HEPATIC AND BIOCHEMICAL REPERCUSSIONS OF A POLYUNSATURATED FAT-RICH HYPERCALORIC AND HYPERLIPIDIC DIET IN WISTAR RATS

Idália M. B. **BURLAMAQUI**[1], Conceição A. **DORNELAS**[2], José Telmo **VALENÇA Jr**[2], Francisco J. C. **MESQUITA**[3], Lara B. **VERAS**[4] and Lusmar Veras **RODRIGUES**[2]

ABSTRACT - ***Context*** - Non-alcoholic fatty liver disease is characterized by lipid deposits in the hepatocytes and has been associated with obesity, dyslipidemia and type-2 diabetes. It is considered a hepatic manifestation of the metabolic syndrome, of which the main component is insulin resistance leading to hyperinsulinemia and increased production of inflammatory cytokines. Saturated fat promotes hypertriglyceridemia and hyperinsulinemia, reduces levels of high-density cholesterol and increases levels of low-density cholesterol, while polyunsaturated fat is associated with hypolipidemic, antiinflammatory and imunoregulating action. ***Objective*** – To evaluate the hepatic and biochemical repercussions of a polyunsaturated fat-rich diet in Wistar rats. ***Methods*** - Twenty-two rats were distributed equally in two groups: GI – standard diet (Biobase Bio-tec Ratos e Camundongos®) providing 3.000 kcal/kg and GII – hypercaloric and hyperlipidic diet providing 4.250 kcal/kg (ω-6:ω-3 = 3:1). The animals were euthanized after 23 weeks of experiment. The weight, biochemical parameters and hepatohistological changes were registered. ***Results*** - Findings were submitted to variance analysis with the level of statistical significance at 5%. The average weight did not differ significantly between the groups at baseline ($P = 0.711$), but was greater in Group II by the end of the experiment ($P = 0.000$). The levels of triglycerides ($P = 0.039$), total cholesterol ($P = 0.015$) and HDL ($P = 0.005$) were higher in Group I than in Group II. Macrovesicular steatosis was significantly more common in Group II than in Group I ($P = 0.03$). ***Conclusion*** - Hypercaloric and hyperlipidic diet rich in polyunsaturated fat promotes weight gain and favors the development of hepatic steatosis while reducing serum levels of triglycerides, total cholesterol and HDL.

HEADINGS – Fatty liver. Obesity. Dyslipidemias. Diabetes mellitus, type 2. Rats.

INTRODUCTION

A fat-rich diet is a risk factor for obesity[16]. Extensive research has shown that dietary fat affects a range of metabolic functions, depending on the quantity and composition of fatty acids[9]. Diets rich in polyunsaturated fat (PUFA), especially ω-3 and ω-9, are associated with hypolipidemic, antiinflammatory and imunoregulating action. On the other hand, saturated fats and trans fats are known to promote hypertriglyceridemia and hyperinsulinemia, reduce levels of high-density cholesterol (HDL) and increase levels of low-density cholesterol (LDL)[18].

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver injury in many countries[3, 13]. Manifestations range from simple steatosis to non-alcoholic steatohepatitis (NASH) to fibrosis, cirrhosis and hepatocellular carcinoma[27]. The prevalence of NAFLD, which is currently 15%−40% among Western populations and 9%−40% among Asian populations[14], has increased dramatically over the past 15 years, mostly due to its association with the world's two largest current epidemics: obesity and type-2 diabetes mellitus (DM2)[1].

Current research on nutritional change is revealing the important role played by NAFLD in metabolic and cardiovascular complications. NAFLD is considered a hepatic manifestation of the metabolic syndrome, of which the main component is insulin resistance followed by hiperinsulinemia[4, 19, 25, 32] and is characterized by increased uptake, synthesis and accumulation of fatty acids in the hepatocytes, leading to lipogenesis and fatty liver[3]. In addition, mitochondrial oxidation defects can cause the fatty acid synthesis to increase to the detriment of triacylglycerol secretion, thereby contributing to hepatic steatosis[30]. Hyperinsulinemia

