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Comparison of high-protein diet versus ketogenic diet as weight reduction diets in obese rats: hepatic and metabolic effects

Sherein F. El-sayed [1],Islam Maher Wahid [2], Islam Mohamed Salem [2], Mona Mostafa Ahmed[3], Reham M. Wahid [1]

1- Physiology Department, Faculty of human medicine, Zagazig University, Zagazig, Egypt.
2- Internal medicine department, endocrinology, Faculty of human medicine, Zagazig University, Zagazig, Egypt.
3- Pathology department, Faculty of human medicine, Zagazig University, Zagazig, Egypt.

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- high-protein diet,
- ketogenic diet
- metabolism
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Abstract

Background/Objectives: Obesity is a worldwide health problem, and adopting a diet is one of the main factors to enhance weight loss and manage obesity implications. However, the recommendation of a certain diet remains controversial. We aimed to compare the hepatic and metabolic effects of both high-protein diet (HPD) and ketogenic diet (KD) in a rat model of obesity.**Methods:** This study included 40 adult male albino rats, which were divided into four groups: group I, control group with a normal diet; group II, obese treated with high-fat diet; group III, obese treated with HPD; and group IV, obese treated with KD. Measurements of the lipid profile, glucose, and insulin serum levels and liver function tests were performed by examining the liver parenchyma for histopathology and oxidative stress (OS) markers. **Results:** High serum levels of glucose, insulin, and lipid profile and homeostasis model assessment of insulin resistance index were observed in the KD group compared with those in the other groups, with high levels of liver enzymes and OS markers and tumor necrosis factor in the hepatic tissue ($p < 0.05$). On the other hand, the HPD showed improved metabolic parameters and liver function with a low expression of OS markers in the hepatic tissue. **Conclusion:** These findings showed that HPD can be recommended as an effective weight loss diet with regard to its metabolic and hepatic effects. However, further clinical investigations are required to determine other HPD effects in obese patients.

Corresponding author: Reham M. Wahid M.D. in Medical Physiology. Address: 16, Abdelaziz alhady St, Mobarak Avenue, Zagazig, Alsharqia, Egypt. Mob: 00201066888752. Dr_rehamwahid@yahoo.com

INTRODUCTION

Obesity is considered one of the critical health problems affecting people worldwide. Excessive fat accumulation with increasing body weight is the major characteristic of this disease[(1)]. Obesity is associated with several liver abnormalities, such as nonalcoholic fatty liver disease (NAFLD), in which steatosis or steatohepatitis occurs[(2)]. Metabolic complications of obesity include type 2 diabetes mellitus (T2DM), metabolic syndrome, dyslipidemia, hypertension, and ischemic heart disease.

Macrophages are found in a great number in the adipose tissues of obese people, and once activated, they secrete various kinds of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 and IL-1[(3)], that promote more generation of reactive oxygen species and nitrogen by macrophages and monocytes, which leads to high oxidative stress (OS)[(4)] that subsequently increases the risk of the development of metabolic syndrome in these patients. Increased OS is also involved in the pathogenesis of different diseases, such as hepatic steatosis, atherosclerosis, and hypertension[(5)].

Lifestyle modification is the first line of treatment of obesity; therefore, targeting diet and physical activity can make a crucial step in the management of obesity[(6)]. Diet remains controversial, and several different types have been recommended for weight loss. However, a lack of scientific evidence is still an issue that cannot allow recommending one diet over another. Ketogenic diet (KD) is used in weight loss programs for neurological disease, NAFLD, and obesity, and despite its known advantages, its metabolic effects are not yet precisely understood.

A previous study has shown the beneficial effects of KD on the improvement of the blood glucose levels in obese individuals and patients with T2DM[(7)]. KD is also used as an effective therapy for epilepsy[(8)]. However, KD is shown to increase hepatic insulin resistance[(9)], which enhances T2DM development, in addition to its effect in impaired glucose tolerance in KD-fed rats[(10)]. A previous study has shown that KD has a negative hepatic effect as it leads to hepatic steatosis in mice[(9)], whereas Cotter et al.[(11)] suggested that ketogenesis can improve fatty liver disease.

A number of studies revealed that high-protein diets (HPDs) are used to prevent obesity development and lose weight[(12)]. French et al.[(13)] reported that HPD reduces weight gain and decreases food intake and liver fat deposition in obese Zucker rats. However, Díaz-Rúa et al.[(14)] showed that the intake of HPD increases liver triacylglycerol deposition, leading to hepatic injury. Therefore, this study was conducted to determine the effects of KD and HPD on the metabolic and hepatic changes associated with experimentally induced obesity in male albino rats.

