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Electrocardiogram Analysis of Hyperlipidemia-Induced Wistar Rats using Wireless Mice Electrocardiogram

(Analisis Elektrokardiogram Tikus Wistar Aruhan Hiperlipidemia menggunakan Elektrokardiogram Tikus Tanpa Wayar)

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ABSTRACT

Coronary heart disease (CHD) is a life-threatening disease caused by obstruction of the coronary arteries that interferes with blood flow known as atherosclerosis. Hyperlipidemia, a risk factor of atherosclerosis, is characterized by excessive concentrations of total cholesterol, LDL, and triglycerides with low concentrations of HDL. A high-fat diet (HFD) contributes to the progression of atherosclerosis, CHD, and other cardiovascular diseases. This study aims to measure electrocardiography (ECG) waves of hyperlipidemia-induced rats. Twenty rats were fed different diets for eight weeks, i.e., the control group (normal diet) and the HFD group (high-fat diet). Their ECG was recorded using a Wireless Mice Electrocardiogram (WIM ECG) for 5-10 min. After eight weeks, the HFD group showed a significantly higher lipid profile concentration (cholesterol: 179.03 mg/dL, triglyceride: 149.11 mg/dL, LDL: 123 mg/dL, HDL: 29.15 mg/dL) than the control. This hyperlipidemic condition causes a significant change in some characteristics of the ECG wave. At week 8, the characteristic ECG wave duration for the HFD groups was RR intervals (176.5 ms), QT intervals (123.5 ms), T waves (33.6 ms), P wave (27.4 ms), QRS interval (64.9 ms), ST-segment (23.7 ms), and heart rate (334 bpm). This study concludes that long-period HFD feeding in rats leads to hyperlipidemia and causes changes in the characteristics of ECG waves.

Keywords: Atherosclerosis; electrocardiogram; high-fat diet; hyperlipidemia; WIM ECG

ABSTRAK

Penyakit jantung koronari (CHD) ialah penyakit yang mengancam nyawa yang disebabkan oleh penyumbatan arteri koronari yang mengganggu aliran darah yang dikenali sebagai aterosklerosis. Hiperlipidemia, faktor risiko aterosklerosis, dicirikan oleh kepekatan berlebihan jumlah kolesterol, LDL dan trigliserida dengan kepekatan HDL yang rendah. Diet tinggi lemak (HFD) menyumbang kepada penjanjangan aterosklerosis, CHD dan penyakit kardiovaskular yang lain. Kajian ini bertujuan untuk mengukur gelombang elektrokardiografi (ECG) tikus yang disebabkan oleh hiperlipidemia. Dua puluh ekor tikus diberi makan diet yang berbeza selama lapan minggu, iaitu kumpulan kawalan (diet biasa) dan kumpulan HFD (diet tinggi lemak). ECG mereka direkodkan menggunakan *Wireless Mice Electrocardiogram* (WIM ECG) selama 5-10 minit. Selepas lapan minggu, kumpulan HFD menunjukkan kepekatan profil lipid yang jauh lebih tinggi (kolesterol: 179.03 mg/dL, trigliserida: 149.11 mg/dL, LDL: 123 mg/dL, HDL: 29.15 mg/dL) daripada kawalan. Keadaan hiperlipidemik ini menyebabkan perubahan ketara dalam beberapa ciri gelombang ECG. Pada minggu ke-8, tempoh ciri gelombang ECG untuk kumpulan HFD ialah selang RR (176.5 ms), selang QT (123.5 ms), gelombang T (33.6 ms), gelombang P (27.4 ms), selang QRS (64.9 ms), segmen ST (23.7 ms) dan kadar denyutan jantung (334 bpm). Kajian ini menyimpulkan bahawa pemberian HFD jangka panjang pada tikus membawa kepada hiperlipidemia dan menyebabkan perubahan dalam ciri gelombang ECG.

Kata kunci: Aterosklerosis; diet tinggi lemak; ECG WIM; elektrokardiogram; hiperlipidemia

Introduction

Coronary heart disease (CHD) is a type of cardiovascular disease (CVD) caused by obstruction of the coronary arteries called atherosclerosis, which blocks the blood flow. CHD is asymptomatic in its early development stages, making it difficult to accurately diagnose coronary artery disease (CAD) (Setyaji, Prabandari & Gunawan 2018). In Indonesia, CHD is one of the leading causes of death at 26.4% (P2PTM Kemenkes RI 2019).