This work was performed in the Surgical Department, Federal University of Ceará Medical School, Fortaleza, CE, Brazil.
[1] Department of Surgery, Federal University of Ceará; [2] Department of Pathology and Forensic Medicine, Federal University of Ceará; [3] School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil; [4] School of Medicine, Medical School, Juazeiro do Norte, CE, Brazil.
Correspondence: Dr. Idália Maria Brasil Burlamaqui – Avenida Beira Mar, 3680 – apt. 2001 – Meireles – 60465-121 – Foratleza, CE, Brasil. E-mail: idaliaburlamaqui87@hotmail.com

also induces oxidative stress with the consequent peroxidation of lipids in the hepatocyte membrane and production of cytokines, especially tumor necrosis factor-alpha (TNF-α)[3, 36].

Recent studies have shown that the adipose tissue secretes not only inflammatory cytokines such as interleukin–6 (IL-6) and TNF-α, but also adiponectin-an, insulin-sensitizing and antiinflammatory adipocytokine with multiple beneficial effects on the clinical complications of obesity[24]. The hepatoprotective action of adiponectin has been described in several clinical and experimental studies. Low serum levels of adiponectin in obese humans constitute an independent risk factor for NAFLD, including hepatic dysfunction of variable severity[39]. Thus, in one study, patients with NASH experienced a reduction of over 50% in adiponectin levels compared to normals[22]. Adiponectin levels are reported to decline by 20%−40% during the transition from simple steatosis to steatohepatitis[22, 37]. In patients with NASH, very low levels of adiponectin have been associated with severe inflammation, suggesting that adiponectin deficiency is an important risk factor for the development of steatosis, steatohepatitis and other forms of liver damage[5].

Since hypercaloric and hyperlipidic diets can induce liver damage, dyslipidemia and DM2, it may be hypothesized that PUFA-rich diets (containing ω-3 and ω-9) offer cardiovascular, hepatic and metabolic protection. Thus, the present study was designed to evaluate the hepatic and biochemical repercussions of a polyunsaturated fat-rich, hypercaloric and hyperlipidic diet in Wistar rats.

METHODS

Animals and diets

The study was previously approved by the Federal University of Ceará (UFC), Fortaleza, CE, Brazil, Ethics Committee for Animal Research (CEPA/UFC) under protocol #11/06 and was conducted according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985).

Twenty-two, 180-250 g, 8-week-old male Wistar rats (*Rattus norvegicus albinus*, Mammalia, Rodentia, Muridae) supplied by the UFC laboratory animal facility were used in the experiments. During the adaptation period, the rats were dewormed with pyrantel pamoate and oxantel pamoate (Basken®) at 1 mL/kg. The animals were randomly assigned to two groups at 8 weeks of life, accommodated in individual cages and euthanized in the 23rd week of the experiment. All animals had access to food and water ad libitum.

Group I (GI): Control group of 11 animals receiving standard diet. The diet (Biobase Bio-tec Ratos e Camundongos®) provided a total of 3.000 kcal/kg (Tables 1 and 2).

TABLE 2. Lipid profile and ratios between ω-3, ω-6 and ω-9 fatty acids in standard diet and hypercaloric and hyperlipidic diet

Type of fat	Standard diet	Hyperlipidic diet
Polyunsaturated	54%	19%
Monounsaturated	24%	29%
Saturated	15%	6%
ω9/ω6	0.4 : 1	1.5 : 1
ω6/ω3	8 : 1	3 : 1
Polyunsaturated/saturated % ω-6 and 9	5.2 : 1 >60% ω-6	7.6 : 1 >50% ω-9

Group II (GII): Treatment group of 11 animals receiving a hypercaloric and hyperlipidic diet. The diet consisted of 15 g standard diet + 20 g Nutri Diabetic® + 5 mL canola oil and was rich in polyunsaturated fat and omega fatty acids, providing a total of 4.250 kcal/kg (Tables 2 and 3).