Materials and methods

This study was simply random and conducted on 40 adult healthy males of local strain albino rats weighing 170–190 g,

The rats were kept in steel wire cages (five/cage) with free access to water and chow at room temperature and were maintained in a 12-h light/dark cycle. The experimental protocol was approved by the Physiology Department and ZU-IACUC (approval number, ZU-IACUC/3/F/151/2019).

This study included 40 rats (n = 10 for each group): group I, control group with a normal diet (61.2 % carbohydrate, 11.5% fat, and 27.2% protein for 10 weeks) [15], obesity was induced by feeding rats high-fat diet (HFD), which consisted of 16.4% protein, 25.6% carbohydrate, and 58.0% fat, for 8 weeks[16]. After induction of obesity, at the 8th week 30 rats were further divided into 3 groups: group II, obese treated with HFD for 10 weeks; group III, obese treated with HPD comprising 33% carbohydrate, 14% fat, and 44% protein for 10 weeks; and group IV, obese treated with KD comprising 0.4% carbohydrate, 95% fat, and 4.5% protein for 10 weeks[17].

Calculation of body mass index

Twenty-four hours after the end of the study and after overnight fasting, rats were anesthetized by ether inhalation, and blood samples were collected from the orbital veins. Both blood glucose and insulin levels were performed by enzyme-linked immunosorbent assay (ELISA). Then, the homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = fasting serum glucose (mg/dl) × fasting serum insulin (μIU/ml)/405. (kits for the estimation of the serum glucose, insulin, cholesterol, TG, and HDL levels were purchased from Biosource Europe S.A. [Belgium]).

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were estimated using rat ALT and AST ELISA kits (Shanghai Sunred Biological Technology, China), and the serum alkaline phosphatase level was estimated using a commercial kit (SPINREACT, Ctra. Sta. Coloma, Spain). The serum total protein and albumin levels were measured using the bromocresol green. In addition, the serum total bilirubin level was estimated as described by Walters and Gerarde[18].

Tissue sampling

Immediately after blood sample collection, rats were killed by decapitation after light ether anesthesia. Then, total hepatectomies were performed, and the livers specimens were then washed with phosphate-buffered saline (pH, 7.4), and a portion of the liver obtained was subjected to homogenization and stored at −70°C for biochemical estimations of hepatic malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD). The other portion (left lobe) was used for histopathological studies. The MDA levels were calculated using a commercial assay kit (Bioxytech MDA-586, OxisResearch), and the markers of OS were measured as described by Nebot et al.[19].

Histopathological examination

The liver was fixed overnight in 10% neutral buffer formalin and embedded in paraffin. Tissue sections were cut at 4-μm thickness. The sections were stained with a hematoxylin and eosin staining kit (Baibo Biotechnology Co., Ltd., Shandong, China) [20], and immunostaining was performed on paraffin sections using a microwave-based antigen retrieval technique as described by Khan et al.[21].

The antibody against TNF-α (Abcam, Cambridge, UK) was used to detect TNF-α. Sections were treated with the EnVision+ DAB kit (Dako, Denmark) according to the manufacturer's instruction, the hepatic specimens were examined by light microscopy, and ImageJ software was used to calculate the ratio of the brown-stained area to the total area.

Statistical analysis

The data presented in our study are expressed as mean ± standard deviation for quantitative data and statistically analyzed using the SPSS program (19) (SPSS Inc., Chicago, IL, USA). The ANOVA (post hoc LSD) test was used to compare the means among more than two groups in quantitative data [22].

A p value of ≤0.05 was considered statistically significant.

Results

Effects of the normal diet, HFD (obesity), HPD, and KD on liver function parameters

There was no significant difference in total bilirubin among all groups ($p > 0.05$) (Table 1). Furthermore, it was observed that the level of the liver enzyme ALT markedly decreased in the HPD group compared with those in both the HFD and KD groups ($p < 0.001$) and significantly increased in the KD group compared with that in the HFD group ($p < 0.001$). On the other hand, there was no significant difference in AST among the HFD, HPD, and KD groups ($p > 0.05$) (Table 1).

Finally, a marked increase in the albumin, globulin, and total protein levels was observed in the HPD group compared with those in both the HFD and KD groups ($p < 0.05$). However, there was a marked decrease in the same parameters in the KD group compared with those in both the HFD and HPD groups ($p < 0.05$) (Table 1).

Effects of the normal diet, HFD (obesity), HPD, and KD on the body weight, plasma glucose and insulin levels, and HOMA-IR

There was a highly significant increase in the fasting glucose and insulin levels and HOMA-IR in the KD group compared with those in the other groups ($p < 0.001$) (Table 2), whereas a significant decrease in both fasting glucose level and HOMA-IR was noted in the HPD group compared with those in both the HFD and KD groups ($p < 0.05$) (Table 2).

Regarding weight change, there was a marked weight loss in both the HPD and KD groups compared with that in the HFD group ($p < 0.001$) (Table 3). However, it was observed that weight loss was higher in the HPD group than in the KD group ($p < 0.001$) (Table 2).

