Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Apr 08; 10(A):793-799.
https://doi.org/10.3889/oamjms.2022.9252
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Physiology*

Islamic Fasting Models but not Only Ramadan Improved Metabolic Parameter in High-Fat-High-Fructose-Induced Rats

Naufal Arif Ismail[1], Miranti Dewi Pramaningtyas[2]*, Titis Nurmasitoh[2]

[1]Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia; [2]Department of Physiology, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia

Edited by: Sinisa Stojanoski
Citation: Ismail NA, Pramaningtyas MD, Nurmasitoh T. Islamic Fasting Models but not Only Ramadan Improved Metabolic Parameter in High-Fat-High-Fructose Induced Rats. Open Access Maced J Med Sci. 2022 Apr 08; 10(A):793-799. https://doi.org/10.3889/oamjms.2022.9252
Keywords: Intermittent fasting; Lipid profile; Metabolism; Ramadan fasting; Rats
***Correspondence:** Miranti Dewi Pramaningtyas, Department of Physiology, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Kaliurang Street KM 14.5, Sleman, Special Region of Yogyakarta 55584 Indonesia. E-mail: miranti.dewi@uii.ac.id
Received: 07-Mar-2022
Revised: 20-Mar-2022
Accepted: 29-Mar-2022

Funding: Partial financial support was received from the Faculty of Medicine of Universitas Islam Indonesia without a grant number.
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Various types of Islamic fasting, apart from Ramadan fasting, have not been studied for their effects on health and metabolic processes.

AIM: This study aimed to evaluate the effect of models of Ramadan, Dawood, and Monday-Thursday fasting on metabolic parameters in high-fat-high-fructose (HFHF)-induced rats.

METHODS: Wistar rats were subjected to normal diet control, HFHF diet alone, and modeling Islamic fastings such as Ramadan, Dawood, and Monday-Thursday fasting models, within a period of 29 days at night that HFHF previously induced for 2 weeks. Serum lipid profile, glucose, uric acid, aspartate transaminase (AST) and alanine transaminase (ALT) for liver function, and urea and creatinine for kidney function were assessed after HFHF induction (pre-test) and after fasting treatment (post-test).

RESULTS: HFHF administration for 2 weeks caused dyslipidemia and increased urea levels significantly. However, other parameters were impaired but not statistically significant. Islamic fasting models demonstrated a significantly improved lipid profile. However, glucose, uric acid, AST, ALT, urea, and creatinine improved after fasting treatment but were not statistically significant.

CONCLUSION: Islamic fasting models have a beneficial effect on improving metabolic parameters. Both Dawood and Monday-Thursday fastings can be considered to promote health and improve metabolic processes as well as Ramadan fasting.

Introduction

The prevalence of obesity, metabolic syndrome, and non-alcoholic fatty liver disease (NAFLD) has increased associated with excessive intakes of calories and fat, such as consumption of high fat in processed foods and high fructose in soft drinks [1], [2], [3]. Consumption of high-fructose levels implicates leading to promote hepatic *de novo* lipogenesis, increasing insulin resistance, inflammation, oxidative stress, and fibrosis. Therefore, high-fat-high-fructose (HFHF) consumption has become a major risk factor in developing metabolic diseases [1], [4].

One of the non-pharmacological methods to improve excessive caloric intake is lifestyle modification. For example, intermittent fasting has been popularly practiced in medical applications and religious rituals [5]. Ramadan fasting is a fasting that every Muslim must carry out according to the instructions of the Holy Qur'an. The practice of fasting Ramadan, which is a type of intermittent fasting, has been going on for more than 1400 years. During Ramadan, they are instructed to refrain from desire, including eating, drinking, as well as sexual intercourse from sunrise to sunset (12–16 h) for 28–30 days in the holy month of Ramadan [6]. In the past two decades, fasting has become an interesting topic, resulting in many studies on the effects of fasting, especially intermittent fasting or time-restricted fasting [7], [8]. Several studies have reported that fasting has been shown to have several beneficial effects on metabolic parameters, both in experimental animals and humans [9]. Based on observational studies in humans, Ramadan fasting has improved lipid profiles by reducing triglyceride (TG), total cholesterol (TC), and low-density lipoprotein (LDL) [10]. A study by Ma *et al*. [5] in experimental animals reported that intermittent fasting had reduced glucose levels and enhanced hepatic metabolism, in line with other studies in which intermittent fasting reduces systemic oxidative stress and increases antioxidant enzymes in the liver as a central metabolic homeostasis organ [11], [12].

