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Effect of Different Types of Ketogenic Diet on Lipid Profile, Atherogenic Ratios, and Cardiac Histopathology in Male Albino Rats

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ABSTRACT

Background and Objective: Dyslipidemia, characterized by abnormal levels of total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), and triglycerides (TG), is a major risk factor for cardiovascular diseases. Although the ketogenic diet (KD) is widely used as a very low-carbohydrate, high-fat therapeutic approach, its effects on lipid metabolism and cardiac health remain controversial. This study aimed to compare the impact of different types of ketogenic diets on serum lipid profile, atherogenic ratios, and cardiac histopathological changes in male albino rats.

Methods: Thirty-five male albino rats were randomly divided into five groups of seven: a control group, a high-fat ketogenic diet (70% coconut oil), a high-fat ketogenic diet (70% animal fat), a high-protein ketogenic diet (35% casein, 60% coconut oil), and a cyclical ketogenic diet (70% coconut oil). The dietary intervention lasted for 50 days. At the end of the intervention, serum lipid parameters (TC, TG, LDL-C, HDL-C) and lipid ratios (TC/HDL-C, TG/HDL-C, LDL-C/HDL-C) were measured. Also, histopathological examination of heart tissue was performed using hematoxylin-eosin (H&E) staining. SPSS25 software was used for statistical analysis ($p<0.05$).

Results: High-fat and high-protein diets significantly increased TC, TG, LDL-C, and VLDL levels in rats, with the coconut oil ketogenic diet showing the most adverse changes. HDL-C levels showed no significant changes across groups. Lipid ratios indicating atherogenic risk were increased dramatically in the high-fat and high-protein groups. Histological examinations of the heart in the coconut oil group showed fat accumulation, inflammatory cell infiltration, and cardiomyocyte destruction, while these changes were less severe in the animal fat group and were rarely observed in the control group.

Conclusion: According to the results obtained, the effects of ketogenic diets are significantly influenced by the macronutrient composition, especially the fat source. High-fat diets, especially those enriched with coconut oil, have significant adverse effects on metabolic od lipids and cardiac tissue, while high-protein and periodic diets are considered comparatively safer, though further studies are needed to confirm their long-term effects. These findings highlight the importance of dietary fat composition when considering ketogenic diets for potential clinical use.

Keywords: ketogenic diet, Serum Lipid Profile, Lipid ratios, Histopathological changes, Albino rats

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1. INTRODUCTION

Dietary patterns play a fundamental role in promoting health, preventing disease, and managing metabolic disorders. In recent decades, the ketogenic diet (KD) has attracted much attention as a nutritional strategy for weight reduction and managing metabolic syndrome. The KD diet is introduced with very low carbohydrates intake, moderate protein, and increased fat, which collectively shift the body's metabolism from glucose utilization toward fat oxidation and ketone body production. This metabolic adaptation, known as nutritional ketosis, alters energy homeostasis and impacts various biochemical pathways [1].Evidence from previous studies has suggested both beneficial and adverse outcomes of ketogenic diets. While some investigations report reductions in body weight and improved glucose control [2]. others indicate unfavorable changes in lipid profiles, oxidative stress levels, or hepatic steatosis [3]. The ketogenic diet is often considered a beneficial intervention for achieving optimal weight, having a positive effect on high blood pressure, and improving metabolic markers such as reducing TG and increasing HDL and, TC. Despite these benefits, its association with increased LDL-C and VLDL levels observed in some ketogenic diet users raises concerns regarding potential cardiovascular risk. Furthermore, the stringent dietary modifications required to achieve ketosis likely limit its feasibility as a long-term sustainable strategy[4]. The basic philosophy of the ketogenic diet is to shift the body's energy source by reducing the calorie content of carbohydrates and replacing it with calories from protein and, in particular, fat[5]. The main objective of this study was to evaluate and compare different formulations of the ketogenic diet on changes in serum lipid profile, lipid ratios, and cardiac histopathology in male albino rats and to gain a better understanding of the metabolic consequences of dietary components through a systematic comparison of these diets and to contribute to increasing the evidence surrounding ketogenic nutrition.

2. METHODOLOGY

2.1. Experiment Animals

This research was a laboratory study conducted at the University of Sulaymaniyah, Dept. of Food Sciences and Quality Control from (27 Dec- 2024 - 15 Feb-2025). The experiment period lasted for 50 days. The study included 35 male albino rats, randomly divided into five groups (n = 7 per group).