Effects of the normal diet, HFD (obesity), HPD, and KD on the lipid profile (HDL, LDL, VLDL, cholesterol, and TG) levels

No differences were observed with respect to the HDL levels among all groups ($p > 0.05$) (Table 4), whereas there was a marked increase in the cholesterol, TG, LDL, and VLDL levels in the KD group compared with those in the others ($p < 0.001$) (Table3).

Although there was a significant decrease in the cholesterol, TG, LDL, and VLDL levels in the HPD group compared with those in both the HFD and KD groups ($p < 0.05$) (Table 3), a marked increase in these parameters was observed in both the HFD and HPD groups compared with that in the control group ($p < 0.05$) (Table 3).

Effects of the normal diet, HFD (obesity), HPD, and KD on the OS enzymes in the liver tissue

The KD group was characterized by a marked decrease in the SOD and CAT levels in the liver tissue compared with those in the other groups ($p < 0.001$). However, it showed a dramatic increase in the MDA level compared with those in the other groups ($p < 0.001$) (Table 4). On the other hand, there was a significant increase in the SOD and CAT levels in the liver tissue of the HPD group compared with those in the HFD group, in addition to a significant

decrease in the MDA levels compared with that in the HFD group ($p < 0.001$) (Table 4). Finally, there was no significant difference between the control and HPD groups regarding the same parameters ($p > 0.05$) (Table 4).

Effects of the normal diet, HFD (obesity), HPD, and KD on the H&E staining and TNF expression in the liver tissue

It was observed that ketogenic diet group showed some fatty degeneration of hepatocytes. However, HPD group showed normal architecture (Figure 1). In addition, TNF was highly expressed in the KD group compared with that in other groups and it was the lowest in the control and HPD groups ($p < 0.001$) (Table 4 and Figure 2).

Table (1) Comparison of the liver function parameters among normal, obese, HPD and KD groups.

	Groups control N=10	Obese (HFD) N=10	HPD N=10	Ketogenic diet N=10	Anova Test	P value
Total bilirubin	0.66±0.08	0.71±0.11	0.64±0.07	0.68±0.08	1.06	**0.37**
ALT	50±2.9	77±4.7[a]	59±3.1[a b]	95±7.1[a b c]	113.5	**0.00****
AST	71±3.2	130±11.2[a]	128±21.2[a]	122±17.8 [a]	39.3	**0.00****
ALP	92±4.8	153±12.7[a]	138±15.9[a b]	183±13.7[a b c]	102.7	**0.00****
Total protein	7±0.4	6.4±0.2[a]	7.1±0.16[b]	5.8±0.2[a b c]	38.3	**0.00****
Albumin	3.7±0.3	3.3±0.2[a]	3.6±0.08[a b]	3.1±0.07[a c]	19.4	**0.00****
Globulin	3.2±0.2	3.1±0.1	3.4±0.2[b]	2.6±0.2[a b c]	15.4	**0.00****
Albumin/globulin ratio	1.1±0.08	1.07±0.06	1.07±0.08	1.2±0.1[b c]	4.3	**0.01***

All variables were expressed using mean (± SD) and compared using Anova test where:

- *P* value = 0.00** was considered statistically highly significant (S).
- *P* value ≤ 0.05* was considered statistically significant(S).
- *P* value >0.05 was considered statistically non-significant
- Post-hoc analysis comparing "Normal Vs Obese Vs HPD Vs Ketogenic" groups

And LSD post hoc test where:

[a] means : significant compared with normal (1) group.

[b] means: significant compared with obese or HFD (2) group.

[c] means: significant compared with HPD (3) group.

Abbreviations:

HPD: High protein diet - KD: ketogenic diet - HFD: high-fat diet - ALT: alanine aminotransferase

AST: Aspartate aminotransferase - ALP: alkaline phosphatase

Table (2): Comparison of the metabolic parameters and weights among normal, obese, HPD and KD groups.

	Groups control N=10	Obese (HFD) N=10	HPD N=10	Ketogenic diet N=10	Anova Test	P value
Fasting insulin	9.8±0.2	16.4±5.2[a]	13.7±1.9[a]	19.5±0.97[a b c]	20.9	**0.00****
Fasting glucose	90.7±5.6	116±12.2[a]	107±8.5[a b]	142.8±5.7[a b c]	66.1	**0.00****
HOMA IR	2.1±0.1	4.7±1.7[a]	3.5±0.68[a b]	6.5±0.5[a b c]	36.3	**0.00****
Weight before (at the 8th week)	192.7±15.5	403.4±13[a]	421±13.9[a b]	425±15.7[a b]	530.6	**0.00****
Weight After (at the 18th week)	213±12.9	470.8±51 [a]	322.4±19.6[a b]	397±18[a b c]	214.8	0.00**
Weight Loss	-20.3±10	-66.4±40[a]	98.6±15[a b]	28.4±31[a b c]	129.8	0.00**

All variables were expressed using mean (± SD) and compared using Anova test where:

- *P* value = 0.00** was considered statistically highly significant (S).
- Post-hoc analysis comparing "Normal Vs Obese Vs HPD Vs Ketogenic" groups

And LSD post hoc test where:

[a] means: significant compared with normal (1) group.