Hyperlipidemia, a condition where blood has too many lipids, is considered the best indicator of atherosclerosis risk. In humans, hyperlipidemia occurs when total cholesterol (TC) exceeds 200 mg/dL, high-density lipoprotein (HDL) is less than 40 mg/dL, low-density lipoprotein (LDL) exceeds 130 mg/dL, and triglyceride (TG) is more than 150 mg/dL. Several factors lead to hyperlipidemia, e.g., genetics, gender, age, obesity, lack of exercise, smoking, alcohol consumption, high blood pressure, and diet. Hyperlipidemia may not exhibit symptoms. However, patients suffering from decades of hypercholesterolemia, a type of hyperlipidemia, are more susceptible to developing CVD (Nelson 2013).

Diet is one of the factors affecting cholesterol levels inside the human body. A high-fat diet (HFD) contributes to advancing atherosclerosis, CHD, and other CVDs. HFD can result in metabolic problems in humans and other mammals, e.g., rodents. Metabolic problems due to HFD are considered more applicable than monogenic ones because monogenic problems are rare in humans (Maulana & Ridwan 2021).

Early detection is a vital step in reducing deaths from CVDs. One early detection technique is electrocardiogram wave analysis (Hammad et al. 2018). Electrocardiograms (ECG) measure heart rhythm and electrical activity, interpreted in an ECG wave (Padsalgikar 2017). The ECG wave displays the heart's electrical activity, enabling it to compare with others. Such information helps understand the purpose and structural traits of the heart, detecting abnormalities of cardiac muscle motion potentials, conduction of electrical impulses, disturbances of heart rate and rhythm, and observing diverse consequences of chemical substances and drugs on the heart (Kumar, Pachori & Acharya 2017). ECG waves can be recorded using devices such as Wireless Mice Electrocardiograms (WIM ECG). The WIM ECG is a non-invasive, cost-effective, and user-friendly ECG waveform recording device used for the practical monitoring of rodents' myocardium, mainly in rats and mice (Nugroho, Chusnia & Suprijanto 2017).

This study aims to determine the ECG waves in HFD rats by using a WIM ECG device as an excellent way to detect practical cardiac modifications due to hyperlipidemia. This study's findings can help demonstrate the ability of ECG waves to detect cardiac characteristics in hyperlipidemic rats. In addition, recorded ECG waves acquired in this study can serve as a reference to assess ECG records in future studies.

Materials and Methods

Experimental animals

Twenty of six weeks old male Wistar rats (*Rattus norvegicus*) weighed 180-200 g were maintained in the animal enclosures of the School of Biological Science and Technology, Bandung Institute of Technology, Indonesia. The animal models have been reared with laboratory management standards in a room with a temperature of 25±4 °C, relative humidity of 70-90%, and a cycle of 12 h of darkness and 12 h of light. Rats were given drinks and food daily on an *ad libitum* basis (Liu & Fan 2017). The acclimatization of the animals was performed for seven days. They were then divided into two groups comprising ten rats with different diets, i.e., the control group with a normal diet and the HFD group with a high-fat diet. This study has been approved by the Research Institute for the Ethics Commission of Padjadjaran University, Indonesia, with approval No. 44/UN6.KEP/EC/2022.

Induction of hyperlipidemia

In the HFD treatment group, hyperlipidemia was induced by feeding a high-fat diet containing 50% standard feed, 0.2% cholesterol powder, 19% duck egg yolk, 3.8% used cooking oil, 27% animal fats, and 0.01% Propylthiouracil (PTU) dissolved in drinking water (Susilowati et al. 2020; Yuan et al. 2019). Subject rats were fed a high-fat diet and PTU *ad libitum* for eight weeks. In the control group, subject rats were fed with standard feed from PT. Charoen Pokphand Indonesia Tbk.

Preparation of rat blood serum

Blood samples were drawn every two weeks for eight weeks through the retro-orbital venous plexus. The collected blood samples were centrifuged at 2500 rpm for 15 min after being allowed to clot for 60 min. The resulting serum was stored at -20 °C for lipoprotein levels measurement.

Measurement of lipid profile

Lipid profile measurements were carried out using the

Enzymatic Endpoint Method with a spectrophotometer. The blood serum lipid content was measured using total cholesterol (TC) and triglyceride (TG) reagents from Reiged Diagnostics, Indonesia, also HDL cholesterol reagent from Glory®Diagnostics, Indonesia. LDL concentration was calculated using Friedewald's Formula:

LDL-c (mg/dL) = TC (mg/dL) – HDL-c (mg/dL) – (TG/5 (mg/dL)) (Knopfholz et al. 2014).

