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Effect of dietary supplementation with olive and sunflower oils on lipid profile and liver histology in rats fed high cholesterol diet

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ABSTRACT

Objective: To compare the effects of high-monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) against the metabolic disorders elicited by a high-cholesterol diet (HC) in rats.

Methods: Using *in vivo* dietary manipulation, rats were fed with different diets containing 4% soybean oil (cholesterol free diet) and 1% HC containing 12% olive oil (HC + OO) enriched with MUFA and 12% sunflower oil (HC + SO) enriched with PUFA for 60 d. Serum lipid levels and hepatic steatosis were evaluated after the treatment period.

Results: Comparatively, rats treated with HC + OO diet experienced a decrease in the serum LDL-C, VLDL-C and CT levels compared to those fed with HC + SO diet ($P < 0.05$). Otherwise, HC + OO provoked significant microvesicular steatosis situated in the hepatic acinar zone 1.

Conclusions: HC + OO diet has high absorption velocity in the acinar zone 1 of liver compared to the HC + SO diet. Based on this, the reduction of the LDL-C, VLDL-C and CT serum levels in the animals treated with HC + OO diet may have been caused by the delay in the FA release to the blood.

1. Introduction

The consumption of westernized diets is involved with the predisposing to the development of a variety of diseases such as obesity, cognitive dysfunction, diabetes, immunologic dyshomeostasis and cancer [1–3]. It has been reported that rich-saturated fatty acids (FAs) and low polyunsaturated fatty acids (PUFAs) diets could favor the development of metabolic abnormalities in several organisms [4,5]. With regard to lipid nutrition, both quantity and type of dietary lipids have a significant impact on it. In addition, numerous reports have highlighted the association of fatty acids and cholesterol excess with changes in the serum lipid levels [6,7].

Within this context, many studies have focused on the evaluation of dietary FA composition, since its variation may cause important alterations in the lipoprotein synthesis and lipid composition of cellular structures [8–10]. Of particular importance, it is noteworthy that the unsaturation level of dietary FA has been related to a lower rate of a variety of diseases [11–15]. The consumption of diets enriched in monounsaturated or polyunsaturated FA as diet therapy against saturated FA induced-increased plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels has been widely accepted in the last three decades [16,17]. In addition, monounsaturated FA ingestion has been associated to lipid levels reduction and maintenance of the serum high density lipoprotein cholesterol (HDL-C) levels in animals and humans [17–20].

The nature of dietary FA and serum lipid profile is linked to a variety of pathological conditions, especially those related to the liver, a vital organ responsible for receiving, storing and directing lipid compounds toward the tissues [6,21]. Considering that appropriate lipid diets could be used as nutritional alternatives to prevent lipid disturbances, this study was undertaken to determine the effects of diets rich in high-

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monounsaturated fatty acids (MUFA) (olive oil rich in oleic acid) and PUFA (sunflower oil rich in linoleic acid) in adult rats fed with cholesterol-augmented diets. The serum triglycerides, total cholesterol, HDL-C, LDL-C, and very low density lipoprotein cholesterol (VLDL-C) were measured. Furthermore, histologic analysis from rats liver was carried out to evaluate the intensity and the topography of lipid deposits into the hepatocytes (steatosis).

2. Material and methods

2.1. Animals

Fifteen male *Wistar* rats (300 g) were used for the experiment. The animals were acquired from the Department of Biochemistry (UFRN, Natal, Brazil) and singly housed in separate boxes placed in a room at 22 °C temperature and a 12 h light/dark cycle. They had free access to food and water. All the animals were allowed to acclimatize for 1 week prior to the start of the study. All protocols used in this study were accepted by the local ethical committee of the local University (URCA). The ethical aspects were carefully followed according to the National Research Council Guide for the Care and Use of Laboratory Animals (1996).

2.2. Experimental design

The animals were randomized into three groups (n = 5), and subjected to different dietary regimens for 60 d, as described below. (1) For casein standard diet (CSD) [22] group, animals received 4% cholesterol-free soybean oil; (2) the animals received 1% high cholesterol diet (HC) + 12% oleic acid rich olive oil (OO; enriched with MUFA) were treated as HC + OO group; (3) the animals received 1% HC + 12% linoleic acid rich sunflower oil (SO; enriched with PUFA) were treated as HC + SO group.

Diets were prepared in pellet form. The oils were manually applied on the pellets. All rats were allowed to free-fed under the respective diets. All diets were stored at −20 °C, and fresh newly diets were daily provided to the rats. Rats from groups fed with HC diets + OO and SO consumed the same caloric values (Table 1).

Table 1
Centesimal composition of the diets.