[b] means: significant compared with obese or HFD (2) group.

[c] means: significant compared with HPD (3) group.

Abbreviations:

HPD: High protein diet - D: ketogenic diet - HFD: high-fat diet

HOMA IR: Homeostatic Model Assessment for Insulin Resistance

Table (3): Comparison of lipid profile parameters among normal, obese, HPD and KD groups.

	Groups				Anova Test	P value
	control N=10	Obese (HFD) N=10	HPD N=10	Ketogenic diet N=10		
Cholesterol	80±7.2	137.9±34.8[a]	115.7±28.8[a b]	211.2±8.7[a b c]	56.5	0.00**
Triglycerides	92.4±8.4	150±33.6[a]	118.8±30.2[a b]	202.4±11.5[a b c]	39.6	0.00**
HDL	50.8±2	49.6±2.5	51.6±3.6	51.1±1	1.2	**0.35**
LDL	10.5±3.8	61.3±31.2[a]	41.2±26.4[a b]	119.5±9.1[a b c]	47.6	0.00**
VLDL	18.4±1.6	31.4±7.1[a]	23.7±6[a b]	40.4±2.2[a b c]	38.2	0.00**

All variables were expressed using mean (± SD) and compared using Anova test where:

- *P* value = 0.00** was considered statistically highly significant (S).
- *P* value >0.05 was considered statistically non-significant.
- Post-hoc analysis comparing "Normal Vs Obese Vs HPD Vs Ketogenic" groups

And LSD post hoc test where:

[a] means: significant compared with normal (1) group.
[b] means: significant compared with obese or HFD (2) group.
[c] means: significant compared with HPD (3) group.

Abbreviations:

HPD: High protein diet - KD: ketogenic diet - HFD: high-fat diet
HDL: high-density lipoproteins - LDL: low-density lipoproteins - VLDL: very low-density lipoproteins

