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Effects of Ramadan Fasting on Plasma Free Fatty Acids in Patients with Non-alcoholic Fatty Liver Disease

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) is a global health concern with the prevalence rate of 10-35%. Several factors are involved in the pathogenesis of NAFLD. This study aimed to evaluate the effects of Ramadan fasting on plasma free fatty acids in patients with NAFLD.

Methods: This cross-sectional study was conducted on 50 NAFLD patients in the holy month of Ramadan during June-July 2014 (Islamic year: 1435) in Mashhad, Iran. Patients were classified into two groups with body mass index (BMI) of <29.9 (n=23) and >30 kg/m2 (n=27). Participants were within the age range of 18-65 years. Inclusion criteria were NAFLD patients with fatty liver diagnosis in ultrasound and minimum fasting duration of 23 days in Ramadan. Levels of plasma free fatty acids (palmitic, elaidic, and oleic fatty acid) were assessed in the blood samples of all patients using gas chromatography with flame ionization detection (GC-FID).

Results: No significant changes were observed in the plasma levels of palmitic, elaidic and oleic fatty acids in overweight patients (BMI: 25-30 kg/m^2). However, plasma levels of elaidic acid significantly increased in obese patients (P<0.001).

Conclusion: According to the results of this study, adherence to the principles of Ramadan fasting could enhance the health status of NAFLD patients. However, it is recommended that future studies be conducted in this regard.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common chronic disorder, which occurs due to the accumulation of triacylglycerol in the cytoplasm of liver cells when the fat deposit in these cells reaches more than 5% in proportion to the weight of hepatocytes (1). NAFLD may start with a simple steatosis and advance to other disorders, such as steatohepatitis (simple steatosis with inflammation), fibrosis, cirrhosis, liver failure, and even carcinoma (2).

Many predisposing factors are involved in the development of fatty liver, including unhealthy dietary habits, modern lifestyle and obesity. Patients with NAFLD may manifest several symptoms of metabolic syndrome and insulin resistance. Prevalence of NAFLD is higher among men compared to women, while the incidence rate has been reported to increase in postmenopausal women (3-9).

Global prevalence rate of NAFLD in adults is estimated at 10-35%, while it has been reported that 7% of children and 35% of adults suffer from NAFLD in Iran (10, 11). Several parameters are involved in the pathogenesis of this disease, including genetic factors, metabolic syndrome, increased liver fatty acids, decreased fatty acid oxidation, and prolonged fasting (12, 13).

Plasma free fatty acids (FFAs) accumulate in the liver of severely obese individuals (14). Mechanism of NAFLD mainly involves the increased flow of FFAs from dietary triglycerides (TGs) to the liver or excess FFAs from the adipocyte tissue to the liver in prolonged fasting, reduced beta-oxidation of FFAs in hepatocyte mitochondria, and decreased release of very low

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density lipoprotein from the liver (12, 13).

Ramadan fasting is a type of starvation associated with no significant changes in energy intake, while altering the mealtime only. During Ramadan, Muslims have two main meals per day: at the sunrise (Sahur) and sunset (Iftar). Ramadan fasting might lead to problems such as dehydration and starvation, which adversely affect metabolic functions. Moreover, prolonged fasting is associated with the changes of sleep-wake cycle, which are likely to transform metabolism and endocrine system (15, 16).

Since fasting is a form of starvation, it has been shown to be involved in the pathogenesis of NAFLD. To the extent of our knowledge, no former studies have investigated the association between plasma FFAs and Ramadan fasting. This study aimed to evaluate the effect of Ramadan fasting on plasma FFAs in patients with NAFLD.

Material and methods

This cross-sectional study was conducted on 50 patients with NAFLD (age range: 18-65 years) during June-July 2014 (Islamic calendar: 25 Shaban-8 Dhul Al-Qi'dah 1435) in Mashhad, Iran.

Inclusion criteria were NAFLD patients with diagnosis of fatty liver based on ultrasound and minimum fasting duration of 10 h. Exclusion criteria of the study were as follows: 1) history of alcohol consumption; 2) consumption of more than 10 g/day of alcohol in women and more than 20 g/day in men; 3) presence of acute inflammation (hs-CRP>5); 4) pregnancy or lactation; 5) BMI of <25 kg/m^2; 6) use of medications; 7) weight loss within the past three months; 8) presence of endocrine and metabolic disorders (e.g., other hepatic disorders, malignancies, and kidney, respiratory and cardiovascular disorders) and 9) fasting duration of less than 23 days in Ramadan.