Euthanasia and collection of blood and liver samples

Following anesthesia by intraperitoneal injection of 80 mg/kg ketamine and 8 mg/kg xylazine, the animals were placed in dorsal decubitus on a wooden board with all four limbs immobilized and submitted to laparotomy by xypho-pubic median incision and exposure of the abdominal cavity. Blood was collected from the abdominal aorta and tested biochemically for glycemia, uric acid, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), triglycerides, HDL and total cholesterol. Subsequently, a 1-cm^3 liver fragment retrieved by left hepatectomy was submitted to anatomopathological analysis at the Department of Pathology and Legal Medicine (School of Medicine, UFC). Finally, euthanasia was induced by aortic bleeding.

Histological study of liver samples

Following fixation in 10% formaldehyde, the liver samples were embedded in paraffin and stained with hematoxylin-eosin (H-E). Hepatic steatosis was classified in four grades based on the histological findings (Table 4)[8].

Statistical analysis

The statistical analysis was performed with the software SPSS v.16.0 for Windows®. All laboratory variables were normal with regard to the distribution of variance homogeneity. Average values were analyzed with Student's *t* test. Fisher's exact test was used to verify the association between diet type and the presence of hepatic steatosis. The level of statistical significance was set at 5% ($P<0.05$).

TABLE 1. Composition of standard (normoglycidic, hypolipidic and high-protein) diet (Biobase Biotec® Ratos e Camundongos)

Food	Amount (g)	Kcal	Carbohydrate (g)	Protein (g)	Lipid (g)
Biobase Bio-tec®	40	120	17.6	8.8	1.6
TOTAL	40	120	17.6	8.8	1.6
Total distribution of calories			70.4	35.2	14.4
Calories (%)			58.66%	29.34%	12.00%

TABLE 3. Composition of hypercaloric and hyperlipidic diet

Food	Amount (g)	Kcal	Carbohydrate (g)	Protein (g)	Lipid (g)
Biobase Bio-tec®	15	45	6.6	3.3	0.6
Nutri Diabetic®	20	80	11	3.2	2.6
Canola oil	5	45	0	0	5
TOTAL	40	170	16.6	6.5	8.2
Total distribution of calories			70.4	26	73.8
Calories (%)			41.36%	15.28%	43.36%

TABLE 4. Classification of steatosis

Grade	Macrovesicular steatosis
0	Absent
1	Mild (>0% to 33%)
2	Moderate (>33% to 66%)
3	Severe (>66%)

RESULTS

Biochemical analysis

The tests revealed statistically significant differences between Group I and Group II with regard to the levels of triglycerides ($P = 0.039$), total cholesterol ($P = 0.015$) and HDL ($P = 0.005$) (Table 5).

Weight distribution

The average weight did not differ significantly between the groups at baseline ($P = 0.711$), but was greater in Group II by the end of the experiment ($P = 0.000$) (Table 6).

Anatomopathological findings

The number of animals with macrovesicular steatosis was significantly greater in Group II (HD) than in Group I ($P = 0.03$) (Table 7; Figures 1 and 2).

TABLE 5. Biochemical findings for Groups I and II

Laboratory Test	Group I Average ± standard diet	Group II Average ± standard diet	*P*-value
Glycemia (mg/dL)	136.09 ± 28.01	150.00 ± 24.91	0.261
Uric Acid (mg/dL)	1.39 ± 0.36	1.15 ± 0.36	0.165
AST (U/L)	87.54 ± 35.27	66.64 ± 19.10	0.129
ALT (U/L)	42.31 ± 12.39	33.36 ± 11.71	0.117
GGT (U/L)	0.40 ± 0.33	0.50 ± 0.68	0.700
CRP	0.5 ± 0.00	0.5 ± 0.00	1.000
Triglycerides (mg/dL)	80.09 ± 22.36	59.44 ± 18.10	0.039*
Total cholesterol (mg/dL)	70.91 ± 18.08	53.44 ± 8.12	0.015*
HDL (mg/dL)	62.55 ± 11.75	48.22 ± 7.25	0.005*

* Statistically significant difference ($P<0.05$)

TABLE 6. Weight distribution (g) of Group I and Group II at baseline and by the end of the experiment

Group	n	Average (g) ± Standard deviation	
		Baseline	Final
I	11	200.8 ± 11.1	385.0 ± 16.4
II	11	203.4 ± 17.0	431.6 ± 20.1

* By the end of the experiment the average weight was greater in Group II than in Group I ($P = 0.000$)