2.2. The laboratory Albino Rats and Experiment Design

The rats were 8 ± 2 weeks old and weighed approximately 120–170 g at baseline. According to the rules for the proper care and use of laboratory animals, all rats were purchased from the animal house for producing lab animals in Tikrit. After two days of acclimation, the animals were randomly divided into five groups of 7, with two rats per group placed in a standard plastic cage, and all rats were coded. The cages were kept in a controlled environment at 25 C and 50% relative humidity and a 12-hour light/dark cycle. All of cages were covered with sawdust which was

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replaced twice a week and the cages had been kept clean and hygienic throughout the experiment. Rats were freely provided ad libitum with drinking water throughout the experiment.

2.3. Standard and Experimental Diet

The standard rats' diet was prepared according to AOAC with some modifications (AOAC 2020) **[6]**. The portions of the ingredients was75% Carbohydrate, 10% Protein (Casien, Applied Nutrition, England), 8% Fat, 6% Vitamins and minerals (Tondor, China), and 1% Cellulose (Applied Nutrition, England). All ingredients are shown in Table 1. Rats were treated with four types of ketogenic diets as: 70% plant oil, 70% animal oil, 60% plant oil (high protein), and cyclic KD with 70% plant oil shown in table 2. All ingredients of both the standard diet and the experimental diet were combined directly with the addition of the commercial vitamins and mineral mixture for survival to make into a dough-like then cut into small pieces to become ready to feed the rats. Feeding was done twice a day, with 18 to 22 g of food being consumed by the test rats per meal. Water was freely available to rats along with the meal.

Table 1. Standard Diet Composition

Ingredients	Percentage%
protein (Pure casein)	10
Corn oil	8
Mineral mixture	5
Sucrose	5
Vitamin mixture	1
Non-nutritive cellulose	1
Corn starch	To complete 100%

Table 2. Formulation of Rats' Feed.

Rats group	Type of keto diet	Ingredients %				
		Fat	Protein	Carbohydrate	Cellulose	Vitamins& Minerals
A	70% plant oil (Coconut)	70	18	10	1	1
B	70 % Animal fat	70	20	10	1	1
C	60% plant oil (coconut), High Protein	60	35	3	1	1
D	70% plant oil (Cyclic ketogenic diet)	70	18	10	1	1
E	St. rats' diet (Control)	8	10	75	1	6

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2.4. Blood Collection and Serum Lipid Profile

At the end of the 50-day research period, after the rats had fasted for 12 hours, chloroform was used to anesthetize them. After opening the abdominal cavity surgically, about 1 ml of blood was collected via heart directly using the sterile syringe, in the belief it's enough to conduct a lipid profile and a biochemical marker analysis of the rats. The blood was placed in the gel tubes for biochemical tests. The serum levels of TG, TC, HDL-C, LDL-C, VLDL-C were clinical chemistry Auto analyzer (Cobas C 311, HITACHI) in Mahan laboratory/ Sulaymaniyah city, Iraq, ISO certified from UK (ISO 15198) and (ISO 9001). Lipid profile ratios (TC/HDL-C, TG/HDL-C, LDL-C/HDL-C) were evaluated.

2.5. Histopathology Assessment of Rats' Heart Tissue

Formalin-fixed Cardiac tissue was taken from 3 groups of rats, each one includes 7 rats: Control group, high fat ketogenic diet (plant oil) group, and high fat ketogenic diet (animal fat) group. This assessment conducted in the Arena laboratory (Tehran city /Iran), For this preparation 5-micron sections with routine H&E staining technique: Cardiac tissues were evaluated Histopathologically following H&E staining and scored for lipid accumulation, inflammatory infiltrates, and fibrosis using a standardized scoring system (0-4).

2.6.Statistical Analysis

Statistical analysis was performed using SPSS software (ver,25). Data were expressed as mean ± standard error of the mean (SEM). ANOVA test was used to compare mean values, and $p<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Lipid Profile

3.1.1.Changes in Total Cholesterol (TC) Levels in Different Ketogenic Diets

According to the results presented in Figure 1 and Table 3, a significant effect of diet type on serum TC levels was observed. Group A with a coconut oil (70%) diet showed a significantly higher TC level (145.1 ± 21.9 mg/dL, ***$p<0.001$) compared to the control group E (86.1 ± 10.3 mg/dL), confirming the clear effect of high-fat diets on increasing TC levels. Group (B) that received the animal fat (70%) diet also had higher TC levels than the control group (103.1 ± 10.8 mg/dL, $p=0.003$), although this increase was less than that of the coconut oil (70%) group (A). In contrast, the high-protein diet group C, although having a higher mean TC than the control group E (109.8 ± 30.2 mg/dL), this difference was not statistically significant ($p=0.041$). The intermittent ketogenic diet group also had a slight increase in TC levels (97.7 ± 18.4 mg/dL), which was not statistically significant ($p=0.087$). These findings are consistent with are consistent with studies reporting no direct relationship between dietary cholesterol intake and serum cholesterol levels[7]. However, as [8] reported, the response to ketogenic diets is highly variable among individuals, and there are increasing reports of significant increases in TC after initiation of these diets. From a clinical perspective, studies have shown an association between higher TC levels and increased

