatively impact the cardiovascular system's ability to function optimally. High LDL levels are associated with the development of atherosclerosis, a condition in which lipid deposits accumulate in arterial walls, leading to plaque formation, narrowing of the arteries, and reduced blood flow (Das and Nishikant 2023). These changes can impair the heart's ability to pump blood effectively, thereby compromising oxygen delivery to tissues.

The link between high LDL levels and cardiovascular dysfunction is well-documented in both human and veterinary medicine (Sánchez-León et al. 2024). Elevated LDL levels are commonly referred to as a major risk factor for cardiovascular diseases, as they contribute to the formation of plaques in the arteries, a process known as atherosclerosis. According to George et al. (2021), in animal models, elevated LDL levels are strongly associated with the development of endothelial dysfunction, which impairs vascular relaxation and increases the risk of hypertension. In such animals, the heart compensates for the decreased blood flow by increasing its rate and force of contraction, which can lead to cardiac stress, left

ventricular hypertrophy, and eventually heart failure (Ioanna et al. 2020).

Furthermore, the role of LDL extends beyond cardiovascular dysfunction to influencing the removal of metabolic waste products (Wang and He 2024). Efficient waste removal is integral to maintaining homeostasis, as the heart's pumping action facilitates the transport of metabolic by-products, such as carbon dioxide and urea, to the lungs and kidneys, respectively, for excretion. High LDL levels, however, have been shown to exacerbate renal dysfunction and reduce the heart's efficiency in circulating blood, further hindering the removal of metabolic waste (Jorge et al. 2021). As LDL contributes to atherosclerotic changes in the blood vessels, the subsequent reduction in perfusion and oxygen delivery to renal tissue can lead to kidney damage and poor waste elimination. This cascade of events further stresses the importance of regulating lipid metabolism to prevent systemic dysfunction.

The liver plays a vital role in detoxification, metabolism, and maintaining homeostasis within the body (Yang et al. 2024). As a key organ in the breakdown and clearance of toxic substances, the liver helps filter blood from the digestive tract before it circulates throughout the body. It is also involved in metabolizing fats, proteins, and carbohydrates, and in the synthesis of critical proteins like albumin and clotting factors. In the control group (KK), the ultrasound image of the liver showed clear boundaries, indicating a normal liver with a hypoechoic appearance. However, in all treatment groups (KP1, KP2, KP3, and KP4), the liver showed abnormal changes. The margins of the liver were hyperechoic in almost the entire liver, suggesting a buildup of fat. Variations in echogenicity and texture in the liver parenchyma can be caused by liver disorders such as liver cirrhosis, liver abscess, chronic hepatitis, and fatty liver (Victor et al. 2021).

Furthermore, the liver is responsible for breaking down and eliminating waste products, including bilirubin, urea, and excess cholesterol. However, liver function is often compromised in various pathological conditions, particularly in states of hypercholesterolemia, obesity, and elevated low-density lipoprotein (LDL) levels, leading to significant liver cell damage.

Hypercholesterolemia, characterized by elevated cholesterol levels, is closely linked to liver dysfunction. In both humans and animals, high cholesterol levels, particularly elevated LDL, contribute to the accumulation of cholesterol in the liver, leading to a condition known as non-alcoholic fatty liver disease (NAFLD). As described by Das and Nishikant (2023), elevated LDL levels can lead to the deposition of lipids within liver cells, impairing their ability to function effectively in detoxification and metabolic processes. This lipid accumulation leads to hepatocyte stress, inflammation, and potential fibrosis, which can progress to more severe liver conditions such as cirrhosis or hepatocellular carcinoma if left untreated. In veterinary medicine, hypercholesterolemia has been observed in various species, including dogs and cats, and is increasingly recognized as a significant contributor to liver disease.

Obesity is another key factor that exacerbates liver dysfunction through its impact on lipid metabolism. Obesity is often associated with increased adiposity, insulin resistance, and dyslipidemia, which include elevated levels of LDL and triglycerides (Bays et al. 2024). According to Isdadiyanto et al. (2020), obese animals are at a higher risk for developing NAFLD, as excessive fat deposits in the liver lead to steatosis, an accumulation of triglycerides within hepatocytes. This condition not only impairs liver detoxification functions but also triggers inflammatory responses, leading to oxidative stress and further damage to liver cells. The relationship between obesity and liver damage is particularly concerning in companion animals, where overfeeding and sedentary lifestyles contribute to a rising prevalence of obesity-related hepatic disorders.