Calculation of the atherogenic index (AI) was done using the method of Schulpis and Karikas:

AI = (Total Cholesterol (mg/dL)-HDL (mg/dL))/HDL (Schulpis & Karikas 1998).

MEASUREMENT OF CHARACTERISTIC ECG WAVES

ECG evaluation was performed using Wireless Mice Electrocardiogram (WIM ECG) device. The ECG characteristics were determined based on P-wave, T-wave, intervals (QRS, QT, and RR), ST segment, and heart rate. The ECG recording detection system (Figure 1) was modified based on the system developed by Nugroho, Chusnia and Suprijanto (2017). This study acquired a specimen room to prevent the movement of the animal model. Anesthesia was not used since it can cause heart rate disturbances and other electrophysiological parameters (Kumar, Pachori & Acharya 2017).

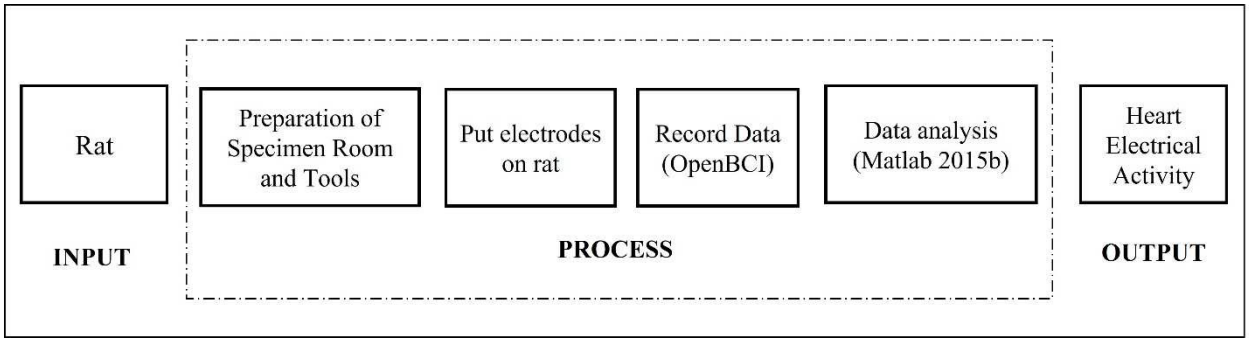


FIGURE 1. Procedures involved in ECG Recording Detection System. First, rats were prepared for the ECG measurement. Second, the process stage was executed by preparing the specimen room and tools, placing electrodes on rats, recording data using OpenBCI software, and analyzing data using Matlab 2015b software. Finally, the heart's electrical activity was measured

DATA ANALYSIS

Results were calculated for mean and standard deviation and subjected to parametric analysis (i.e., one-way ANOVA with Tukey's posthoc) and non-parametric analysis (i.e., Mann-Whitney test). The difference between the means was considered statistically significant when the P value was less than 0.01.

RESULTS AND DISCUSSION

FOOD CONSUMPTION AND BODY WEIGHT

Diet is one of the essential factors needed for organisms' health. A nutritional imbalance in food intake will negatively impact health (Cena & Calder 2020). A high-fat diet (HFD) could lead to obesity, atherosclerosis, CHD, and other CVDs. In this study, the control group's food consumption was significantly higher than the HFD group. In the HFD treatment group, the average daily food consumption was 11.53 g, while the control group was 26.54 g (Figure 2(A)). Previous studies also reported a similar finding: Feeding HFD caused a decrease in rats' food consumption due to the low recognition rate of HFD food; therefore, the rats eat in small quantities. However, it still impacted weight gain (Han et al. 2018) since the energy produced by high-fat diets is higher than standard diets, contributing to obesity (Moreno-Fernández et al. 2018; Wali et al. 2020; Wang et al. 2015). Indeed in our study, both groups showed a significant weight gain (Figure 2(B)**)**. The weight of the HFD group increased from 193.57 g to 317.79 g, while the control group increased from 186.23 g to 338.3 g when compared, the weight of both groups is relatively the same.