In addition to Ramadan fasting, in Islam, other types of fasting are "Sunnah", which means it is recommended but not obligatory, for example, Dawood and Monday-Thursday fasting. Dawood fasting is alternate days fasting: fasting on the first day, allowed to eat and drink on the second day, then the third

day performed fast again like the first day, and so on. Meanwhile, Monday-Thursday fasting is carried out just on Mondays and Thursdays repeatedly. The duration of Dawood and Monday-Thursday fasting is from sunrise to sunset but can be done throughout the year except for the month of Ramadan and other days where fasting is prohibited in Islam [6].

Although there have been many fasting studies on metabolism, especially Ramadan fasting, there has been no study evaluating the effect of Dawood, Monday-Thursday, and Ramadan fastings on improving metabolic parameters and comparing their effects in one study. Therefore, the present study aimed to evaluate the effect of Ramadan, Dawood, and Monday-Thursday fasting on metabolic parameters of HFHF-induced rats.

Methods

Study design and fasting model

This is an experimental study with a pre-test and post-test randomized control group design. Ethical clearance met the eligibility of international and national guidelines of ethical standards and procedures and was ethically approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Number: KE/FK/0058/EC/2021). The resource equation formula was used to estimate sample size, resulting in 3–5 rats each [13]. A total of 25 male Wistar rats aged 8 ± 1 week old, weighing 190 ± 40 g, were provided by the Laboratory of Physiology, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia. The timeline of the study is shown in Table 1. Before starting the treatment, the rats were acclimatized for 7 days, light/dark cycle of 12 h, maintaining a temperature of 22°C ± 2 °C, and humidity of 55% ± 10%. All rats were given free access to regular feed (60% carbohydrate, 16% protein, 21% vitamins and minerals, and 3% fat) and tap water. After the acclimatization phase, the rats were randomly divided into five groups as follows:

1) ND: Normal diet
2) HFHFD: HFHF diet
3) RFM: Ramadan fasting model, fasted every day
4) DFM: Dawood fasting model, the first day performed fasting (no food and drink), the second day is not performed fasting (free to eat and drink), and the third day is performed fasting again and repeated so on
5) MTFM: Monday-Thursday fasting model, fasted only on Mondays and Thursdays.

HFHF was administered to HFHFD, RFM, DFM, and MTFM groups for 14 days, then retro-orbital blood collecting of all groups was performed for pre-test data. Fasting treatment groups continued according to their respective models with 14 h (17:00–07:00) fasting duration within a period of 29 days. All groups were given regular feed and tap water in this phase and after. In the end, the rats were sacrificed and blood collected for post-test data. The blood collecting process was under general anesthesia of 50–75 mg/kg intramuscularly of Zoletil® 50 (Virbac SA, Carros, France).

High-fat-high-fructose administration

HFHF was made by mixing 40% quail egg yolk and 60% palm oil (Indofood, Jakarta, Indonesia), administered by oral gavage, and given free access to 30% fructose in 900 ml of tap water [14], [15], [16]. Quail eggs were obtained from a local market in Sleman, Yogyakarta, Indonesia, and then the yolk was separated. Palm oil was used for cooking fried foods five times at a temperature of 100°C–150°C to prepare oxidized palm oil.

Metabolic parameters

The collected blood was then centrifuged at 8000 rpm for 10 min to obtain serum. Serum was used to analyze all metabolic parameters except glucose, which uses blood. Serum lipid profile levels, including high-density lipoprotein (HDL), LDL, TG, and TC, were determined using the cholesterol oxidase-peroxidase aminoantipyrine method. Glucose levels were determined using EasyTouch® GCU (WuXi Xinda Medical Device, Jiangsu, China), which is also accurate and precise [17]. Uric acid levels were determined using the 2,4,5-tribromo-3-hydroxybenzoic acid method. The optimized UV-test method was used to determine aspartate transaminase (AST) and alanine transaminase (ALT) levels. Urea levels were determined using the urease-glutamate dehydrogenase method, and creatinine levels were determined using a kinetic test without deproteinization according to the Jaffe method. All parameters, except glucose, were analyzed using commercial reagent kits supplied by DiaSys (Holzheim, Germany) and the procedure following standard manufacturer's instructions.