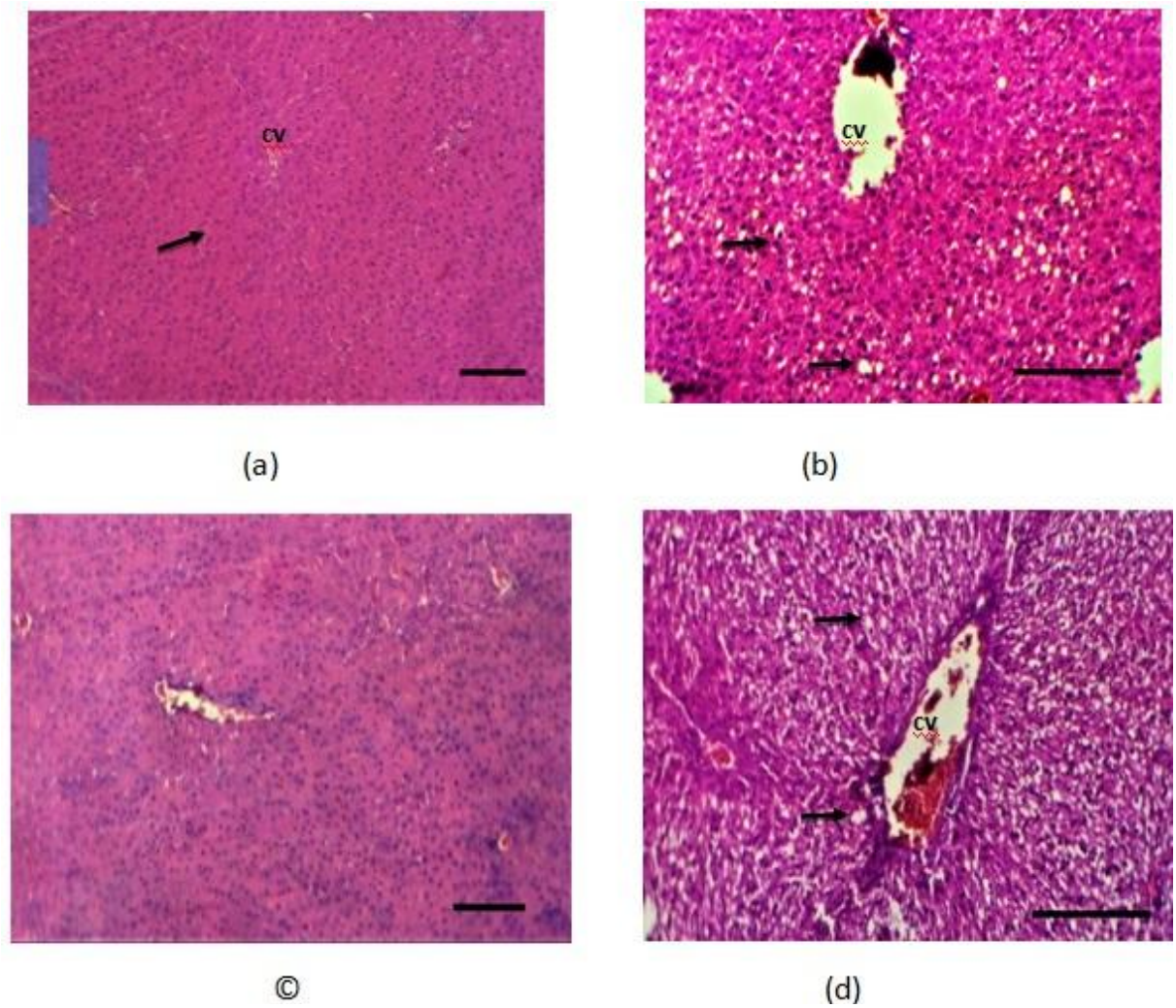

Figure (1) Representative images of H&E staining of liver sections (a) control showing normal central vein (cv) and hepatocytes arrow (x200 in original magnification) (b) HFD group showing mild fatty degeneration in form of vacuoles in some hepatocytes arrow(x400 in original magnification) (c) HPD group showing normal liver architecture (x200 in original magnification) (d) ketogenic diet group showing some fatty degeneration of hepatocytes arrow and dilated central vein (cv) (x400 in original magnification) Scale bar 50 micrometers).

Table (4): Comparison of the oxidative stress enzymes and TNF expression in liver tissue of normal, obese, HPD and KD groups.

All variables were expressed using mean (± SD) and compared using Anova test where:

	Groups				Anova Test	P value
	control N=10	Obese (HFD) N=10	HPD N=10	Ketogenic diet N=10		
SOD liver	34.4±3.4	18.3±2 [a]	34±2 [b]	8.2±3 [a b c]	241.3	**0.00****
MDA liver	45.9±7.6	110.6±11 [a]	43.2±14 [b]	182.6±15 [a b c]	371.8	**0.00****
CAT liver	65±4.2	35.3±2 [a]	63.6±2 [b]	20.3±3 [a b c]	566.8	**0.00****
TNF expression area%	0.35± 0.15	22.3±2.3	0.93±0.29	51.6±4.8	809.1	**0.00****

- *P* value= 0.00** was considered statistically highly significant (S).
- Post-hoc analysis comparing "Normal Vs Obese Vs HPD Vs Ketogenic" groups

And LSD post hoc test where:

[a] means: significant compared with normal (1) group.

[b] means: significant compared with obese or HFD (2) group.

[c] means: significant compared with HPD (3) group.

Abbreviations:

HPD: High protein diet - KD: ketogenic diet - HFD: high-fat diet - SOD: superoxide dismutase