Study protocol was approved by the Research Ethics Committee of Mashhad University of Medical Sciences (code: 930229). Participation in the research was voluntary, and written informed consent was obtained from all the subjects prior to the study.

Anthropometric measurements

Weight and height of the participants were measured by a trained individual using standard methods. Body weight was measured with minimum clothing and no shoes using a digital scale with accuracy of 100 g. Height was measured using a tape, while the participants were standing in a normal position without shoes, and recorded to the nearest 0.1 cm. In addition, BMI of the subjects was calculated as weight (kg)/height^2 (m^2) (15).

Measurement of plasma free fatty acids

Plasma FFAs were detected as previously described. In this process, plasma lipids were extracted using a mixture of chloroform and methanol (17). To carry out potassium hydroxide (KOH) derivatization, the extracted lipid from the samples was added to a mixture composed of 100 ml of methanolic KOH (2 mol/l) and 200 ml of n-hexane.

Afterwards, the mixture was incubated at room temperature for 5 min before the addition of sodium bisulfate (40 mg), and the supernatant was collected (18). Finally, the supernatant was analyzed via gas chromatography with flame ionization detection (GC-FID) (Varian 450-GC, USA) in order to determine the plasma levels of FFAs (palmitic, elaidic and oleic fatty acids).

Data analysis

Study results were presented as mean ± standard deviation, and data analysis was performed in SPSS version 11.5 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used to assess the normal distribution of data, and comparison of the mean values with a significant difference was performed using paired samples t-test (normally distributed variables) before and after Ramadan. In all statistical analyses, P value of less than 0.05 was considered significant.

Results

In total, 50 NAFLD patients were enrolled in this study, including 33 men and 17 women within the age range of 18-65 years (mean age: 40.52±10.90 years). Mean duration of fasting in the participants was 27.3±5 days (range: 25-30 days).

According to the information in Table 1, BMI patients were not significantly reduced during Ramadan, while the difference. Results of the assessment of plasma FFAs based on the BMI of

Table 1. Effect of fasting on body mass index (BMI) before and after Ramadan

Variable	Before fasting (Mean±SD)	After fasting (Mean±SD)	P-value
BMI (kg/m^2)	31.38±4.9	31.0768±4.7	0.113

Table 2. Effects of fasting on plasma palmitic, elaidic, and oleic fatty acid levels (ppm) before and after Ramadan based on BMI

		Before fasting (Mean±SD)	After fasting (Mean±SD)	P-value
BMI<29.9 kg/m^2	Palmitic acid (ppm)	77.68±17.91	80.65±18.61	0.45
	Oleic acid (ppm)	42.8±8.9	46.4±7.5	0.38
	Elaidic acid (ppm)	20.94±1.06	28.96±8.20	0.26
BMI>30 kg/m^2	Palmitic acid(ppm)	79.23±18.52	83.61±19.2	0.63
	Oleic acid (ppm)	45±9	46.4±15.1	0.65
	Elaidic acid (ppm)	24.83±2.07	35.86±9.8	0.001*

participants during Ramadan are presented in Table 2. According to the information in this table, no significant changes were observed in the plasma levels of palmitic, elaidic, and oleic fatty acids in overweight patients (BMI: 25-30 kg/m^2). However, plasma levels of elaidic acid significantly increased in obese patients (P<0.001).

Discussion

In the present study, no significant increase was observed in the plasma levels of FFAs (palmitic, elaidic, and oleic fatty acids) in overweight NAFLD patients before and after Ramadan. However, plasma level of elaidic acid significantly increased in obese patients at the end of Ramadan.