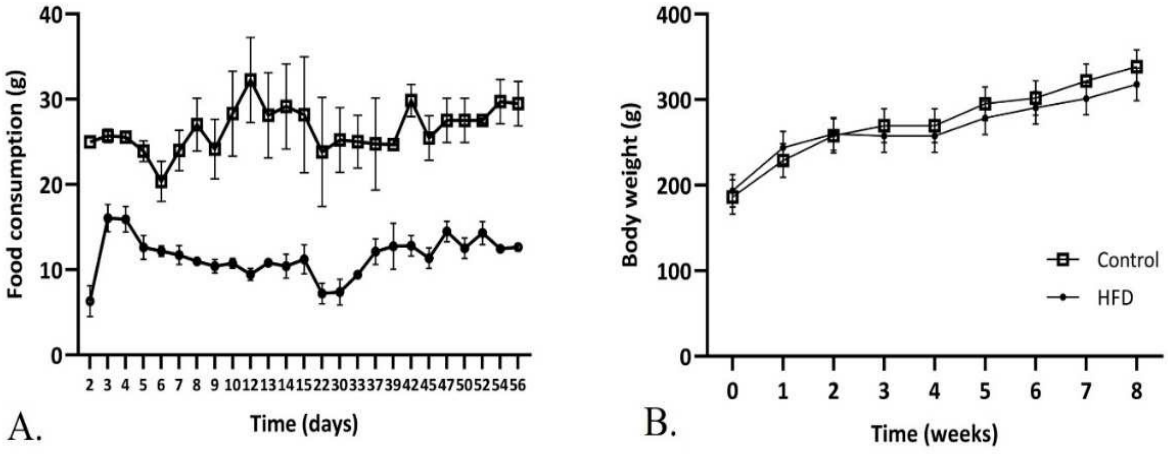
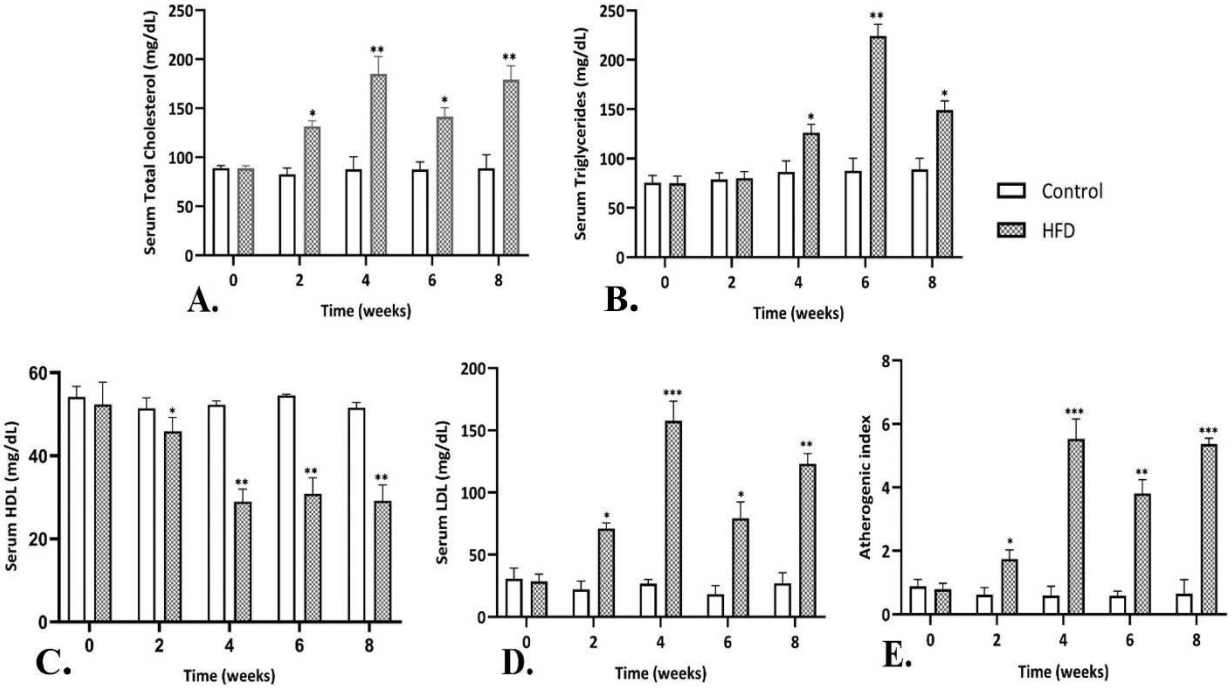


FIGURE 2. Comparison of mean ± SEM of (A) food consumption and (B) body weight of rats (*Rattus norvegicus*) in HFD and control groups. N=10 for each group

THE EFFECT OF HFD ON LIPID PROFILE

The lipid profile observed at weeks 0, 2, 4, 6, and 8 showed a significant increase in TG concentrations (Figure 3(A)). TG is a precursor for cholesterol formation, indicated by an increase in the concentration of TC and LDL (Zhang et al. 2020). The TC and LDL concentrations significantly increased over time, contrary to decreasing HDL concentration (Figure 3(A)-3(D)). Similar findings were also demonstrated by Maulana and Ridwan (2021), where prolonged high-fat diet feeding in Wistar rats caused an increase in TC, TG, and LDL and decreased HDL. According to Astuti (2019), LDL concentration exceeding 27 mg/dL is categorized as a high concentration, and HDL concentration less than 35 mg/dL indicates a low concentration.