MDA: malondialdehyde - CAT: catalase - TNF: Tumor necrosis factor

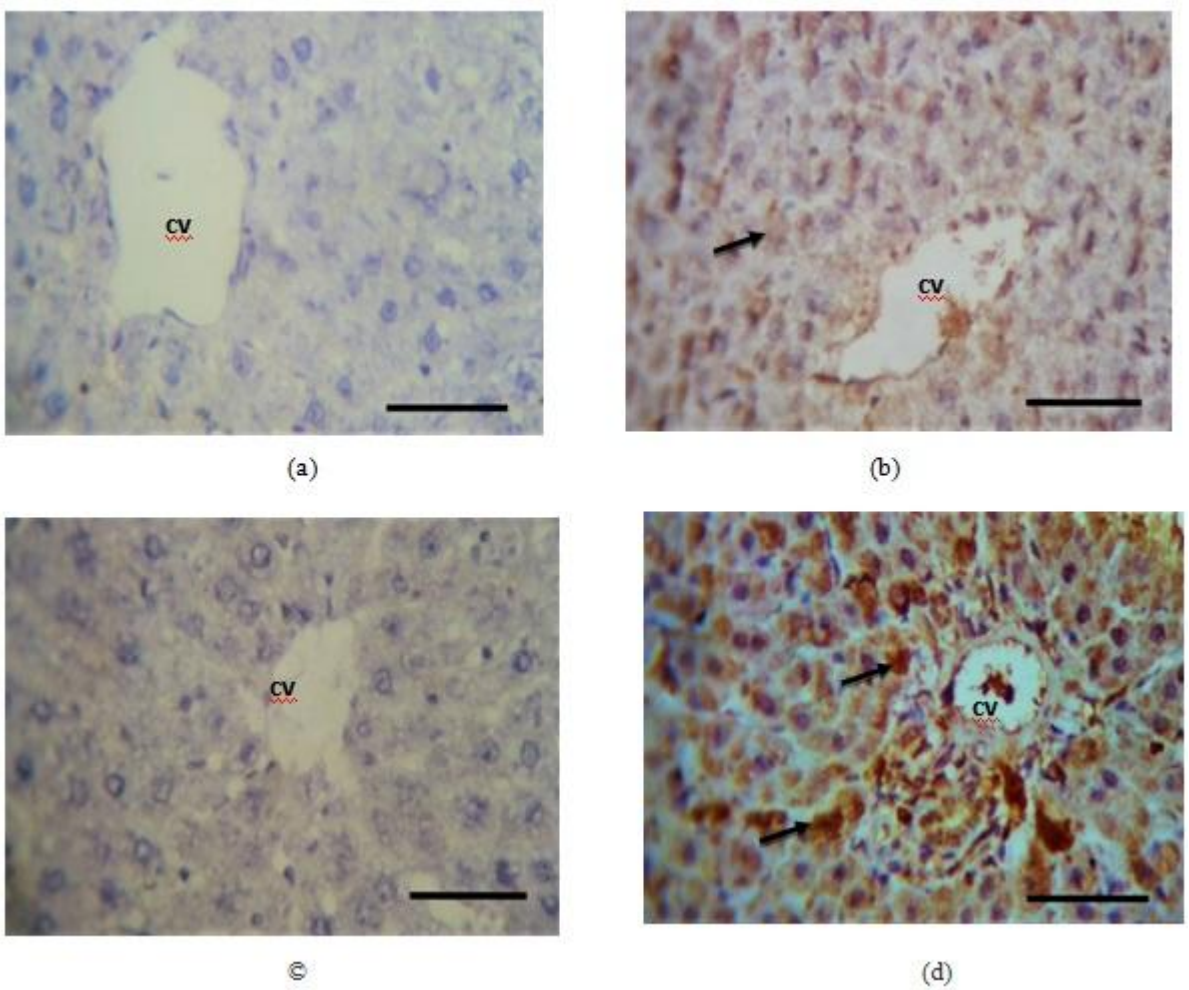

Figure (2) Representative immunohistochemistry images of TNF-α expression in liver sections (a) control showing 0.35± 0.15 area % TNF expression (b) HFD group showing 22.3±2.3 area % TNF expression with the brown color of TNF stain arrow(c) HPD group showing 0.93±0.29 area % TNF expression (d) ketogenic diet group showing 51.6±4.8 area % TNF expression with the brown color of TNF stain arrow (x400) Scale bar 50 micrometers.cv: central vein

Discussion

Obesity is a worldwide problem. It leads to several diseases, such as diabetes mellitus, hepatic steatosis, cardiovascular diseases, and cancer. It is also associated with increased OS and chronic inflammation[3]. The first line of obesity management is changing lifestyle, which requires increasing physical activity and changing diet[6]. Several different types of diets have been used for the reduction of weight, but we could not recommend one diet over another. It is one of the more controversial issues.

KD is one of the most popular regimens used in weight loss programs for obesity, fatty liver, and neurological disease. There were controversial results on the effects of KD on the control of blood glucose levels in patients with diabetes and obese individuals[10]. In addition, Cotter et al.[11] suggested that ketogenesis can improve fatty liver disease. However, another study has shown that KD leads to increase liver fat content in mice[9].

HPD is another diet commonly used as an alternative weight reduction regimen[12]. The results of several experimental studies remain controversial, and Díaz-Rúa et al.[14] reported that the intake of HPD for a long time increases intrahepatic lipid (IHL) accumulation, leading to liver injury. However, French et al.[13] reported that HPD decreases food intake, helps in weight loss, and decreases IHL content in obese Zucker rats.

In light of the previous discrepancy, our study was performed to explore the effects of KD and HPD on the metabolic and hepatic changes associated with experimentally induced obesity in male albino rats.

Our study showed that there was a significant weight loss in both the HPD and KD groups compared with that in the HFD group. However, it was observed that weight reduction was more observed in the HPD group than in the KD group. Kinsey- Jones et al.[23] found that HPD increases satiety effect and reduces hunger, leading to decreased food intake. In addition, increased amino acids in the diet lead to stimulation of the satiety center in the hypothalamus and nucleus tractus solitarius via the vagus nerve[12]. Increased dietary protein has also been shown to increase resting, diet-induced, and total energy expenditure, enhancing weight loss [24]. Also, Lim et al.,2022 revealed that consumption of HPD for 8weeks increases satiety effect and loss of body weight in obese women independent of percentage of carbohydrate in HPD [25]. .