As mentioned earlier no studies have been performed before on the plasma levels of FFAs in fasting NAFLD patients during Ramadan. In a study in this regard, plasma levels of FFAs were compared between two groups of obese and overweight NAFLD patients, and the saturated fatty acids were reported to have the most significant association with NAFLD compared to other unsaturated fatty acids Furthermore, palmitic acid was identified as the most important plasma FFA associated with the progression of fatty liver disease (14) also plasma level of elaidic acid in obese patients was significantly higher compared to the overweight group, while the differences in other plasma FFAs were not considered significant between these groups. This is in congruence with the results of the current study. Therefore, it could be concluded that high plasma levels of palmitic acid and elaidic acid might be associated with increased risk of NAFLD.

Another study was conducted on four patients with fatty liver disease to determine the origin of liver fat. According to the findings, level of palmitic acid in liver cells increased after five days of fasting (18-20), which is similar to the results of the present study. In the mentioned research, the adipose tissue was reported as the main origin of liver fat due to denovo- lipogenesis in hepatocytes.

In this regard, Muller et al. (1975) investigated the effect of fasting on the amount of liver fat and reported no increase in this index after fasting for 12 h. However, fasting for 36 h was found to enhance the release of fatty acids from the tissue, as well as their transfer to hepatocytes (21). On the same note, findings of Yasuhara et al. (1991) indicated that fasting for 24 h increased fatty acid flow from the adipose tissue to hepatocytes in rats, leading to mild steatosis (22). In the present study, plasma FFAs showed no increase during Ramadan fasting, which could be attributed to the shorter fasting duration (14 h).

Based on the results of the aforementioned studies, it could be inferred that patterns of plasma fatty acid changes vary in patients with fatty liver disease while fasting in Ramadan. This is due to the fact that in fasting individuals, lake of food intake might lower plasma insulin levels, thereby leading to the release and flow of FFAs from the adipose tissue to the plasma (23-25).

Prolonged starvation is associated with increased production of ketone bodies, which mostly accumulate in the blood. Eventually, beta oxidation of FFAs reduces in the liver in order to decrease the production of ketones, which leads to the storage of fatty acids in the liver, predisposing individuals to NAFLD (26-28).

Ramadan fasting begins at sunrise, and the Sahur meal provides the required energy for the early hours of fasting. Continuous fasting is associated with blood glucose drop and increased level of stress hormones; after that, the body needs higher energy supply from gluconeogenesis and lipolysis.

Gluconeogenesis and lipolysis discontinue after

fast break at Iftar, and adequate proteins and phospholipids are supplied by the diet; therefore, construction of lipoproteins and their secretion into the blood increase in the liver (29-31).

One of the limitations of the present study was lack of a control group. Moreover, we did not measure total plasma FFAs of NAFLD patients, as well as some influential factors in FFA plasma levels, including dietary patterns and physical activity.

Conclusion

This was the first study to investigate the effect of Ramadan fasting on the plasma levels of FFAs. According to the results, level of elaidic acid increased in Ramadan in NAFLD patients, which could be due to the consumption of high-fat foods or altered body metabolism during starvation. However, these findings must be confirmed in larger sample sizes and patients with different disease stages. Therefore, it is recommended that future studies in this regard evaluate the impact of fasting, and physical activity on the total plasma levels of FFAs in overweight and obese individuals.