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cardiovascular mortality. [9] A previous review also reported a significant association between high total cholesterol levels and an increased risk of cardiovascular disease. Also, a 10-year prospective study by [10] showed that the highest risk of ischemic heart disease was observed at total cholesterol levels above 185 mg/dL. However, it should be noted that due to methodological variations in different studies, there are significant discrepancies between the reported results. These discrepancies may be due to various confounding factors, including differences in the genetics of the participants, the exact composition of the diets, the duration of the studies, and the measurement methods. The significant increase in TC in the high-fat groups, especially the high-unsaturated fat groups, particularly the coconut-oil group, could raise concerns about the increased risk of cardiovascular disease. Also, the relatively normal cholesterol levels in the high-protein and intermittent ketogenic groups suggest that these diets may be comparatively safer options in terms of lipid profiles.

3.1.2. Changes in Serum Triglyceride (TG) Levels in Different Ketogenic Diets

The results in Figure 1, Table 3 of a serum TG level in albino rats showed a pattern of significant changes under the influence of different diets. The groups fed high-fat diets showed the highest TG levels. The group A receiving the coconut oil (%70) diet (191.41 ± 72.65 mg/dL) and the group receiving the animal fat (%70) diet (118.41 ± 13.52 mg/dL) showed a significant increase compared to the control group (E) (74.10 ± 9.93 mg/dL) ($p < 0.0001$ and $p = 0.0002$, respectively). According to the findings, high-fat diets, regardless of the type of fat, can act as a strong risk factor for hypertriglyceridemia, although the magnitude of this effect varies with the type of fat in the diet [11, 12]. In contrast, the high-protein diet (82.50 ± 10.64 mg/dL) and intermittent ketogenic diet (69.77 ± 7.00 mg/dL) groups, showed slight increases compared to the control group, but these differences were not statistically significant ($p = 0.310$ and $p = 0.999$, respectively). These results suggest that it is not simply the increased fat content of the diet that disrupts the lipid profile, but rather the type of macronutrients and composition of the diet play a decisive role in this regard [13]. Human studies have also reported similar findings. A study of 48 obese patients showed that very low-calorie KD (VLCKD) with different protein sources (whey, plant, and animal) all led to significant improvements in the lipid profile, reduced waist circumference, and reduced triglyceride levels [14]. These improvements were observed regardless of the protein source, indicating an overall positive effect of low-carbohydrate diets on metabolic health. Possible mechanisms for these effects include increased fat oxidation, decreased production of triglyceride-rich lipoproteins, and improved insulin sensitivity. Studies also show that replacing carbohydrates with healthy fats may improve lipid profiles [15, 16]. [17] Noted, medium-chain triglycerides (MCTs) are metabolized differently and may be a better option in ketogenic diets. These findings highlight the importance of paying attention to the quality of fat consumed as well as its quantity[18]**.** However, the conflicting metabolic of ketogenic diets should also take into consideration considered. As reported by [19], significant increases in serum TG, TC, and LDL in individuals treated with a ketogenic diet associated with cardiovascular complications. On the other hand, [20] reported that a ketogenic diet can improve hepatic steatosis, reduce liver fatty acid content, and increase triglyceride metabolism.

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3.1.3. Changes in HDL Levels in Different Ketogenic Diets

The statistical analysis of HDL-C levels showed that none of the diets studied had a significant effect on HDL level (Figure1, Table3). The mean HDL-C level in all experimental groups ranged from 43 to 53 mg/dL, with no significant difference compared to the control group (45.14 ± 5.47 mg/dL). This finding suggests that although high-fat diets clearly affected TC and TG levels, they did not significantly affect HDL-C levels, known as the "good" cholesterol. This result could indicate independent mechanisms of regulation of this lipoprotein in the body that are resistant to dietary changes. However, it is noteworthy that the 70% animal-fat group exhibited the lowest mean HDL-C levels, which may hold potential clinical relevance despite the lack of statistical significance.