Table 1: Timeline of the study

Animals	Preparation	Randomization	Treatment	Assessment	Treatment	Assessment
Wistar rats (*n* = 25)	Acclimatization	ND (*n* = 5)	Standard diet	Pre-test	Standard diet	Sacrifice and post-test
		HFHFD (*n* = 5)	HFHF + standard diet		Standard diet	
		RFM (*n* = 5)	HFHF + standard diet		RFM + standard diet	
		DFM (*n* = 5)	HFHF + standard diet		DFM + standard diet	
		MTFM (*n* = 5)	HFHF + standard diet		MTFM + standard diet	
Day	0–7		8–22	23	24–53	54

ND: Normal diet, HFHF: High-fructose high-fat, HFHFD: HFHF diet, RFM: Ramadan fasting model, DFM: Dawood fasting model, MTFM: Monday-Thursday fasting model.

Statistical analysis

All data analyses were made using SPSS version 25 (IBM, Illinois, USA). All data were displayed as the mean ± standard deviation (SD) of five rats in each group. Statistical analysis was determined using the one-way ANOVA followed by Tukey's post hoc test, or non-parametric data were determined using Kruskal–Wallis followed by Mann–Whitney as a *post hoc* test for multiple comparisons on pre-test and post-test, respectively, to determine whether there was an adequate change in metabolic parameters or not. Data with $p < 0.05$ were considered statistically significant.

Results

The effect of high-fat-high-fructose administration on metabolic parameters

The effect of HFHF administration for 2 weeks on metabolic parameters is shown in Table 2. Comparative analysis between groups showed that LDL, TG, and TC levels in the HFHFD, RFM, DFM, and MTFM groups were significantly higher ($p < 0.05$) compared to the ND group after HFHF administration. This result indicates that HFHF induces dyslipidemia adequately.

After 2 weeks of HFHF administration, HFHFD, RFM, DFM, and MTFM groups showed higher glucose levels than the ND group although the comparison between groups was not statistically significant ($p > 0.05$). This finding suggested that HFHF administration was insufficient to cause a drastic increase in glucose levels.

Compared with the ND group, uric acid levels after HFHF administration showed significant results ($p < 0.05$) only in the HFHFD group. Meanwhile, uric acid in RFM, DFM, and MTFM groups slightly increased compared to the ND group ($p > 0.05$). This result indicates that HFHF administration did not disrupt uric acid properly.

Liver function characterized by AST and ALT levels was analyzed. The results showed that the comparison between groups of those two parameters was insignificant ($p > 0.05$) after administering HFHF for 2 weeks in all HFHF administered groups compared to the ND group. This result indicates that HFHF does not adequately alter liver function.

Kidney function characterized by urea and creatinine was also analyzed. After administering HFHF, the comparative analysis showed that urea levels in the HFHFD, RFM, DFM, and MTFM groups were significantly higher ($p < 0.05$) than in the ND group. However, creatinine was not statistically significant ($p > 0.05$) between groups despite HFHF administered groups showing higher levels than the ND group. It is demonstrated that HFHF disrupted kidney function, reflected by increased urea levels but not creatinine levels.

The effect of Islamic fasting models' treatment in improving metabolic parameters

Metabolic parameters result after Islamic fasting models' treatment is shown in Table 3. Islamic fasting models' treatment significantly ($p < 0.05$) decreased LDL, TG, and TC levels and increased HDL in the RFM, DFM, and MTFM groups compared to the HFHFD group. These results indicate that the Islamic fasting model improves the lipid profile adequately.

Glucose levels in the MTFM, RFM, and DFM groups were lower but not statistically significant ($p > 0.05$) than in the HFHFD group after Islamic fasting model treatments. This finding suggested that fasting treatment decreased glucose levels but was still inadequate.

After the Islamic fasting models' treatment, uric acid levels in all fasting treated groups were lower but insignificant ($p > 0.05$) than in the HFHFD group. Thus, it indicated that uric acid was not decreased adequately due to fasting treatments.

Islamic fasting models' treatment slightly improved liver function but was not statistically significant ($p > 0.05$), characterized by lower levels of AST and ALT in all fasting treated groups compared to the HFHFD group. This finding indicated that fasting treatment improves liver function inadequately.

After the Islamic fasting models' treatment, urea and creatinine levels were lower in fasting treated