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References

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980; 55(7):434-8.
2. Luyckx FH, Lefebvre PJ, Scheen AJ. Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence ofweight loss. Diabetes Metab. 2000; 26(2):98-106.
3. Lewis JR, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. Dig Dis Sci. 2010; 55(3):560-78.
4. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology. 1999; 116(6):1413-9.
5. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology. 1999; 116(6):1413-9.
6. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest. 2008; 118(3):829-38.
7. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. Am J Clin Nutr. 2007; 86(2):285-300.
8. Petta S, Muratore C, Craxi A. Non-alcoholic fatty liver disease pathogenesis: the present and the future. Dig Liver Dis. 2009; 41(9):615-25.
9. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM. 2010; 103(2):71-83.
10. Alavian SM, Mohammad-Alizadeh AH, Esna-Ashari F, Ardalan G, Hajarizadeh B. Non-alcoholic fatty liver disease prevalence among school-aged children and adolescents in Iran and its association with biochemical and anthropometric measures. Liver Int. 2009; 29(2):159-63.
11. Amirkalali B, Poustchi H, Keyvani H, Khansari MR, Ajdarkosh H, Maadi M, et al. Prevalence of non-alcoholic fatty liver disease and its predictors in north of Iran. Iran J Public Health. 2014; 43(9):1275-83.
12. Sass DA, Chang P, Chopra KB. Nonalcoholic fatty liver disease: a clinical review. Dig Dis Sci. 2005; 50(1):171-80.
13. Wilfred de Alwis NM, Day CP. Genes and nonalcoholic fatty liver disease. Curr Diab Rep. 2008; 8(2):156-63.
14. de Almeida IT, Cortez-Pinto H, Fidalgo G, Rodrigues D, Camilo ME. Plasma total and free fatty acids composition in human non-alcoholic steatohepatitis. Clin Nutr. 2002; 21(3):219-23.
15. Arabi SM, Hejri Zarifi S, Nematy M, Safarian M. The effect of Ramadan fasting on non-alcoholic fatty liver disease (NAFLD) Patients. J Fasting Health. 2015; 3(2):74-80.
16. Nematy M, Alinezhad-Namaghi M, Rashed MM, Mozhdehifard M, Sajjadi SS, Akhlaghi S, et al. Effects of Ramadan fasting on cardiovascular risk factors: a prospective observational study. Nutr J. 2012; 11:69.
17. Bligh EG, Dyer WJ. A Rapid, Method of total lipid extraction and purification. Can J Biochem Physiol. 1959; 37(8):911–7.
18. Firl N, Kienberger H, Hauser T, Rychlik M. Determination of the fatty acid profile of neutral lipids, free fatty acids and phospholipids in human plasma. Clin Chem Lab Med. 2013; 51(4):799–810.
19. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005; 115(5):1343-51.
20. Sprangers F, Romijn JA, Endert E, Ackermans MT, Sauerwein HP. The role of free fatty acids (FFA) in the regulation of intrahepatic fluxes of glucose and glycogen metabolism during short-term starvation in healthy volunteers. Clin Nutr. 2001; 20(2):177-9.

21. Aoki TT, Muller WA, Brennan MF, Cahill GF Jr. Metabolic effects of glucose in brief and prolonged fasted man. Am J Clin Nutr. 1975; 28(5):507-11.
22. Yasuhara M, Ohama T, Matsuki N, Saito H, Shiga J, Inoue K, et al. Induction of fatty liver by fasting in suncus. J Lipid Res. 1991; 32(6):887-91.
23. Saudek CD, Felig P. The metabolic events of starvation. Am J Med. 1976; 60(1):117-26.
24. Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. Science. 1991; 254(5031):573-6.
25. Fan Y, Jimenez Del Val I, Muller C, Lund AM, Sen JW, Rasmussen SK, et al. A multi-pronged investigation into the effect of glucose starvation and culture duration on fed-batch CHO cell culture. Biotechnol Bioeng. 2015; 112(10):2172-84.
26. Holness MJ, Hegazy S, Sugden MC. Signalling satiety and starvation to beta-Cell insulin secretion. Curr Diabetes Rev. 2011; 7(5):336-45.
27. Mustonen AM, Karja V, Kilpio M, Tammi R, Tammi M, Rouvinen-Watt K, et al. Manifestations of fasting-induced fatty liver and rapid recovery from steatosis in voles fed lard or flaxseed oil lipids. Nutrients. 2013; 5(10):4211-30.
28. Owen OE, Caprio S, Reichard GA Jr, Mozzoli MA, Boden G, Owen RS. Ketosis of starvation: a revisit and new perspectives. Clin Endocrinol Metab. 1983; 12(2):359-79.
29. Azizi F. Islamic fasting and health. Ann Nutr Metab. 2010; 56(4):273-82.
30. Unalacak M, Kara IH, Baltaci D, Erdem O, Bucaktepe PG. Effects of Ramadan fasting on biochemical and hematological parameters and cytokines in healthy and obese individuals. Metab Syndr Relat Disord. 2011; 9(2):157-61.
31. Asemi Z, Samimi M, Taghizadeh M, Esmaillzadeh A. Effects of Ramadan fasting on glucose homeostasis, lipid profiles, inflammation and oxidative stress in women with polycystic ovary syndrome in Kashan, Iran. Arch Iran Med. 2015; 18(12):806-10.