3.1.4. Changes in LDL Levels in Different Ketogenic Diets

According to the results presented in Table 3, serum LDL-C levels in albino rats, unlike HDL-C, LDL-C levels were significantly affected by diet type ($p < 0.0001$). Group A (70% coconut oil) showed the highest LDL-C levels (29.21 ± 7.31 mg/dL), followed by the high-protein group (Group C) (25.80 ± 8.75 mg/dL) ($p < 0.0001$ and $p = 0.001$, respectively). The group receiving the intermittent ketogenic diet (20.51 ± 3.89 mg/dL) also showed a significant increase compared to the control group (12.91 ± 1.08 mg/dL) ($p = 0.003$). Interestingly, the group B receiving the animal fat (%70) diet (15.84 ± 3.97 mg/dL) was the only group that, despite a numerical increase, did not show a significant difference from the control group ($p = 0.083$) (Figure 1). These findings suggest that the mechanisms by which different diets affect LDL-C levels may be very complex and directly related to dietary composition. The marked increases in LDL-C observed in the coconut-oil and high-protein groups may indicate a potential elevation in cardiovascular risk. The results obtained are consistent with previous studies showing the effect of high-fat diets on increasing LDL-C levels[21-23]. Differences in response to ketogenic diets may be influenced by a variety of factors, including metabolic characteristics, the exact composition of the diet, and the duration of the study. For example, recent reports suggest that the greatest increases in LDL-C levels often occur in individuals with low BMI who had moderate LDL-C levels prior to the KD [8, 24]. Findings from human studies in this area are conflicting. While some studies, such as[25], have reported that a ketogenic diet increases LDL-C levels in healthy young women, [26] found no significant difference in lipid profile changes between KD and non-KD. Other studies have also reported conflicting results [27], reporting a reduction in LDL with a high-protein, moderate-carbohydrate diet compared to a KD.

3.1.5. Changes in VLDL Levels in Different Ketogenic Diets

As shown in Table 3, the analysis of VLDL-C levels revealed a pattern similar to that of triglycerides, indicating a pronounced effect of high-fat diets on lipoprotein metabolism. VLDL-C levels were significantly increased in both the coconut oil (70%) group A (33.14 ± 9.23 mg/dL) and the animal fat (70%) group B (23.71 ± 2.08 mg/dL) ($p < 0.0001$ for each). This finding aligns

with the established role of VLDL as the main carrier of TG in the blood. In contrast, the high-protein (17.23 ± 2.06 mg/dL) and intermittent ketogenic (13.59 ± 1.92 mg/dL) groups did not show significant differences from the control group (14.94 ± 1.81 mg/dL) (Figure 1). These results indicate that increased hepatic VLDL production and secretion may represent the main mechanism of hypertriglyceridemia induced by high-fat diets. Additionally, the greater severity of VLDL elevation in the coconut oil (%70) group A again emphasizes the important role of lipid type in regulating lipoprotein metabolism. The mechanism of these effects can be explained in cholesterol metabolism. As previously reported[28] and [29], cholesterol is derived from two main sources: endogenous (internal synthesis) and exogenous (dietary origin). Endogenous cholesterol is synthesized primarily in the liver under conditions of excess calorie intake. In this process, acetyl-CoA, a byproduct of glucose metabolism (glycolysis), is converted primarily to TG and TC. These lipids are then packaged into VLDL and released into the circulation. VLDL particles are further converted to IDL and then to LDL, thereby transporting cholesterol to peripheral tissues. In contrast, exogenous cholesterol enters the gastrointestinal tract through the diet. This dietary cholesterol is packaged into particles called chylomicrons and transported through the lymphatic system to the liver. Finally, dietary cholesterol can also enter the blood in the form of VLDL particles or remnant chylomicrons. This process explains why high-fat diets lead to increased VLDL-C levels. Interestingly, in this study, no significant difference in HDL levels was observed among the different groups ($p>0.05$). However, the ketogenic group showed a significant increase in TC, TG, LDL-C and VLDL-C levels compared with the other groups. This differential lipid profile suggests that different diets can have selective effects on different parts of the lipid profile. These findings are of clinical importance because increased VLDL-C levels are known to be a contributing factor in cardiovascular disease. Increased hepatic production and secretion of VLDL not only leads to hypertriglyceridemia, but may also be associated with the formation of small, dense LDL particles, which are highly atherogenic.