*Significantly different vs the control group. **Significantly different vs the HFD group at week 2 & 6 (The TC & LDL chart) & the HFD group at week 4 & 8 (The TG chart) & HFD group at week 2 (The HDL & AI chart). ***Significantly different vs the HFD group at week 8 (The LDL chart) & the HFD group at week 6 (The AI chart)

FIGURE 3. The mean (±SEM) effect of HFD feeding on the serum levels of (A) TC, (B) TG, (C) HDL, (D) LDL, and (E) AI in rats for eight weeks. N=10 for each group. The overall one-way analysis of variance with Tukey's post hoc test showed significant differences when $p<0.01$

The HFD treatment group showed a significant AI increase (Figure 3(E)), with the highest value at week 4 (5.5). AI is a strong marker for predicting the risk of atherosclerosis and coronary heart disease (Niroumand et al. 2015). The value of AI is highly dependent on HDL concentration; if HDL concentration is smaller, AI's value will be higher, and the risk of atherosclerosis will be greater (Koca et al. 2021).

Long-period HFD feeding can directly or indirectly affect lipid metabolism (Wali et al. 2020). HFD diet includes immoderate saturated fatty acids (SFAs), which can lower the polyunsaturated fatty acids (PUFAs) in hepatocyte cells if consumed for an extended period (Maulana & Ridwan 2021). PUFAs have important roles in maintaining cell membrane fluidity, inhibiting inflammatory processes, and reducing TG synthesis inside the liver (Adermark et al. 2021). Decreased levels of PUFAs, particularly in hepatocytes, cause oxidative stress and inflammatory response, activating sterol regulatory element-binding protein-1c (SREBP-1c) (Wali et al. 2020; Wiktorowska-Owczarek, Berezińska & Nowak 2015). SREBP-1c is a transcription element that regulates genes that play a role in the synthesis and absorption of free fatty acids (FFAs), cholesterol, and TG by stimulating the expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) proteins. Both proteins increase the production of Fatty Acids (FA), which can be esterified into TG (Maulana & Ridwan 2021). Further, TG is transformed into VLDL, and VLDL will become LDL. The subsequent increase in LDL concentration is accompanied by a decrease in HDL concentration, indicating the pathogenesis of atherosclerosis (Zhang et al. 2020).

Under abnormal conditions, accumulated LDL is oxidized by free radicals. Macrophages that engulf the oxidized LDL will become foam cells and form plaque in developing atherosclerosis. The higher accumulation of macrophages and cholesterol in macrophages will lead to enlargement and instability of plaque, which increases the progression of atherosclerosis (Wali et al. 2020).

OxLDL can stimulate atherosclerosis through complicated inflammatory and immunogenic mechanisms resulting in lipid and endothelial cell dysfunction and foam cell formation. OxLDL forms lipoprotein aggregates and can cause inflammation in endothelial cells by recruiting monocytes in circulating blood vessels to these areas, resulting in atherosclerosis (Yang et al. 2018). In addition, HFD feeding causes modifications in cardiac lipid composition, resulting in cardiac lipotoxicity and impacting cardiac function. Other research has mentioned that HFD has an immediate negative effect on the myocardium, resulting in dysfunctional diastolic, left ventricular hypertrophy, and rapid growth in LV mass, in addition to causing cardiac pumping disorders (Zhang et al. 2021).