Table 2: Effect of high-fructose high-fat on metabolic parameters

Parameter	Group					p[#/##]
	ND (*n* = 5)	HFHFD (*n* = 5)	RFM (*n* = 5)	DFM (*n* = 5)	MTFM (*n* = 5)	
HDL (mg/dl)	36.46 ± 3.71	21.24 ± 5.74[a]	23.12 ± 6.31[a]	27.54 ± 3.78[a]	21.36 ± 2.76[a]	**0.000**[#]
LDL (mg/dl)	23.66 ± 3.75	44.72 ± 6.20[a]	49.92 ± 2.37[a]	50.42 ± 6.46[a]	48.78 ± 6.56[a]	**0.000**[#]
TG (mg/dl)	41.02 ± 4.49	93.24 ± 12.78[a]	101.88 ± 13.47[a]	99.88 ± 21.84[a]	95.18 ± 7.68[a]	**0.000**[#]
TC (mg/dl)	66.02 ± 8.07	97.80 ± 9.92[a]	95.00 ± 8.57[a]	96.54 ± 8.31[a]	96.30 ± 10.94[a]	**0.000**[#]
Glu (mg/dl)	106.60 ± 28.85	128.00 ± 19.01	124.40 ± 28.22	125.80 ± 15.27	140.20 ± 16.98	0.257[#]
UA (mg/dl)	1.65 ± 0.21	2.22 ± 0.21[a]	1.74 ± 0.13[b]	1.97 ± 0.15	1.82 ± 0.21[b]	**0.001**[#]
AST (u/l)	118.78 ± 9.67	126.54 ± 29.65	118.12 ± 9.19	112.22 ± 13.56	130.28 ± 44.23	0.803[#]
ALT (u/l)	54.62 ± 7.40	68.36 ± 8.40	66.32 ± 11.72	64.64 ± 6.48	66.34 ± 12.68	0.216[#]
Urea (mg/dl)	13.32 ± 3.71	53.86 ± 5.00[a]	53.82 ± 12.13[a]	49.74 ± 4.93[a]	59.72 ± 12.21[a]	**0.011**[##]
Cre (mg/dl)	0.36 ± 0.91	0.48 ± 0.16	0.41 ± 0.06	0.47 ± 0.11	0.51 ± 0.09	0.234[#]

[#]Comparison between groups using the one-way ANOVA test then continued Tukey's post hoc test, [##]Comparison between groups using the Kruskal–Wallis test then continued Mann–Whitney test, [a]Significantly different compared to the ND group, [b]Significantly different compared to the HFHFD group. Bold indicates statistically significant, P < 0.05. Data are expressed by mean ± SD. This assessment was carried out after HFHF administration (pre-test). HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride, TC: Total cholesterol, Glu: Glucose, UA: Uric acid, AST: Aspartate transaminase, ALT: Alanine transaminase, Cre: Creatinine, SD: Standard deviation, ND: Normal diet, HFHF: High-fructose high-fat, HFHFD: HFHF diet, RF: Ramadan fasting, DF: Dawood fasting, MTF: Monday-Thursday fasting.

Table 3: Effect of Islamic fasting models on metabolic parameters of high-fructose high-fat-induced rats

Parameter	Group					p[#/##]
	ND (*n* = 5)	HFHFD (*n* = 5)	RFM (*n* = 5)	DFM (*n* = 5)	MTFM (*n* = 5)	
HDL (mg/dl)	40.72 ± 6.38	25.52 ± 4.36[a]	43.36 ± 9.00[b]	38.04 ± 7.61[b]	36.18 ± 6.21[b]	**0.031[##]**
LDL (mg/dl)	21.88 ± 6.21	45.66 ± 3.21[a]	25.18 ± 4.93[b]	30.46 ± 4.60[b]	31.58 ± 5.84[b]	**0.000[#]**
TG (mg/dl)	37.60 ± 6.59	87.10 ± 20.43[a]	36.84 ± 5.10[b]	38.14 ± 5.15[b]	43.60 ± 14.78[b]	**0.000[#]**
TC (mg/dl)	63.90 ± 6.35	93.58 ± 5.88[a]	64.26 ± 8.17[b]	70.36 ± 10.52[b]	73.00 ± 16.79[b]	**0.001[#]**
Glu (mg/dl)	105.60 ± 30.05	122.40 ± 33.50	92.60 ± 16.99	107.20 ± 5.72	96.40 ± 26.44	0.384[#]
UA (mg/dl)	1.35 ± 0.12	1.87 ± 0.27[a]	1.50 ± 0.24	1.66 ± 0.10	1.45 ± 0.49	0.051[#]
AST (u/l)	119.90 ± 35.95	118.62 ± 23.87	95.98 ± 11.71	102.32 ± 34.23	125.42 ± 11.50	0.340[#]
ALT (u/l)	51.98 ± 12.07	69.22 ± 10.87	58.38 ± 7.87	58.26 ± 15.53	59.36 ± 9.48	0.250[#]
Urea (mg/dl)	17.70 ± 3.37	54.24 ± 3.18[a]	39.04 ± 15.89[a]	36.20 ± 2.57[a, b]	42.54 ± 9.14[a]	**0.000[#]**
Cre (mg/dl)	0.37 ± 0.12	0.54 ± 0.16	0.33 ± 0.14	0.31 ± 0.15	0.35 ± 0.14	0.158[##]