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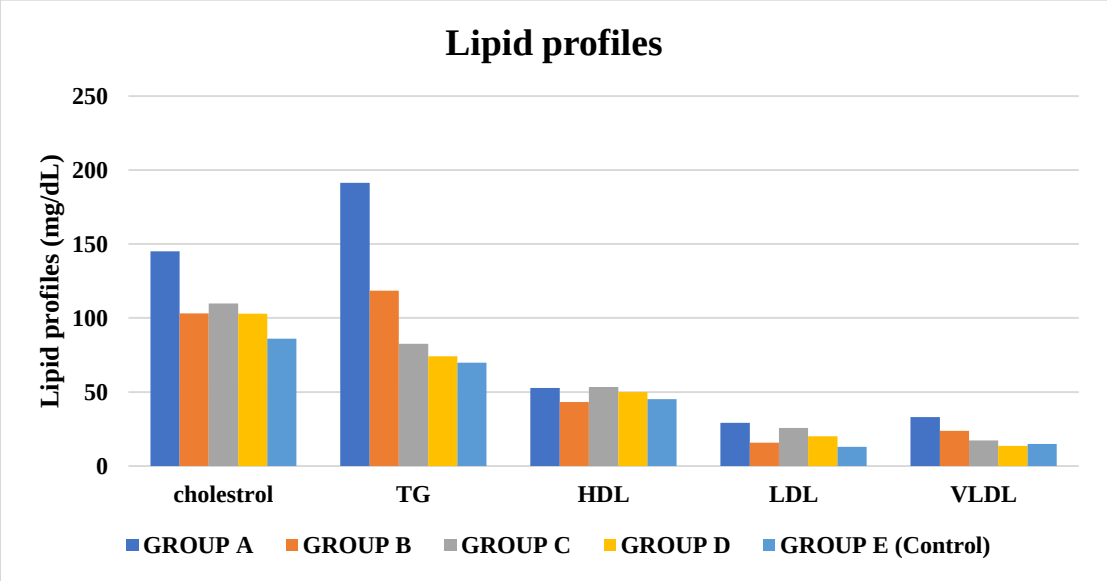

Figure 1. Lipid profiles in different ketogenic diet groups

Table 3. Lipid profiles in different ketogenic diet groups

Rats' Group	Diet name	cholesterol	triglyceride	HDL-C	LDL-C	VLDL
A	High Fat (70%coconut oil)	145.1 ± 21.9 ***	191.41 ± 72.65 ***	52.79 ± 7.37^{ns}	29.21 ± 7.31 ***	33.14 ± 9.23 ***
B	High Fat (70% animal fat)	103.1 ± 10.8 **	118.4 ± 12.9 **	43.33 ± 16.68 ^{ns}	15.84 ± 3.97 ^{ns}	23.71 ± 2.08 ***
C	High Protein (60% Coconut oil, 35% Casien)	109.8 ± 30.2^{ns}	82.5 ± 10.9 ^{ns}	53.48 ± 14.79 ^{ns}	25.80 ± 8.75 **	17.23 ± 2.06 ^{ns}
D	Cyclic Ketogenic (70% coconut oil)	102.89 ± 16.90^{ns}	69.8 ± 7.7 ^{ns}	49.97 ± 9.94 ^{ns}	20.51 ± 3.89 **	13.59 ± 1.92 ^{ns}
E	Control (Standard)	86.1 ± 10.3	74.1 ± 9.8	45.14 ± 5.47	12.91 ± 1.08	14.94 ± 1.81

Note: Symbols of statistical significance (p-value) compared to the control group: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not statistically significant ($p \geq 0.05$), -: baseline value of the control group (no comparison).

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3.2. Atherogenic Ratios

3.2.1. Changes in TC/HDL-C Ratio in Different Ketogenic Diets

The results of the comparison of the TC to HDL ratio between the study groups showed significant differences in the lipid profile. Group A with a high-fat diet showed a significantly higher ratio (2.75 ± 0.38, ***p<0.001), which was a significant increase compared to the control group (1.92 ± 0.26) indicating an adverse effect of this diet on lipid balance. Group B also had a higher ratio than the control group (2.11 ± 0.24, *p=0.042), although this increase was less than that of group A. Groups C and E showed a higher mean ratio (2.07 ± 0.35 and 1.96 ± 0.41, respectively), but these differences were not statistically significant (p=0.087 and p=0.215) (Table 4) (Figure 2).