Nutrients	CSD	HC + OO	HC + SO
Protein[a]	147.340	147.340	147.340
Lipid[b]	40.000	120.000	120.000
Fiber	50.000	50.000	50.000
Sacharose	100.000	100.000	100.000
Mineral mixture	35.000	35.000	35.000
Vitamin mixture	10.000	10.000	10.000
L-cistin[c]	1.800	1.800	1.800
Bitartarate of colin	2.500	2.500	2.500
Buthyl hidroquinone	0.008	0.008	0.008
Synthetic cholesterol	0.000	10.000	10.000
Starch	613.540	523.360	523.360

[a] Casein containing 95% of protein. [b] Addition of 4% soybean oil (Sb−O), 12% olive oil rich in oleic acid (HC + OO) and 12% sunflower oil rich in linoleic acid (HC + SO). [c] Used to complement the sulphurated amino acid of the casein.

2.3. Body weight and food consumption

The food intake and the growth of animals were monitored every 3 d by recording the body weight.

2.4. Lipid profile determination

Twenty-four hours after the last day of treatment, the animals were anesthetized after an overnight fast (16 h). The animals underwent cardiac puncture to collect blood. Blood samples were transferred into anticoagulant-free vials and allowed to stand for 30 min to clot. Afterwards, the vials were centrifuged at 300 × g for 10 min and the resultant serum was used for further analysis of TG, TC, HDL-C, VLDL-C and LDL-C. Serum lipid levels were colorimetrically measured by routine procedures, using commercial kits from Labtest Diagnóstica S/A (Minas Gerais, Brazil). All analyzes were performed according to the manufacturer's instructions in triplicate.

2.5. Histologic analysis by HE staining

The animals were sacrificed by decapitation and submitted to a thoracic and abdominal incision to obtain the liver fragments. Portions of liver were fixed in 10% buffered formol for 24 h, dehydrated in a gradual series of alcohols and diaphanous in xylene for paraffin embedding. Later, paraffin blocks were sectioned at 4 μm and stained with hematoxylin and eosin for histological examination by optical microscopy. Histopathological assessment of liver damage was recorded through the presence and intensity of steatosis, measured by scores, which varied from light +, mild ++ and severe +++. The pattern (macrovesicular and/or microvesicular) was also analyzed. Its distribution was determined across the hepatic acinar zones (1, 2, or 3).

2.6. Statistical analysis

The data were analyzed by Two-way ANOVA followed by Kruskal–Wallis test when appropriate (GraphPad Prism Software, San Diego, CA, USA). The histopathological results were analyzed by Kruskal–Wallis, Wilcoxon and Mann–Whitney U Tests. Cochran Q test was used to determine the location in the acinar hepatic zones. The value of $P < 0.05$ was considered to indicate a statistically significant difference among the groups.

3. Results

3.1. Food ingestion and weight gain

Significant alterations in the weight gain and food consumption among the rats from the different treatment regimens were observed (Table 2). The highest food ingestion values were noticed in the animals that received the standard diet (free-cholesterol diet) during the 60 d of treatment. Compared to the standard group, a decrease of 9.14 and 9.45% in the food consumption was observed for the animals fed the HC + SO and HC + OO diets, respectively (Table 2). In addition, the animals ingesting the HC + SO and HC + OO diets obtained a substantial increase in the weight gain of 47.13% and 30.89% respectively, in comparison to the standard group (Table 2).

Table 2
Values of food ingestion (g) and the animals weight variation average, according to the experimental diets.

Group	Ingestion of food	Initial weight	Final weight	Weight gain
CSD	1000.20 ± 24.82	302.51 ± 4.59	354.60 ± 6.80	52.09 ± 11.39
HC + OO	905.68 ± 28.69	280.86 ± 8.72	349.04 ± 10.97	68.18 ± 19.69
HC + SO	908.75 ± 26.97	272.30 ± 7.42	348.94 ± 13.14	76.64 ± 20.56

Data were expressed as mean ± Standard Deviation (SD).

Table 3
Effect of diets on serum lipid (mg/dL) values of rats.

Group	TG	CT	HDL-C	VLDL-C	LDL-C
CSD	61.06 ± 6.76	95.68 ± 6.40	49.72 ± 6.26	12.21 ± 0.95	33.73 ± 3.15
HC + OO	33.01 ± 6.03[a]	75.85 ± 9.02[a]	43.07 ± 5.17[a]	6.59 ± 1.01[a]	21.01 ± 3.31[a]
HC + SO	56.33 ± 6.22[b]	104.99 ± 10.69[b]	42.77 ± 2.47[a]	11.26 ± 1.79[b]	50.95 ± 4.55[ab]

Data were expressed as mean ± SD. Compared to CSD, [a]$P < 0.05$; compared to HC + OO, [b]$P < 0.05$.