THE EFFECT OF HYPERLIPIDEMIA ON ECG WAVE CHARACTERISTICS

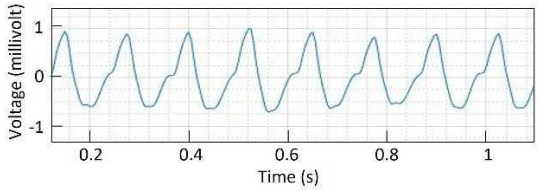
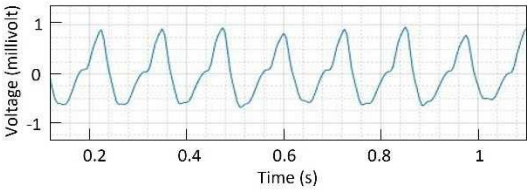
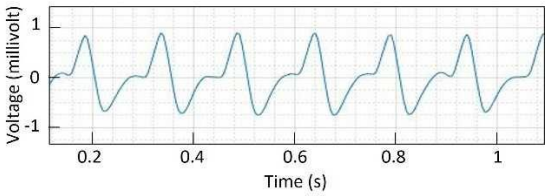
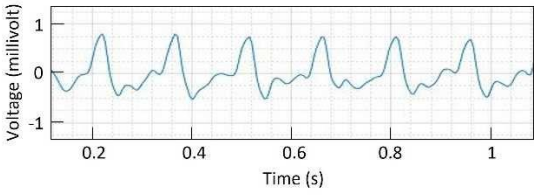
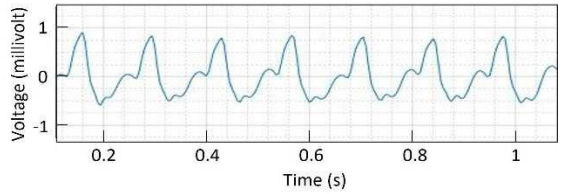
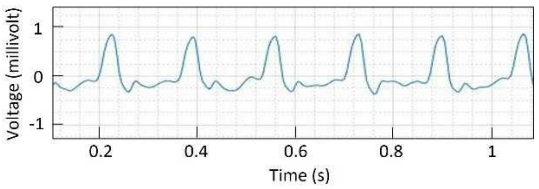
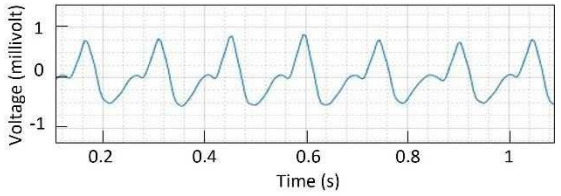
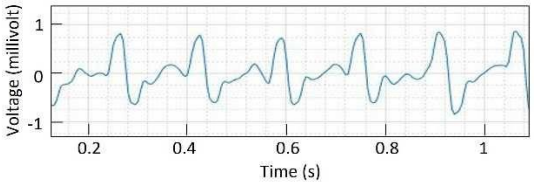
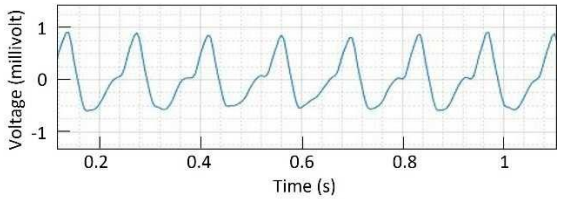
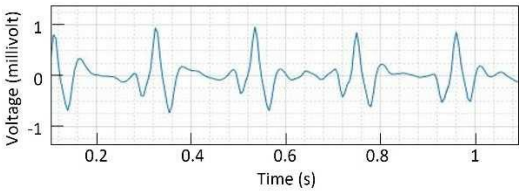
In this study, the rats' ECG wave characteristics were determined before blood withdrawal to examine the lipid profile. The observations of the ECG waveform (Table 1) showed that the control group has a longer wave density on the same scale as the HFD group. The analysis of ECG waves using MATLAB 2015b software to determine the duration of each characteristic, i.e., heart rate, RR interval, P and T wave, QRS and QT interval, and ST-Segment, is presented in Figure 4.

This study showed that the hyperlipidemia condition influenced the duration of the characteristic ECG waves. In the 8th week, the duration of the characteristic ECG waves in the HFD group and the control group were RR interval (176.5 & 130.3 ms), QT interval (123.5 & 104.7 ms), T wave (33.6 & 26.7 ms), P waves (27.4 & 23.3 ms), QRS interval (64.9 & 63.5 ms), and ST segment (23.7 & 23 ms; Figure 5). The characteristics of T wave duration, RR and QT interval in the HFD group were significantly higher than in the control group. Meanwhile, the P wave duration, QRS interval, and ST segment were relatively similar between both groups. This observation indicates that HFD feeding causes modifications inside the heart system, particularly in conducting and contractile muscle cells (Park & Fishman 2017). Conducting cells are muscle cells that control and coordinate the heart rate. Meanwhile, contractile cells produce energy for the heart to have strong contractions so it can pump blood throughout the body (Martini, Nath & Bartholomew 2015).