3.2.2. Changes in TG/HDL-C Ratio in Different Ketogenic Diets

According to the results in Table 4 and Figure 2, the comparison the TG to HDL-C ratio between different study groups shows significant differences in lipid profile. Group A with high-fat diet showed the significantly highest ratio (3.59 ± 0.96, ***p<0.001), which is a significant increase compared to the control group (1.64 ± 0.10) indicating an adverse effect of this diet on lipid balance. Group B also had a higher ratio than the control group (2.43 ± 0.49, **p=0.003), although this increase was less than that of group A. In contrast, groups C and E did not differ significantly from the control group (1.64 ± 0.56 with p=0.321 and 1.45 ± 0.38 with p=0.157, respectively). Interestingly, group E with a periodic ketogenic diet showed an even lower mean than the control group, although this difference was not statistically significant. These findings are of clinical importance because the TG/HDL-C ratio is known to be a marker of insulin resistance and a predictor of cardiovascular risk [30]. As epidemiological studies have shown, this ratio can provide valuable information about metabolic risk. As [31] reported, the effect of LDL concentration on the cardiac arrest is “U” shaped, with the greatest risk occurring at the maximum and minimum values of LDL concentration. Also, [32] noted that very low LDL values are also associated with a higher risk of mortality. LDL particle size may also be important in determining cardiovascular risk. As [33] has noted, smaller LDL particles are potentially more harmful than larger particles. This highlights the importance of considering the quality of lipoprotein particles in addition to their quantity. On the other hand, HDL levels are considered the “good cholesterol” and are associated with a reduced risk of cardiovascular disease [24]. However, as [34], [35] have noted, both very high and very low HDL concentrations may be undesirable. TG levels are also known to be a significant risk factor, and there is a linear relationship between high triglyceride concentrations and ischemic stroke. However, [10] demonstrated that very low triglyceride concentrations may also be associated with an increased risk of stroke. Based on these findings, while high-fat diets (especially type 1) can adversely increase the TG/HDL-C ratio, intermittent ketogenic diets may have neutral or even favorable effects on this index. Overall, these results suggest that while high-fat ketogenic diets may unfavorably elevate atherogenic lipid ratios, intermittent ketogenic regimens may exert neutral or potentially beneficial effects, particularly in metabolic-risk populations.

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3.2.3. Changes in LDL-C/HDL-C Ratio in Different Ketogenic Diets

The results of the LDL-C/HDL-C ratio, a reliable indicator of atherogenic risk, showed a pattern similar to other lipid parameters. The coconut oil (%70) group A (0.55 ± 0.11) and the high-protein diet group C (0.51 ± 0.19) showed significantly the highest LDL-C/HDL-C ratio ($p < 0.0001$ and $p = 0.001$, respectively). The intermittent ketogenic diet group (0.42 ± 0.08) also showed a significant increase compared to the control group E (0.29 ± 0.04) ($p = 0.003$). Interestingly, the animal fat (%70) group B (0.33 ± 0.08) did not show a significant difference from the control group(E), despite the numerical increase (Table 4) (Figure 2). These findings suggest that a coconut oil (%70) diet may exert adverse effect on the balance between atherogenic and protective lipoproteins. The elevations observed in the coconut-oil and high-protein groups indicate that these diets may increase atherogenic risk. These results suggest that the assessment of lipid ratios can provide more valuable clinical information than absolute lipid levels. As reported by [36] and [37], KD may be reduce total cholesterol and increasing HDL-C. Also, as noted by [12, 38] these diets can have a significant effect on blood triglyceride levels. These findings emphasize the importance of considering lipid ratios rather than absolute levels in assessing cardiovascular risk. They also suggest that diet type can have a significant impact on these ratios and should be considered in dietary recommendations for individuals at high cardiovascular risk.

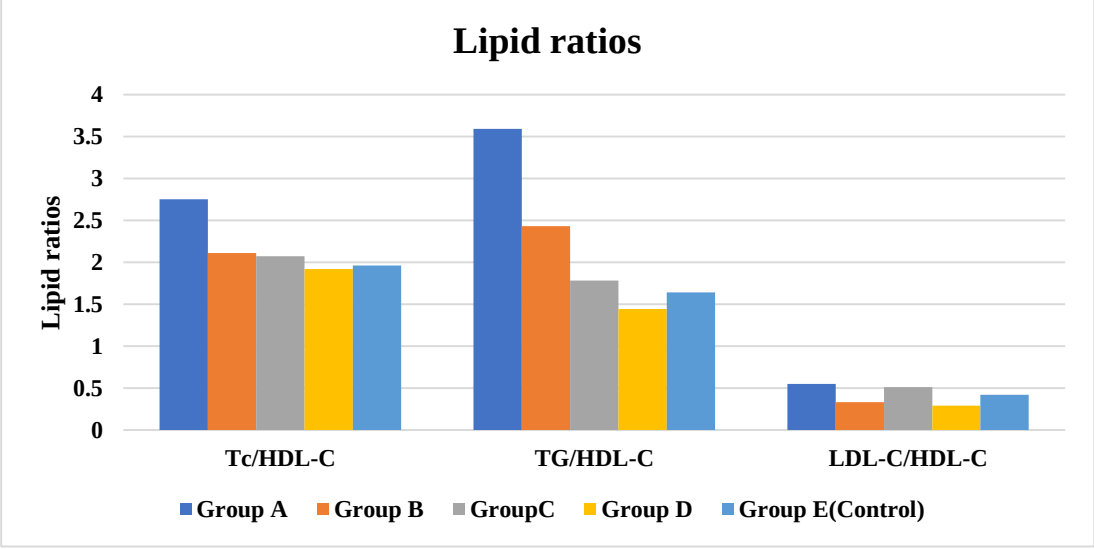

Figure 2. Lipid ratios in different ketogenic diet groups

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Table 4. Lipid Ratios in Different ketogenic Diet groups