On the other hand, KD has been shown to increase energy expenditure in mice[26]. In KD, carbohydrate restriction increases gluconeogenesis, which is energy demanding[27] and results in increasing hepatic oxygen consumption for triglyceride-fatty acid recycling and for gluconeogenesis increasing energy expenditure and weight loss [28]. Ketosis induced by KD decreases appetite[29]. Another study has described that weight reduction results from the effect of proteins in reducing food intake[30]. In mice, KD decreases inflammation in the adipose tissue with increased uncoupling protein 1 levels and high energy expenditure[31].

In the present study, there was a significant increase in the fasting glucose and insulin levels and HOMA-IR in the KD group compared with those in the other groups. Histopathological examination of the liver from

the KD group showed fatty degeneration of hepatocytes with intracytoplasmic vacuoles.

In line with our study, Grandl et al.[32] showed that KD exacerbates systemic glucose intolerance compared with HFD, and that impaired glucose tolerance is due to the inability of insulin to suppress glucose output from the liver and increasing hepatic insulin resistance. Bielohuby et al.[10] revealed that KD-fed rats exhibit increased hepatic and peripheral tissue insulin resistance as a result of elevated intramyocellular lipids leading to glucose intolerance.

In this condition, decreased hepatic insulin sensitivity was due to increased diacylglycerol contents in the liver, which stimulate protein kinase Cε with decreased insulin-activated insulin receptor substrate-2 tyrosine phosphorylation by the insulin receptor kinase, decreasing the action of insulin in glucose output control from the liver and stimulating hepatic glycogen production[33].

On the contrary, another study has shown that feeding mice with KD for the long term improved insulin sensitivity[34]. Basciani et al.[35] revealed that KD leads to the improvement of the metabolic parameters over a short period with a significant reduction in body weight, HOMA-IR index, and insulin, LDL, and total cholesterol levels in all obese patients.

Furthermore, the present study showed that there was a marked decrease in the fasting glucose and insulin levels and HOMA-IR in the HPD group compared with those in the KD and HFD groups.

In line with our study, Johnston et al.[36] reported that HPD significantly reduces HOMA-IR in adults at risk for insulin resistance in comparison with a normal and lower protein diet with the same level of caloric restriction. In addition, Wang et al.[37] revealed that HPDs promote glucagon-like peptide-1 (GLP-1) release, which increases the satiating effect of HPDs. GLP-1 is an incretin that can decrease blood glucose levels by increasing insulin secretion in a glucose-dependent manner. GLP-1 also stimulates β-cell proliferation and neogenesis while inhibiting apoptosis[38].

In addition, Wojcik et al.[39] showed that an HPD that contained a mixture of plant-based and animal protein sources is effective for decreasing hepatic steatosis and improving insulin sensitivity independent of weight loss in obese Zucker rats.

Chen et al.[40] reported that a higher intake of animal and total protein is associated with T2DM and enhances insulin resistance. High-protein intake (from animal sources) was associated with lower fiber and vitamin intakes and higher fat intake, which may have been related to the positive association between incidents of T2DM and animal protein ingestion[41]. However, the intake of total plant protein such as protein from grains, legumes, and nuts was associated with a lower risk of insulin resistance and T2DM[40].

In our study, there was a marked increase in the cholesterol, TG, LDL, and VLDL levels in the KD group compared with those in the other groups, whereas no differences were observed with respect to the HDL levels among all groups.

Zamani et al.[42] reported a marked increase in the serum TG, cholesterol, and LDL levels in patients treated with the KD that could lead to early cardiovascular death. In addition, [43]showed that the KD used as weight reduction

regimen in postpartum obese mice resulting in significant increase of hepatic lipid content, total cholesterol, LDL cholesterol serum levels and impaired glucose tolerance. On the contrary, Choi et al.[44] showed that the supplementation of ketogenic drinks for 2 weeks improves blood lipid profile in obese adults.

Furthermore, there was a significant decrease in the cholesterol, TG, LDL, and VLDL levels in the HPD group compared with those in the KD and HFD groups. This result is consistent with that of the study by Gulati et al.[45] who showed that the intervention with HPD leads to a marked weight reduction and improvements in lipid profile and liver enzymes with a decrease in inflammatory marker levels in obese individuals. Lipoprotein lipase activation in the adipose tissue is mainly responsible for the clearance of TG from VLDL and chylomicrons. LPL is primarily activated by insulin, and a previous study has shown that HPD may exert beneficial metabolic effects via improvement in insulin action[46].

There is no significant difference among the HFD, HPD, and KD groups regarding bilirubin and AST. Furthermore, the KD group was characterized by a marked increase in the level of the liver enzyme ALT compared with those in the other groups. Histopathological examination of the liver from the KD group showed fatty degeneration of hepatocytes with intracytoplasmic vacuoles.