3.2. Biochemical parameters

The biochemical measurements of the serum lipid components from the animals treated with the diets were analyzed after the treatment period. HC + OO diet caused significant serum TG and CT reduction of 41.39% and 27.75% in comparison to those animals that received HC + SO diet, respectively ($P < 0.05$) (Table 3). No difference was obtained in standard and HC + SO groups. HDL-C plasma levels was found to be significantly lower in the animals fed HC + OO and HC + SO diets in comparison with the standard group (13.3% and 13.9%, respectively). The serum VLDL-C levels were significantly diminished in HC + OO fed rats when compared to standard and HC + SO diets (46.02% and 41.47%, respectively). The animals ingesting the HC + OO diet showed a significant reduction in the LDL-C levels, from 37.70% to 58.76% in comparison to those animals that ingested the standard and HC + SO diets, respectively ($P < 0.05$).

3.3. Liver histopathology

The considerable effects of lipid diets in compromising the integrity and functionality of the liver prompted us to examine some histological aspects after the treatments. Analysis of hepatic histoarchitecture revealed that liver specimens from the animal group ingesting the standard diet had no significant fatty changes (Figure 1A). On the other hand, liver samples from animals fed HC + OO and SO diets demonstrated important

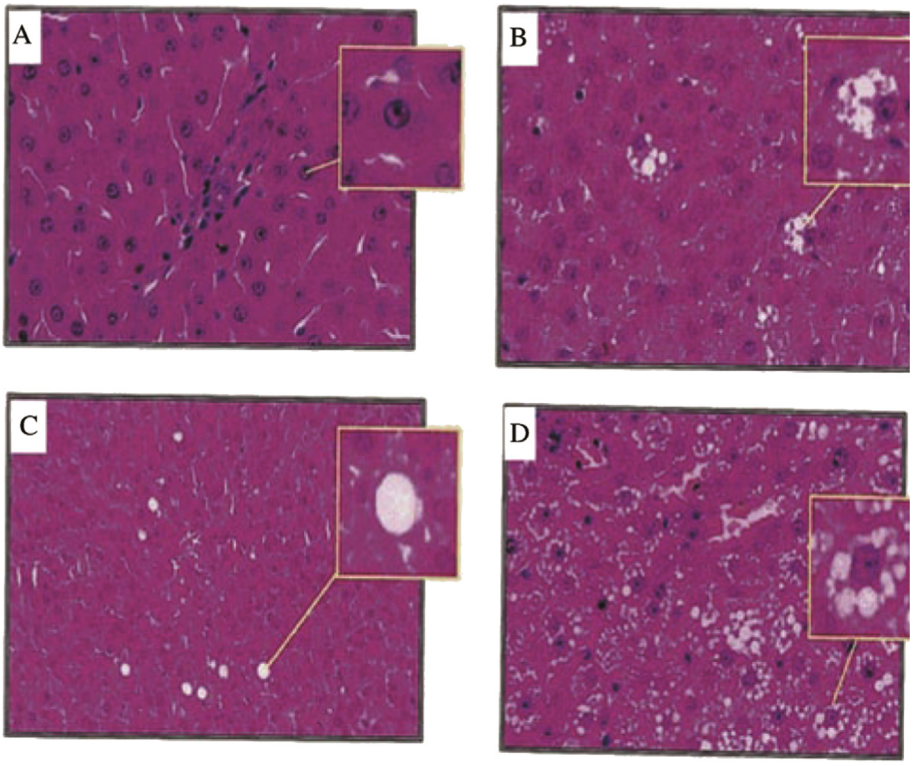

Figure 1. Microphotographs of rats liver. A) Liver microscopy from an animal ingesting standard diet, showing acinar zone 1 without steatosis (400×). B) Microscopic aspect of the liver from the animal ingesting HC + SO diet, showing acinar zone 2 with slight macrovesicular steatosis (+) (200×). C) Microscopic aspect of the liver from the animal ingesting HC + SO diet, showing acinar zone 2 with mild microvesicular steatosis (++). Some hepatocytes are steatosis-free (400×). D) Microscopy of the liver from the animal ingesting a HC + OO diet showing severe microvesicular steatosis (+++), compromising all the hepatocytes situated at acinar zone 1; the portal tract is delimited by a rectangle (H&E ×400).

Table 4
Patterns, intensity and topography of hepatic steatosis observed after ingestion of three different diets.