Rats' Group	Diet Type	TC/HDL-C	TG/HDL-C	LDL-C/HDL-C
A	High Fat (70% coconut oil)	2.75 ± 0.38 ***	3.59± 1.01**	0.55 ± 0.12 ***
B	High Fat (70% animal fat)	2.11 ± 0.24 *	2.43± 0.51*	0.33 ± 0.09ns
C	High Protein (60% Coconut oil, 35% Casein)	2.07 ± 0.35ns	1.78 ± 0.63ns	0.51 ± 0.20 **
D	Cyclic Ketogenic (70% coconut oil)	1.92 ± 0.26ns	1.44± 0.38ns	0.29 ± 0.04
E	Control (Standard)	1.96 ± 0.41	1.64± 0.12	0.42 ± 0.09 **

Note: Symbols of statistical significance (p-value) compared to the control group: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, ns: not statistically significant ($p \geq 0.05$), -: baseline value of the control group (no comparison).

3.3. Histopathology of the Heart of Rats Under Different Fat Diets

In this study, the effects of three diets [control, coconut oil (%70), and animal fat (%70)] on the heart tissue of rats were evaluated through histopathological examinations, the results of which are as follows: 70% fat (coconut oil) group (A-C): (A) overall architecture of the heart with disrupted tissue pattern (100x), (B) increased intercellular space and inflammatory infiltrate and mild fat accumulation in cardiomyocytes (400x), and (C) significant inflammatory cell infiltration with evidence of cardiomyocyte degeneration and necrosis (400x). 70% fat (animal fat) group (D-F): (D) relatively preserved cardiac architecture (100x), (E) mild inflammatory infiltrate with preserved cellular structure and mild cytoplasmic lipid droplets (400x), and (F) occasional inflammatory cells with generally healthy cardiomyocytes (400x). Control group (G-I): (G) normal cardiac tissue architecture (100x), (H and I) regular arrangement of cardiomyocytes with minimal inflammatory cells and occasional physiological lipid droplets (400x).

3.3.1. Lipid Accumulation in Cardiomyocytes

In the control group E, lipid accumulation was observed at its minimal level (score 1) with scattered droplets in less than 5% of the cells. This is normal and consistent with the basal metabolism of the cells. Interestingly, the group A (70% coconut oil) showed the highest lipid accumulation (score 2 in some samples), involving 5-25% of cardiomyocytes. This increase may be due to the impaired metabolism of unsaturated fatty acids, which may contribute to metabolic disturbances or reduced cellular efficiency. In contrast, the (animal fat) (%70) group B, despite initial expectations, had a similar status to the control group E, indicating possible improvements in metabolic efficiency without implying a specific underlying mechanism.

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3.3.2. Inflammatory Responses

The control group (E), as expected, showed the lowest level of inflammation (score 0-1). The coconut oil (%70) group A showed the most pronounced inflammatory response (score 2-3) with the formation of multiple inflammatory foci of lymphocytes and macrophages. Mild inflammation (score 1–2) was observed in the animal fat (70%) group (B), being less severe than in both the coconut oil (70%) group (A) and the control group (E).

3.3.3.Fibrotic Changes

The high-fat 70% (coconut oil) group showed a slight but measurable increase in collagen deposition (score 1 in some samples), which may suggest the beginning of ventricular remodeling, although further evidence is required to confirm this process. These changes may be secondary to inflammatory activity and cellular injury. In contrast, both the control and 70% (animal fat) groups maintained a more normal pattern of collagen distribution.