The link between LDL levels and liver cell damage is especially evident in conditions such as atherosclerosis and hepatic lipidosis. LDL, known for its role in cholesterol transport, when elevated, contributes to the deposition of cholesterol and other lipids in tissues, including the liver. This accumulation results in the formation of foam cells within the liver, contributing to lipid peroxidation and cellular damage. As reported by George et al. (2021), in animal models of hyperlipidemia, the chronic presence of high LDL levels has been shown to induce hepatocellular apoptosis, fibrosis, and a reduction in the liver's detoxification capacity. The resulting damage leads to a vicious cycle, where impaired liver function further exacerbates metabolic disturbances, including hyperlipidemia and obesity, creating a challenging clinical scenario.

One of the most significant effects of liver dysfunction due to hypercholesterolemia, obesity, and elevated LDL levels is the reduction in the liver's ability to detoxify harmful substances from the blood. The liver's detoxification pathways rely on the efficient metabolism of endogenous and exogenous toxins, such as drugs, ammonia, and metabolic waste products. In states of liver damage caused by lipid accumulation and inflammation, these pathways are often disrupted. As noted by Sharon et al. (2021), animals with liver damage from chronic hyperlipidemia often show elevated blood ammonia levels, a condition known as hepatic encephalopathy, which is a direct consequence of the liver's inability to detoxify waste products properly. This accumulation of toxins can lead to severe neurological symptoms and systemic disturbances, complicating the management of affected animals.

Additionally, the ability of the liver to regulate cholesterol levels is critical in preventing the accumulation of excess cholesterol in the body (Xiao et al. 2023). Hypercholesterolemia, coupled with liver dysfunction, can result in a feedback loop where the liver is less able to regulate cholesterol levels, exacerbating lipid buildup and leading to further liver damage. The liver's role in detoxification is compromised as a result, and the body becomes more vulnerable to systemic complications associated with lipid accumulation, including cardiovascular disease and liver failure. The work of Suowen and Jianping (2020) further highlights how the liver's compromised function due to lipid overload also affects its ability to synthesize key proteins involved in lipid metabolism and detoxification, such as apolipoproteins and enzymes necessary for lipid processing.

The spleen, a critical lymphoid organ in mammals, plays essential roles in immune response, blood filtration, and the regulation of hematopoiesis. It consists of two primary components: the red pulp, which is involved in the filtration of blood and removal of aged or damaged red blood cells, and the white pulp, which is integral to immune functions, particularly in the activation of lymphocytes and the initiation of immune responses. Feeding a high-fat diet revealed differences in the ultrasound images of the spleen between the treatment group (KP) and the control group (KK). The treatment groups KP1, KP2, KP3, and KP4 showed that the spleen boundaries were not clearly visible, unlike the KK group, which displayed spleen boundaries. The presence of a hyperechoic appearance in almost the entire cavity of the spleen area in the KP groups indicates the presence of fat, making the spleen appear abnormal with a hypoechoic area. In contrast, the KK group exhibited a hypoechoic area in the spleen area, indicating a normal spleen condition. These results suggest that the high-fat diet altered spleen morphology.

As a vital organ in both immune defense and blood homeostasis, the spleen's function can be significantly affected by various systemic conditions, including hypercholesterolemia, obesity, and elevated low-density lipoprotein (LDL) levels. Recent research has highlighted the connection between these conditions and damage to the spleen's cellular components, which can result in compromised immune responses and altered blood filtration processes.

Hypercholesterolemia, characterized by elevated cholesterol levels, including LDL, has been shown to negatively impact lymphoid organs, including the spleen. The spleen's red and white pulps are susceptible to lipid-related stress, particularly when elevated LDL levels lead to the accumulation of cholesterol and lipoproteins in the bloodstream. As observed by George et al. (2021), high LDL levels can induce a pro-inflammatory state in various organs, including the spleen, leading to an increased infiltration of immune cells such as macrophages and lymphocytes. This inflammatory response, often referred to as atherosclerotic inflammation, can disrupt the normal structure and function of the spleen. In the white pulp, this may result in altered immune cell activation and reduced efficiency in the body's defense against pathogens, while in the red pulp, it can impair the spleen's ability to filter and clear damaged red blood cells.