Group		Macrovesicular	Microvesicular
CSD		–	–
HC + OO	Intensity	+	+++[a]
	Liver acinar zone	1	1
HC + SO	Intensity	+	++[ab]
	Liver acinar zone	2	2

Compared to CSD, [a]$P < 0.05$; compared to HC + OO, [b]$P < 0.05$. (–) absent; (+) slight; (++) moderate; (+++) severe.

histological alterations resulting from lipid storage in the hepatocytes cytoplasm. The analysis of steatosis patterns distributed through different acinar zones of liver indicated that the appearance of microvesicular steatosis was significantly different between all the groups following the dietary treatments ($P < 0.05$) (Table 4). No significant changes were noted with respect to macrovesicular steatosis patterns.

Although animals fed HC + OO diet showed the lowest serum TG, TC and LDL-C levels among the groups, they had severe microvesicular steatosis affecting mainly the hepatic acinar zone 1, diverging from the topography of the lipid storage seen in the hyperlipidemic conditions. Specifically, these animals showed significant (severe) microvesicular fatty changes (+++), predominantly located in acinar zone 1, containing slight areas of macrovesicular lipid storage in this same topography (Figure 1D; Table 4). Regarding the group ingesting the HC + SO diet, the presence of mild microvesicular (++) and slight macrovesicular (+) steatosis was detected, predominantly located in the acinar zone 2 (Figure 1B and C; Table 4).

4. Discussion

Encouraging results have been obtained regarding the benefices of diets rich in unsaturated fatty acids [5,11,12,23,24]. Here, we used high cholesterol diet-fed rats as a model to investigate and compare the effects caused by high-mono and poly-unsaturated fatty acids diets on lipid metabolism and hepatic steatosis in chronically treated rats. Collectively, we showed that oleic acid rich olive oil, containing MUFA, was able to ameliorate the serum lipid profile when compared to cholesterol-free and linoleic acid rich sunflower oil diets. In particular for the values found for TG levels, our results are in discordance with previous reports, which demonstrated that rats concomitantly fed with 0.4% cholesterol and 12% oleic acid diets had a higher concentration in serum TG levels compared to those fed with a PUFA rich corn oil diet [25]. Although lower food ingestion was verified in HC + OO and HC + SO groups, the high percentage of dietary lipids may have contributed to the increased body weight verified in the animals fed with these diets.

Previous studies revealed that a diet rich in cholesterol and oleic acid reduced the serum LDL-C levels in hamsters, which could be a consequence of the increased cholesterol esterification in the liver [26,27]. However, these results were not observed when the animals ingested only dietary cholesterol [26]. In our experimental protocol, HC + OO diet triggered severe microvesicular steatosis affecting mainly the hepatic acinar zone 1. Typically, 2 major histologic patterns of steatosis within hepatocytes define the fatty disorders. Microvesicular steatosis occurs when the cytoplasm is replaced by bubbles of fat with the nucleus remaining centrally placed [28]. Most severely, macrovesicular steatosis is distinguished by a preponderance of large droplet steatosis, in which the cytoplasm is replaced by a large bubble of fat that displaces the nucleus to the edge of the cell [28]. It is well documented that microvesicular steatosis is influenced by the velocity, intensity and duration of lipid storage [26,29]. These variants are closely implicated with the lipid turnover [26]. In cases of non-alcoholic steatohepatitis caused by hyperlipidemic conditions, steatosis generally has its initiation along the perivenular area of the liver (acinar zone 3) and later affects all the other acinar zones. In those cases, obesity and Type 2 Diabetes are considered the usual associations [30].

We hypothesized that a selective esterification occurring in hepatic acinar zone 1 could explain the largest lipid storage observed in this region of the liver from the animals fed a HC + OO diet. Otherwise, linoleic acid (HC + SO) may have undergone higher and selective esterification in hepatic acinar zone 2, near the regions where the lipids are released from the hepatic cells to the blood. This could explain the significant increase of plasma LDL-C levels found in these animals when compared to HC + OO group. Moreover, the greater distance between hepatic periportal (acinar zone 1) and perivenular (acinar zone 3) areas may favor the TG-delayed release to the plasma in the animals treated with HC + OO diet. Comparatively, we suggest that the topographic distribution of the hepatic fat observed in the animals that consumed the HC + OO diet has an important role on the significant reduction of the serum LDL-C, VLDL-C and CT levels compared to those fed with the linoleic acid (HC + SO) diet.

In conclusion, our results indicate that the high percentage of lipids composing HC + OO and HC + SO diets may have contributed to the microvesicular steatosis onset, most severely observed in the HC + OO group. Furthermore, MUFA diet had high absorption velocity in the acinar zone 1 of the liver compared to the PUFA diet. Based on this, we believe that the significant reduction of the serum LDL-C, VLDL-C and CT levels in the animals treated with HC + OO diet may have been caused by the delay in the FA release to the blood.

Conflict of interest statement

The authors have declared that there is no conflict of interest associated with this publication.

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