As shown in Figure 6, the average heart rate at week 0 of the HFD group was 436.5 bpm, while the control group was 432 bpm. The heart rate of HFD groups differed significantly from the control ($p<0.01$). In the HFD group, after feeding with the high-fat diet, the average heart rate decreased significantly over time and reached its lowest duration at week eight at 339 bpm. In contrast, the heart rate of the control group was relatively stable.

Previous studies reported that the normal heart rate of Wistar rats was 300-500 bpm (Botelho et al. 2019; Liu & Fan 2017). In this study, the control and HFD groups were in the normal heart rate categories. However, the

TABLE 1. Comparison of electrocardiogram (ECG) waveforms between the control group and HFD for eight weeks

Control	Time (weeks)	HFD
HFD		
	0	
	2	
	4	
	6	
	8	

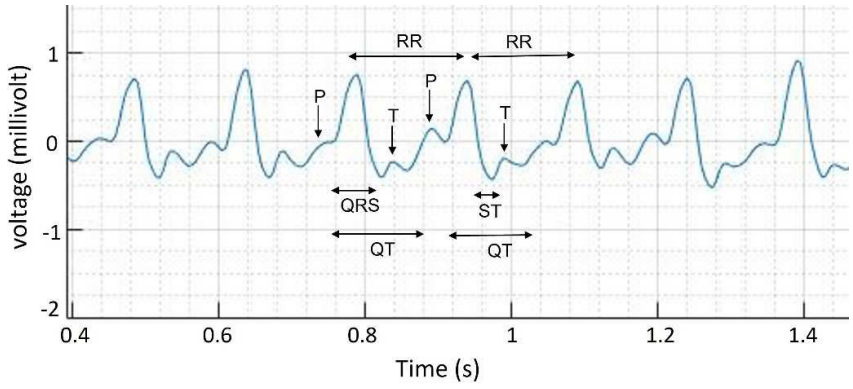
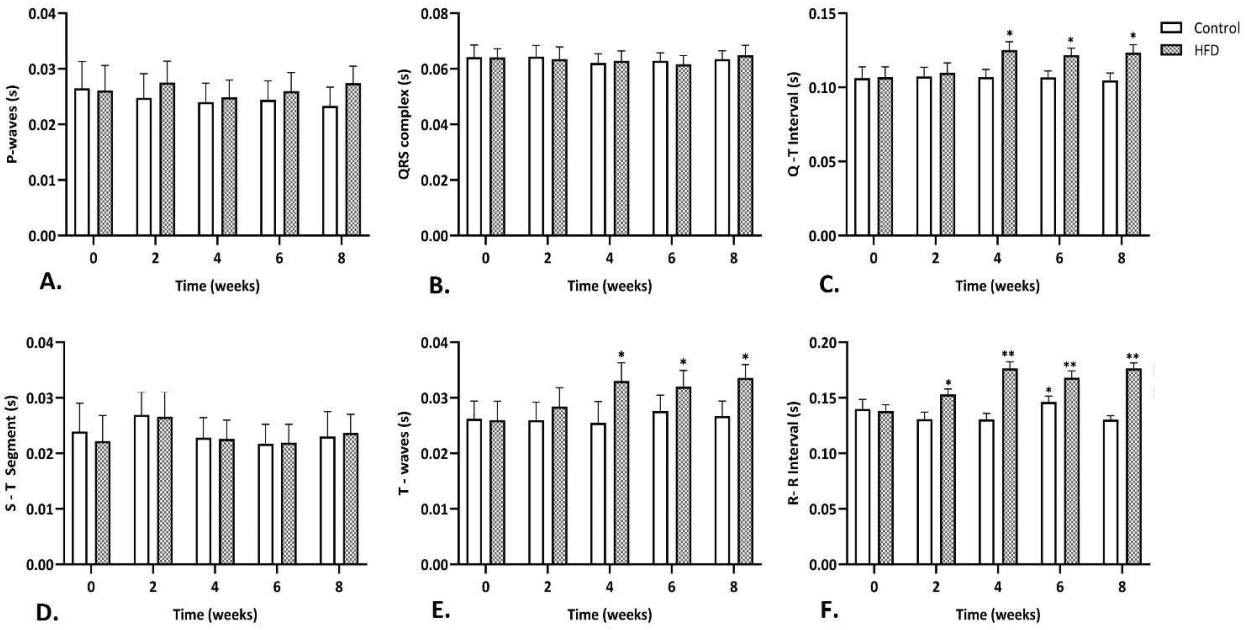