TABLE 7. Fisher's exact test

Group	Absence of steatosis	Presence of steatosis
I	9 (81.8%)	2 (18.2%)
II	3 (27.3%)	8 (72.7%)*

$P = 0.03$

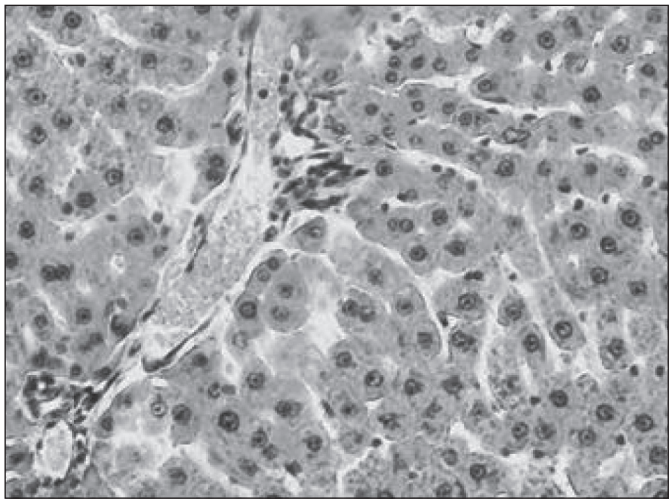

FIGURE 1. Histopathological section of liver tissue from rat 2, Group I, showing macrovesicular steatosis grade 0 (H-E; 400X)

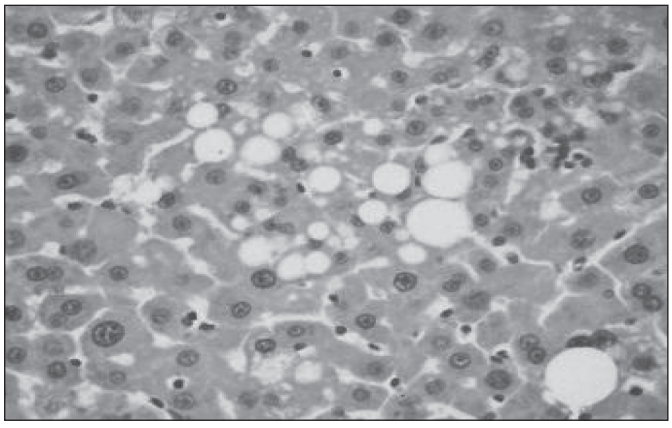

FIGURE 2. Histopathological section of liver tissue from rat 9, Group II, showing macrovesicular steatosis grade 1 (H-E; 400X)

DISCUSSION

Recent research has drawn attention to the association between NAFLD and a number of health problems, including obesity, dyslipidemia, type-2 diabetes and cancer. The pathogenesis of NAFLD is not completely understood, but insulin resistance, oxidative stress and inflammation all play an important role in the development and progression of the disorder[23, 26, 29]. In addition, unusually high levels of circulating free fatty acids have been correlated with NAFLD severity[15].

Changes in dietary fat composition can affect metabolic functions and lead to changes in body weight and composition[28]. In the present study, animals fed with a PUFA-rich diet displayed significant weight gain, as observed in other studies[18]. PUFA-rich diets have been associated with reduced food intake[17], probably, because animals receiving high-fat diets, as opposed to high-calorie diets, require less energy for lipid deposition[34].

In addition, epidemiological studies have found an inverse relation between the incidence of cardiovascular disease and the intake of ω-3 PUFA[21, 31].

The effect of different types of PUFA on body adiposity has been the object of much controversy. PUFA-rich diets with large amounts of ω-3 and ω-6 were recently reported, respectively, to reduce lipolysis and increase dietary lipid uptake in the adipose tissue. Both fatty acids caused changes in liver metabolism and favored lipid deposition[17].

The levels of triglycerides, total cholesterol and HDL were lower in animals receiving the enhanced diet (Table 5). Positive effects of ω-3 fatty acids on the reduction of triglycerides and total cholesterol serum levels have been reported in several other studies using animal or human models[2, 33]. Omega-3 fatty acids can inhibit the activity of diacylglycerol acyltransferase-a catalyst of triglyceride synthesis[35]. Rats fed with ω-3 fatty acid have been shown to have reduced total cholesterol serum levels due to increased biliary cholesterol excretion[6].