In line with our study, Douris et al.[26] and [43] showed that the IHL content increases in KD-fed mice in both short and long terms. The ALT and AST levels increased up to twofold with an increased IHL content, and KD induced inflammation of the liver and increased IHL content[31].

In another study, KD reduced weight and improved the blood glucose levels but had a risk of elevation of liver enzymes, induction of hepatic steatosis, and hypercholesterolemia. Immediate discontinuation of the diet leads to the improvement of hypercholesterolemia and reduced liver enzymes 2 weeks later[47].

On the contrary, Luukkonen et al.[48] reported that KD can improve hepatic steatosis. KD markedly decreased liver fat content with an enhanced breakdown of liver TGs as a result of remodeling of hepatic mitochondrial oxidative flux and decreased lipogenesis. In addition, Holland et al.[34] described that there is a marked reduction in the ALT levels, hepatic inflammatory markers, and hepatic TG accumulation in KD-fed rats. They used a KD with 20% protein, versus <10% in other studies.

In the present study, there was a marked decrease in the level of the liver enzyme ALT in the HPD group compared with those in the other groups. Histopathological examination of the liver from the HPD group showed the normal liver architecture. A marked increase in the albumin, globulin, and total protein levels was observed in the HPD group compared with those in both the HFD and KD groups.

In addition, Drummen et al.[24] observed a significant reduction in the IHL content in obese individuals after following hypocaloric HPD for 8 and 20 weeks with a marked reduction in the AST and ALT levels. In rats, HPD helps in decreasing the IHL content as a result of inhibition of lipogenesis with enhanced lipid oxidation and utilization in the liver[49].

Furthermore, Xu et al.[50] reported that the intake of HPD for 3 weeks has been shown to reduce the IHL content in obese individuals with

improvements in the serum glucose, insulin, lipid, and liver enzyme levels. They concluded that HPD decreased the IHL content by stimulating fatty acid β-oxidation and increasing mitochondrial activity.

In contrast, Díaz-Rúa et al.[14] revealed that the intake of HPD for a long time promotes lipid synthesis and hepatic triacylglycerol deposition. In addition, HPD enhanced mRNA and protein levels of HSP90 in the liver, which is a marker of hepatic injury with increased OS, inflammatory markers, and changes in the acid–base balance.

Although the pathophysiology of NAFLD is complicated and includes a close interaction between host genetics and environmental factors, the generation of OS plays a significant role in the progression of NAFLD[51].

We noticed in our study that the KD group was characterized by a marked decrease in the levels of antioxidant enzymes SOD and CAT in the liver tissue compared with those in the other groups. However, it showed a dramatic increase in the MDA (OS marker) levels compared with those in the other groups. Similarly, Arsyad et al.[52] found that following a KD for 60 days decreases the antioxidant enzyme (SOD) serum level and induces metabolic acidosis and anemia in rats. However, Parry et al.[53] showed that OS markers are not significantly different in the skeletal muscle, liver, or brain of KD-fed rats when compared with the standard diet-fed rats.

In the present study, there was a significant increase in the SOD and CAT levels and decrease in the MDA level in the liver tissue of the HPD group compared with those in both the HFD and KD groups with no significant difference between the control and HPD groups regarding the same parameters. These findings were in line with another study where HPD can reduce serum levels of inflammatory and OS markers in NAFLD[54].

It was observed in our study that TNF-α was highly expressed in the liver of the KD group compared with those in the other groups and it was the lowest in the control and HPD groups.

In humans, the ingestion of KD for 4 weeks is accompanied by increased levels of inflammatory markers and cholesterol[55]. In mice, KD induces inflammation of the liver and increases the IHL content, whereas inflammation is decreased in white adipose tissue[31]. On the other hand, Monda et al.[56] revealed that the KD intervention can improve the serum levels of IL-6, IL-10, TNF-α, and adiponectin. It also has beneficial effects on the inflammatory state. In addition, Charlot et al. showed that feeding obese mice with KD for 6 weeks decreases the liver weight, hepatic steatosis and IL-6 gene expression. But KD had no effect on IL-1, TNF-α and IL-10 gene expression, compared to HFD [57].

Conclusion and recommendations

As in the aforementioned, both HPD and KD can be adopted as weight loss diets, but it was observed that HPD can enhance more weight loss than KD within the same time interval. In addition, HPD had a beneficial impact on the metabolism of glucose and insulin and was associated with improved liver function tests and low OS and levels of inflammatory markers in the liver of a rat model of obesity.

Therefore, we recommend further investigations and clinical applications in humans to determine all effects of HPD to be adopted as a safe diet and regimen for weight reduction in obese patients.

Conflict of interest

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