3.3.4. Cellular Damage and Necrosis

The most important and concerning finding was the observation of cardiomyocyte degeneration and necrosis in the high-fat 70% (coconut oil) group. This phenomenon, evident in the images as cellular swelling and disorganization of cell lines, may reflect tissue injury consistent with histopathological alterations. The findings of this study clearly demonstrate that not all high-fat diets have the same effects on cardiac tissue, and that fat composition is a more determining factor than the percentage of fat in the diet alone. In this study, the diet rich in coconut oil (70%) resulted in the most severe histopathological damage, including disruption of tissue architecture, significant inflammatory infiltration, lipid accumulation in cardiomyocytes, and cell necrosis. In contrast, the group receiving the same percentage of animal fat showed much less damage, similar to the control group. This apparent paradox—between the reported beneficial properties of medium-chain triglycerides (MCTs) and the observed negative results—may related to considering the unique composition of coconut oil. Although it contains MCTs, the very high content of certain saturated fatty acids, such as lauric acid (C12:0) and myristic acid (C14:0), may have dominant toxic effects [39]. These fatty acids are well known to be ligands for the activation of Toll-like receptor 4 (TLR4), which triggers a key proinflammatory signaling pathway and leads to the production of cytokines such as TNF-α and IL-6 [40].This mechanism is well consistent with the significant infiltration of inflammatory cells observed in the coconut oil group. Furthermore, myristic acid is specifically involved in the synthesis of ceramides, particularly C14-ceramide, a lipotoxic metabolite known to induce apoptosis and mitochondrial dysfunction in cardiomyocytes [41].This could provide a strong explanation for the evidence of necrosis and cell degeneration observed exclusively in this group. Alternatively, the different metabolism of animal fat (mainly containing long-chain triglycerides or LCTs) may explain the lower damage. Unlike MCTs, which enter mitochondria rapidly and unregulated, the oxidation of LCTs is dependent on the carnitine palmitoyltransferase (CPT) system, which may exert a level of control and prevent metabolic overload and the production of reactive oxygen species (ROS) [42]. Although both high-fat diets can increase oxidative stress, the fatty acid composition of coconut oil may make the heart particularly vulnerable to this damage. Several studies have investigated the potential protective effects of ketogenic diets, which often contain high amounts of MCTs, on the heart. For example,

[43] showed that a ketogenic diet can improve cardiac function in a model of diabetic cardiomyopathy by suppressing inflammation and improving energy metabolism. Similarly, [40] reported that such a diet can prevent neointimal hyperplasia in vessels by suppressing oxidative stress and inflammation. The findings of this study support the beneficial role of ketone bodies, particularly beta-hydroxybutyrate (β-HB), both as an effective energy source and as an anti-inflammatory signaling molecule [44].

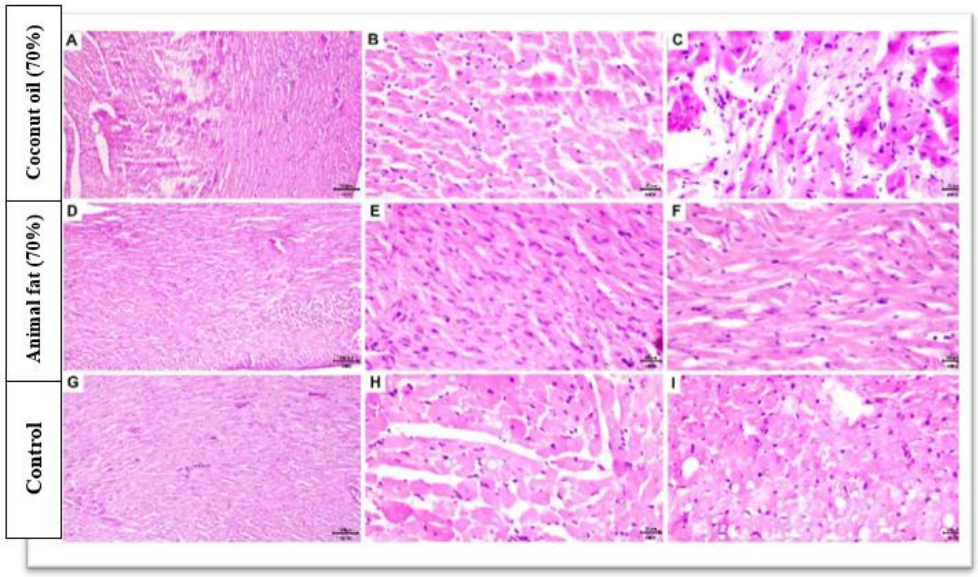

Figure (3) Histopathology of the Heart of Rats Under Different Fat Diets

CONCLUSIONS:

According to the results obtained, the effects of ketogenic diets are significantly influenced by the macronutrient composition, especially the fat source. High-fat diets, especially those enriched with coconut oil, have significant adverse effects on metabolic od lipids and cardiac tissue, while high-protein and periodic diets are considered comparatively safer, though further studies are needed to confirm their long-term effects. These findings highlight the importance of dietary fat composition when considering ketogenic diets for potential clinical use.

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Conflict of interests

Authors declare that they don't have any conflict of interests.

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