FIGURE 4. Identified ECG wave

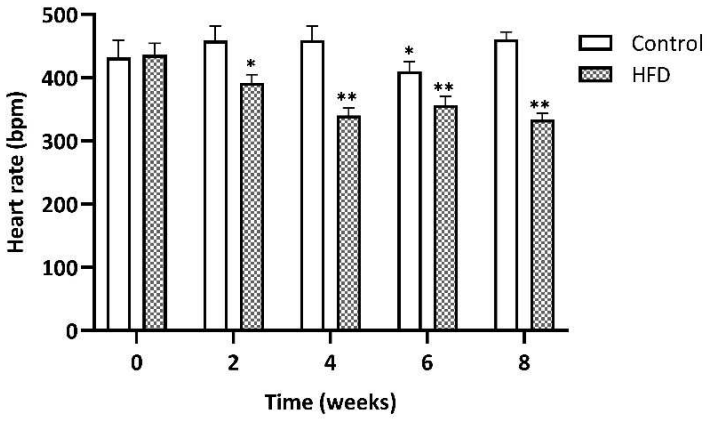


*Significantly different vs the control group. **Significantly different vs the HFD group at week 2 & control group at week 6 (The R-R interval chart)

FIGURE 5. The mean (±SEM) effect of HFD feeding on the duration of (A) P waves, (B) QRS intervals, (C) Q-T intervals, (D) S-T segment, (E) T waves, and (F) R-R intervals in rats for eight weeks. N=10 for each group; The overall one-way analysis of variance with Tukey's post hoc test showed significant differences at $p<0.01$

HFD group did experience a low decrease in heart rate, similar to the findings of Hua et al. (2013) study. This decreased rate indicates a dysfunctional heart due to the HFD feeding disturbing cardiac contractions. Diet or eating behavior can directly change cardiac physiology or indirectly affect the contraction ability of the heart (Hua et al. 2013).

HFD is a major factor contributing to the development of obesity, hyperglycemia, hypertension, hypertriglyceridemia, and hyperlipidemia (Koene et al. 2016). HFD can cause changes in the lipid composition of the heart, resulting in cardiac lipo-toxicity and affecting heart function (Zhang et al. 2021). Other studies reported the negative direct impact of HFD on the myocardium, resulting in diastolic dysfunction, left ventricular hypertrophy (LVH), and a rapid increase in LV mass, leading to impaired cardiac pumping (Abdurrachim et al. 2014; Avelar et al. 2007; Guzzardi & Iozzo 2011).



*Significantly different vs the control group. **Significantly different vs the HFD group at week 2 & control group at week 6

FIGURE 6. Comparison of mean (±SEM) heart rate between control and HFD group at 0 to 8 weeks. N=10 for each group. The overall one-way analysis of variance with Tukey's post hoc test showed significant differences at $p<0.01$

HFD directly causes an increase in the concentration of fatty acids (FA) in the circulatory system, liver, and heart. The FA, which is also metabolized in cardiac cells, enters cardiomyocytes through specific transporters fatty-acids binding protein (FABP) and fatty-acid transport proteins (FATP). FA is converted to acyl-CoA with the aid of acyl-CoA synthetase (ACS). Acyl-CoAs are used for oxidation or non-oxidative pathways, including esterification and TG synthesis. In addition, it also forms lipotoxic intermediate compounds, i.e., ceramides, ROS, and diacylglycerol (DAG). These lipotoxic compounds disrupt calcium (Ca^{2+}) homeostasis. It also causes functional damage to mitochondria and increases the production of ROS, pro-apoptotic, pro-inflammatory molecules, and myocardial dysfunction. All mentioned factors contribute to impaired heart contraction and pumping (Abdurrachim et al. 2014; Wali et al. 2020).

Conclusions

The high-fat diet given to rats for eight weeks causes rats to become hyperlipidemia. Hyperlipidemia leads to changes in the ECG waves, which can be longer than normal. Using a specimen room in ECG wave recording successfully supports the ECG recording in rats without anesthesia. The findings of this observation demonstrate the potential function of ECG waveform duration values in detecting functional cardiac conditions in hyperlipidemia rats. In addition, the results of this study can be used as preclinical studies on heart disorders, supporting research on heart disorders in small mammals in Indonesia to grow and advance.

Acknowledgements

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