[#]Comparison between groups using the one-way ANOVA test then continued Tukey's post hoc test, [##]Comparison between groups using the Kruskal–Wallis test then continued Mann–Whitney test, [a]Significantly different compared to ND group, [b]Significantly different compared to the HFHFD group. Bold indicates statistically significant, P < 0.05. Data are expressed by mean ± SD. This assessment was carried out after Islamic fasting models treatment (post-test). HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride, TC: Total cholesterol, Glu: Glucose, UA: Uric acid, AST: Aspartate transaminase, ALT: Alanine transaminase, Cre: Creatinine, SD: Standard deviation, ND: Normal diet, HFHFD: High-fructose high-fat diet, RF: Ramadan fasting, DF: Dawood fasting, MTF: Monday-Thursday fasting.

groups but did not differ significantly (p > 0.05) from the HFHFD group. It was shown that fasting treatment improves kidney function inadequately.

Discussion

This is the first study to directly compare the three types of fasting in Islam in experimental animals. Our study reveals that 2 weeks of HFHF administration formulated from a mixture of oxidized palm oil and quail egg yolk as well as fructose content in tap water led to impairment in metabolic parameters, including dyslipidemia, increased glucose and uric acid levels, also disrupted liver and kidney function despite certain parameters were not statistically significant. This finding is in line with previous studies [2], [16], [18], [19], [20]. Meanwhile, Islamic fasting models treatment appeared to have beneficial effects that improve lipid profiles, lower glucose and uric acid levels, and improve liver and kidney function, despite certain parameters showing insignificant statistical results. It should be noted that the rats' studies are not fully translatable in humans due to the complexity of the diet and the influence of environmental factors. However, interestingly, based on the available evidence shows that the use of HFHF has a synergistic effect in inducing metabolic syndrome in experimental animals [5]. In addition, rats are nocturnal animals that are more suitable for fasting to be carried out at night [5], [21].

Findings on lipid profiles reveal that HFHF administration causes dyslipidemia. A previous study in rats given HFHF for 2 weeks showed lipid profile impairment with a significant increase in TG and non-esterified fatty acids (NEFA), which reflected an increase in hepatic *de novo* lipogenesis [2]. The fasting model intervention in the present study demonstrated that all Islamic fasting models improve lipid profiles in the groups previously induced by HFHF. A significant decrease in LDL, TG, and TC levels occurred in all fasting models' treatment groups, while HDL levels also increased significantly in the RFM, MTFM, and DFM groups. The improvement in lipid profile reflects a reduction in hepatic *de novo* lipogenesis as a result of the Islamic fasting models treatment that HFHF previously impaired [22]. These findings suggest that more frequent fasting intensity, i.e., in Ramadan fasting, contributes to greater improvements in lipid profiles, reflected by higher HDL levels, and lower LDL, TG, and TC levels than other fasting types. In line with previous studies, the longer intermittent fasting duration did not affect TG and free fatty acids (FFA) changes [23]. These lipid profile findings demonstrated that all Islamic fasting models treatment showed significant improvements, evidenced that Dawood fasting and Monday-Thursday fasting can be considered to be hypolipidemic agents as well as Ramadan fasting.

Adequate HFHF administration resulted in insulin resistance and increased glucose levels associated with dyslipidemia [2], [18]. In this study, glucose levels were higher but not significant in all HFHF administered groups compared to the ND group, indicating that HFHF administration for 14 days was still not adequate to increase glucose levels drastically but very likely in the process of developing metabolic syndrome. In accordance with a previous study that showed HFHF administration for 2 weeks showed insignificantly increased glucose levels but significantly increased plasma insulin levels, homeostasis model assessment of insulin resistance, and liver insulin-resistance index, indicating insulin resistance was developed [2]. Another study by Calvo-Ochoa *et al*. [24] revealed a similar result, in which HFHF administration to rats for 7 days resulted in hyperphagia, obesity, and insulin resistance. The possible mechanism is that the administration of HFHF modulates hippocampal insulin signaling by lowering phosphorylation of insulin receptor substrate-1 (IRS-1) and Akt as well as increased activation of S6 kinases. As a result, IRS-1 is inhibited from recognizing insulin receptors resulting in insulin resistance and increased glucose levels [2], [24]. Fructose supplementation also increased the SREBP1c expression and fatty acid synthesis, leading to the suppression of liver insulin signaling [25]. The treatment of Islamic fasting models in this study showed a decrease in glucose levels to a tolerable limit in all fasting treated groups. However, this finding was unlike improvements in lipid profile linear with fasting intensity. Glucose improvement did not appear to be affected by more frequent fasting intensity. The glucose