The relationship between obesity and splenic dysfunction is another area of growing concern. Obesity is associated with chronic low-grade inflammation, insulin resistance, and dyslipidemia, which includes elevated LDL levels. In obese animals, excessive adiposity has been linked to alterations in immune function and the structure of lymphoid organs, including the spleen. According to Sharon et al. (2021), in obese animal models, the spleen undergoes structural changes, including the expansion of both the red and white pulp areas. The excess fat tissue and the subsequent increase in circulating lipids contribute to the development of a pro-inflammatory environment, which disrupts the spleen's normal filtration and immune functions. The accumulation of lipids in the spleen can lead to oxidative stress and cellular damage, further contributing to the dysfunction of the organ. The resulting altered immune responses make the organism more susceptible to infections and less able to regulate the removal of senescent red blood cells effectively.

LDL levels, in particular, have been implicated in both immune dysfunction and splenic damage. Elevated LDL levels promote the accumulation of cholesterol in various tissues, leading to foam cell formation in the spleen and other organs. As reported by Suowen and Jianping (2020), the presence of these foam cells can disrupt normal cellular processes within the white pulp, where lymphocytes are activated in response to pathogens. The accumulation of lipid-laden macrophages can also hinder the ability of the spleen to efficiently filter blood, impairing its role in hematopoiesis and the clearance of abnormal blood cells. Furthermore, lipid-induced oxidative stress in the spleen results in cellular damage and apoptosis of splenic cells, contributing to a decline in immune function and the filtration capacity of the organ.

A significant consequence of these changes is a reduction in the spleen's ability to perform its immunological and hematological functions effectively. The spleen's immune function, primarily mediated by the white pulp, involves the activation of T and B lymphocytes in response to pathogens. In conditions such as hypercholesterolemia and obesity, the damage to lymphocytes and other immune cells within the white pulp may lead to a decreased ability to mount effective immune responses. This results in compromised host defense, making animals more susceptible to infections and diseases. Furthermore, the red pulp, responsible for blood filtration and the removal of damaged erythrocytes, becomes less effective in maintaining homeostasis when the spleen's function is impaired by lipid accumulation and inflammation. As noted by Ioanna et al. (2020), the impaired function of the red pulp in the context of hyperlipidemia may result in the persistence of senescent red blood cells and an increased risk of anemia in affected animals.

In veterinary practice, the understanding of how hypercholesterolemia, obesity, and high LDL levels contribute to splenic dysfunction is critical for the diagnosis and management of related diseases. Early detection of splenic damage in animals with these risk factors is essential for preventing further complications. Strategies such as dietary modifications aimed at controlling lipid levels, weight management to reduce obesity, and medications to lower LDL levels are crucial

for preserving spleen function and overall health. Emerging therapies targeting inflammation and oxidative stress within the spleen may also offer promising avenues for protecting against the adverse effects of hyperlipidemia and obesity on splenic health (Sara et al. 2022).

In conclusion, the spleen's role as a critical immune and hematological organ can be compromised by conditions such as hypercholesterolemia, obesity, and elevated LDL levels. These conditions promote inflammation, lipid accumulation, and oxidative stress within the spleen, leading to damage of both the red and white pulp. This damage impairs the spleen's ability to filter blood and mount effective immune responses, resulting in a heightened risk of infections and metabolic disturbances. Therefore, managing lipid levels and reducing obesity in animals is essential for protecting the function of the spleen and maintaining overall health.

Conclusion

This study provides an overview of the effect of a high-fat diet on ultrasonographic (USG) images of the heart, liver, and spleen. Administering a high-fat diet composition ranging from 0.5 to 2% of the total body weight of rats has demonstrated the presence of fat in the heart, liver, and spleen. This is characterized by a hyperechoic appearance, indicating the presence of fat, and unclear boundaries between the cavities in these organs.

Author's Contribution: Dwi Kesuma Sari: Conceptualized and designed the study and collected data, methodology, review and editing. Rini Amriani: Conceptualization, methodology, data curation, writing-review, and editing. Nurul Sulfi Andini: Conceptualization, methodology, writing. Nur Alif Bahmid: Methodology and writing. Muhammad Ardiansyah Nurdin: Data curation, Asmi Citra Malina A.R.Tasakka: Literature review. Ika Yustisia: Literature review. Vetnizah Juniantito: Literature review. A. Magfirah Satya Apada: Literature review. A.N.R Relatami: Methodology and literature review. Rian Hari Suharto: Writing and literature review. All authors have read, reviewed, and approved the final manuscript.

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