C-reactive protein (CRP) is produced in the liver. However, high CRP serum levels are considered a non-specific indicator of inflammation. Contrary to expectations, all the animals in our study had CRP levels below 0.5 mg/dL. Higher levels were expected, especially in Group II, due to the increased intake of calories and fat, greater weight and consequently greater probability of inflammation. The low CRP levels observed may be attributed to the antiinflammatory action of ω-3 fatty acids[38].

The study diet was expected to produce a protective effect on the liver, but the histological analysis showed steatosis to be statistically more predominant in Group II than in Group I (Table 7). Possibly, the lower adiponectin levels in the significantly heavier animals of Group II may not have been sufficiently insulin-sensitizing and antiinflammatory to prevent the development of NAFLD.

Studies using rats have shown that lipid deposition in the liver is related to oxidative stress and that lipid peroxidation increases in proportion to the severity of hepatic steatosis[12]. This may explain why hepatic steatosis was observed in a much greater proportion of the animals receiving hypercaloric and hyperlipidic food (72.7%) than in animals receiving the standard diet (18.2%). In other words, the risk of steatosis was greater in the treatment group because lipid peroxidation favors the development of NASH[11, 23].

Interestingly, though hepatic steatosis is often associated with slightly or moderately increased levels of ALT and AST[10], both parameters remained normal in Group II.

Confirming the findings of other researchers[7, 20], our study shows that although diets rich in polyunsaturated fatty acids improve the serum lipid profile, they can also cause undesirable changes in hepatic metabolism and lipid deposition.

CONCLUSIONS

These results show that a hypercaloric and hyperlipidic diet rich in polyunsaturated fat promotes weight gain and favors the development of hepatic steatosis while reducing serum levels of triglycerides, total cholesterol and HDL.

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RESUMO - ***Contexto*** **-** A doença hepática gordurosa não-alcoólica caracteriza-se por depósito de lipídios nos hepatócitos. Desperta grande interesse por sua associação com obesidade, dislipidemias e diabetes mellitus tipo 2. É considerada a manifestação hepática da síndrome metabólica, cujo principal componente é a resistência à insulina, com consequente hiperinsulinemia e produção aumentada de citocinas inflamatórias. Dietas ricas em gorduras saturadas promovem hipertrigliceridemia, diminuição do colesterol de alta densidade, aumento do colesterol de baixa densidade e hiperinsulinemia, enquanto dietas ricas em gordura poliinsaturada podem apresentar efeitos hipolipidêmicos, antiinflamatórios e imunorreguladores. ***Objetivo*** **-** Investigar as repercussões hepáticas e bioquímicas da dieta rica em gordura poliinsaturada em ratos Wistar. ***Métodos*** **-** Os animais (22) foram distribuídos nos grupos GI-dieta padrão (Biobase Bio-tec Ratos e Camundongos®) com 3000 kcal/kg e GII-dieta hipercalórica e hiperlipídica, com 4250 kcal/kg, relação ω-6: ω-3 = 3:1. Foram mortos após 23 semanas de administração das dietas. Avaliaram-se peso, exames bioquímicos e alterações histológicas do fígado. ***Resultados*** **–** Foram utilizados testes de análise de variância com nível de significância de 5% (P<0,05). Não houve diferença significante na média de peso entre os grupos (P = 0,711) no início, entretanto GII apresentou maior média que GI ao final do experimento (P = 0,000). GI mostrou níveis significantemente mais elevados de triglicerídeos (P = 0,03), colesterol total (P = 0,039) e HDL (P = 0,015) do que GII. O GII apresentou maior média de esteatose macrovesicular do que GI (P = 0,005). ***Conclusão*** **-** A dieta hipercalórica e hiperlipídica, rica em gordura poliinsaturada, promove esteatose hepática e incremento de peso, contudo reduz os níveis séricos de triglicerídeos, colesterol total e HDL.

DESCRITORES - Fígado gorduroso. Obesidade. Dislipidemias. Diabetes mellitus tipo 2. Ratos.

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