levels are sequenced from the lowest to highest by RFM, MTFM, and DFM. Nevertheless, our findings suggest that fasting for 14 h in Islamic fastings is still a good glucose control agent. A study by Rakvaag *et al*. [23] in HFD-induced mice and given intermittent fasting treatment resulted in higher insulin levels in mice that were fasted for 12 and 18 h compared to 6 h. However, glucose and β-hydroxybutyric concentrations were higher at 18 h than at 12 h fasting, indicating a decrease in insulin sensitivity and ketogenic state due to extending the fasting duration to 18 h [23]. In line with other studies, intermittent fasting treatment daily for 15 h (17.00-08.00) in 30 days to normal and diabetics sand rats which fed a hypercaloric diet had improved glucose tolerance [26].

Uric acid increases have been proposed associated with HFHF administration. However, our result showed no significant increase in uric acid levels after HFHF administration. A possible explanation is that the period of HFHF administration, which is only 2 weeks, is insufficient to develop hyperuricemia. A previous study supported our finding that uric acid increased after HFHF was administered to rats for 12 weeks [18]. In another study, the administration of HFD for 90 days showed an increase in uric acid compared to the normal group [27]. Along with the Islamic fasting models treatment in the present study, there was a decrease in uric acid levels in the fasting treated groups, although statistically insignificant. This finding indicates the inadequate improvement effect of Islamic fasting models to lower uric acid although previous HFHF administration did not show alteration. In addition, some studies report that there has been a temporary uric acid increase during the Ramadan fast [28], [29].

HFHF consumption is a major risk factor for developing NAFLD and lowering liver function [1], [3]. However, in this study, the AST and ALT levels did not differ significantly across the whole group, demonstrating that the administration of HFHF for 2 weeks had not developed NAFLD. In previous studies, mice given HFHF for 20 weeks produced lipid accumulation in the liver and a significant increase over the normal group [4]. NAFLD development was related to insulin resistance, where insulin normally encourages fat synthesis and reduces lipolytic levels. Therefore, in insulin-resistant conditions, the increased lipolytic rate in peripheral adipose tissue leads to increased delivery of plasma TGs to the liver [30]. Compensatory hyperinsulinemia and high caloric intake stimulate *de novo* lipogenesis and FFA entry into hepatocytes by inhibiting fatty acid oxidation and stimulating fatty acid synthesis, which eventually develops lipid accumulation in the liver [3]. There is no significant alteration of liver function parameters in the present study after Islamic fasting models treatment. A study by Ma *et al*. [5] reported intermittent daily fasting for 12 h at night for 1 month significantly reduced liver weight, no significant reduction in AST and ALT, and a significant increase in adenosine triphosphate, nicotinamide adenine dinucleotide phosphate, and succinate. This result demonstrated that intermittent fasting does not affect the function of the liver but increases its metabolism [5].

HFHF administration showed an increase in urea levels but not in creatinine which indicated that administration of HFHF for 2 weeks potentially decreased kidney function. Previous studies by Thongnak *et al.* [4] reported that HFHF induces moderate levels of glomerular and tubule lesions as well as elevated levels of urea and creatinine, indicating kidney injury has progressed after receiving HFHF for 20 weeks. Increased lipid metabolism is associated with the administration of HFHF through increased lipogenesis and decreased lipolysis, resulting in fat accumulation in the kidneys, thereby decreasing its function [4]. Regarding the treatment of Islamic fasting models within a period of 29 days, the findings demonstrated that the urea and creatinine levels were insignificant slightly lower in fasting-treated groups. Fasting treatments were likely to show inadequate kidney function improvement.

This study has several limitations, such as a short HFHF induction time, the number of days of Dawood and Monday-Thursday fasting is not equivalent to Ramadan fasting and is still in animal models. Nevertheless, this study demonstrated improved metabolic parameters by the Islamic fasting models previously impaired by HFHF in rats.

Conclusion

In conclusion, the data obtained from this study indicate that HFHF caused impairment in metabolic parameters, including dyslipidemia, slightly increased glucose and uric acid levels, also disrupted liver and kidney function despite certain parameters were not statistically significant. Meanwhile, fasting in Islam provides a protective effect against HFHF-induced diseases. Not only Ramadan fasting but both Dawood and Monday-Thursday fasting can also be considered in promoting and maintaining metabolic health.

Acknowledgments

The highest appreciation goes to Endah Sari, Siti Anita, Falah Ghani, Syifa Hanani, Hilda Auliya, Rana Aulia, and Alfian Novanda, who have assisted in this study.

Ethical Approval

Ethical approval was issued by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Number: KE/FK/0058/EC/2021).

Authors' Contributions

NAI conceived and designed the study, conducted research, provided research materials, collected and organized data, analyzed and interpreted data, wrote the initial and final draft of the article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

References

1. Jarukamjorn K, Jearapong N, Pimson C, Chatuphonprasert W. A high-fat, high-fructose diet induces antioxidant imbalance and increases the risk and progression of nonalcoholic fatty liver disease in mice. Scientifica (Cairo). 2016;2016:5029414. https://doi.org/10.1155/2016/5029414
   PMid:27019761
2. Crescenzo R, Bianco F, Coppola P, Mazzoli A, Tussellino M, Carotenuto R, *et al.* Fructose supplementation worsens the deleterious effects of short-term high-fat feeding on hepatic steatosis and lipid metabolism in adult rats. Exp Physiol. 2014;99(9):1203-13. https://doi.org/10.1113/expphysiol.2014.079632
   PMid:24972835
3. Pappachan JM, Babu S, Krishnan B, Ravindran NC. Non-alcoholic fatty liver disease: A clinical update. J Clin Transl Hepatol. 2017;5(4):384-93. https://doi.org/10.14218/JCTH.2017.00013
   PMid:29226105
4. Thongnak L, Chatsudthipong V, Kongkaew A, Lungkaphin A. Effects of dapagliflozin and statins attenuate renal injury and liver steatosis in high-fat/high-fructose diet-induced insulin-resistant rats. Toxicol Appl Pharmacol. 2020;396:114997. https://doi.org/10.1016/j.taap.2020.114997
   PMid:32259528
5. Ma J, Cheng Y, Su Q, Ai W, Gong L, Wang Y, *et al*. Effects of intermittent fasting on liver physiology and metabolism in mice. Exp Ther Med. 2021;22(3):950. https://doi.org/10.3892/etm.2021.10382
   PMid:34335892
6. Muslim IA-H, Sahih Muslim, Book 6: Book of Fasting (Kitab Al-Sawm). Riyadh: Maktaba Dar-us-Salam; 2007.
7. Cho Y, Hong N, Kim K, Cho S, Lee M, Lee Y, *et al.* The effectiveness of intermittent fasting to reduce body mass index and glucose metabolism: A systematic review and meta-analysis. J Clin Med. 2019;8(10):1645. https://doi.org/10.3390/jcm8101645
   PMid:31601019
8. Trepanowski JF, Bloomer RJ. The impact of religious fasting on human health. Nutr J. 2010;9:57. https://doi.org/10.1186/1475-2891-9-57
   PMid:21092212
9. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. Ageing Res Rev. 2017;39:46-58. https://doi.org/10.1016/j.arr.2016.10.005
   PMid:27810402
10. Rahbar AR, Safavi E, Rooholamini M, Jaafari F, Darvishi S, Rahbar A. Effects of intermittent fasting during ramadan on insulin-like growth factor-1, interleukin 2, and lipid profile in healthy muslims. Int J Prev Med. 2019;10:7. https://doi.org/10.4103/ijpvm.IJPVM_252_17
    PMid:30774841
11. Nurmasitoh T, Utami SY, Kusumawardani E, Najmuddin AA, Fidianingsih I. Intermittent fasting decreases oxidative stress parameters in Wistar rats (*Rattus norvegicus*). Univ Med. 2018;37(1):31-8. https://doi.org/10.18051/UnivMed.2018.v37.31-38
12. Mulyaningrum U, Muttaqina AF, Idninda AN, Pulungan N, Agustiningtyas I, Fidianingsih I. Effect of dawood fasting on the increased level of antioxidant enzymes. Open Access Maced J Med Sci. 2021;9(A):1-6. https://doi.org/10.3889/oamjms.2021.4175
13. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. Malays J Med Sci. 2017;24(5):101-5. https://doi.org/10.21315/mjms2017.24.5.11
    PMid:29386977
14. Softic S, Gupta MK, Wang GX, Fujisaka S, O'Neill BT, Rao TN, *et al.* Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. J Clin Invest. 2017;127(11):4059-74. https://doi.org/10.1172/JCI94585
    PMid:28972537
15. Adam SK, Soelaiman IN, Umar NA, Mokhtar N, Mohamed N, Jaarin K. Effects of repeatedly heated palm oil on serum lipid profile, lipid peroxidation and homocysteine levels in a post-menopausal rat model. Mcgill J Med. 2008;11(2):145-51
    PMid:19148313
16. Go RE, Hwang KA, Kim YS, Kim SH, Nam KH, Choi KC. Effects of palm and sunflower oils on serum cholesterol and fatty liver in rats. J Med Food. 2015;18(3):363-9. https://doi.org/10.1089/jmf.2014.3163
    PMid:25393932
17. Dai KS, Tai DY, Ho P, Chen CC, Peng WC, Chen ST, *et al.* Accuracy of the easytouch blood glucose self-monitoring system: A study of 516 cases. Clin Chim Acta. 2004;349(1-2):135-41. https://doi.org/10.1016/j.cccn.2004.06.010
    PMid:15469866
18. Zhuhua Z, Zhiquan W, Zhen Y, Yixin N, Weiwei Z, Xiaoyong L, *et al.* A novel mice model of metabolic syndrome: The high-fat-high-fructose diet-fed ICR mice. Exp Anim. 2015;64(4):435-42. https://doi.org/10.1538/expanim.14-0086
    PMid:26134356
19. Lontchi-Yimagou E, Tanya A, Tchankou C, Ngondi J, Oben J. Metabolic effects of quail eggs in diabetes-induced rats: Comparison with chicken eggs. Food Nutr Res. 2016;60:32530. https://doi.org/10.3402/fnr.v60.32530
    PMid:27717410
20. Moreno-Fernández S, Garcés-Rimón M, Vera G, Astier J, Landrier JF, Miguel M. High fat/high glucose diet induces metabolic syndrome in an experimental rat model. Nutrients. 2018;10(10):1502. https://doi.org/10.3390/nu10101502
    PMid:30322196
21. Bucaktepe PG, Akdağ M, Dasdag S, Celepkolu T, Yılmaz MA,

Demir V, *et al.* Catecholamine levels in a Ramadan fasting model in rats: A case control study. Biotechnol Biotechnol Equip. 2016;30:706-12. https://doi.org/10.1080/13102818.2016.1172510

22. Nieminen P, Rouvinen-Watt K, Harris L, Huitu O, Henttonen H, Mustonen AM. De novo lipogenesis is suppressed during fasting but upregulated at population decline in cyclic voles. Exp Biol Med. 2016;241(8):882-7. https://doi.org/10.1177/1535370216633312
PMid:26892709
23. Rakvaag E, Lund MD, Wiking L, Hermansen K, Gregersen S. Effects of different fasting durations on glucose and lipid metabolism in sprague dawley rats. Horm Metab Res. 2019;51(8):546-53. https://doi.org/10.1055/a-0897-2496
PMid:31121611
24. Calvo-Ochoa E, Hernández-Ortega K, Ferrera P, Morimoto S, Arias C. Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. J Cereb Blood Flow Metab. 2014;34(6):1001-8. https://doi.org/10.1038/jcbfm.2014.48
PMid:24667917
25. Samuel VT. Fructose induced lipogenesis: From sugar to fat to insulin resistance. Trends Endocrinol Metab. 2011;22(2):60-5. https://doi.org/10.1016/j.tem.2010.10.003
PMid:21067942
26. Malaisse WJ, Belkacemi L, Selselet-Attou G, Louchami K, Sener A. Intermittent fasting modulation of the diabetic syndrome in sand rats. II. *In vivo* investigations. Int J Mol Med. 2010;26(5):759-65. https://doi.org/10.3892/ijmm_00000523
PMid:20878099
27. Gujjala S, Putakala M, Nukala S, Bangeppagari M, Ramaswamy R, Desireddy S. Renoprotective effect of caralluma fimbriata against high-fat diet-induced oxidative stress in wistar rats. J Food Drug Anal. 2016;24(3):586-93. https://doi.org/10.1016/j.jfda.2016.01.013
PMid:28911565
28. Khan N, Rasheed A, Ahmed H, Aslam F, Kanwal F. Effect of ramadan fasting on glucose level, lipid profile, HbA1c and uric acid among medical students in Karachi, Pakistan. East Mediterr Heal J. 2017;23(4):274-9. https://doi.org/10.26719/2017.23.4.274
PMid:28634977
29. Ooi SL, Pak S. Short-term intermittent fasting for weight loss: A case report. Cureus. 2019;11(4):4482. https://doi.org/10.7759/cureus.4482
PMid:31249759
30. Morigny P, Houssier M, Mouisel E, Langin D. Adipocyte lipolysis and insulin resistance. Biochimie. 2016;125:259-66. https://doi.org/10.1016/j.biochi.2015.10.024
PMid:26542285