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Research Article

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Analysis of sub-chronic coconut oil consumption in rats meal with different lipid ratios in the diet

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ABSTRACT

Coconut oil has been the subject of great interest as edible oil used for weight reduction and treatment of diabetes, hyperlipidemia, and epilepsy. To evaluate the metabolic impact of its intake, Wistar male rats were randomized into four groups based on the presence of soybean or coconut oil, and on their allowed concentration of fats (control, NormoCoco – both with 7% fat; KetoSoy, and KetoCoco, both with 67% fat) for eight weeks. Body weight, blood biochemical markers and liver histology were evaluate. When weight gain was adjust for energy intake, the KetoSoy group was the sole group with less weight gain than the control. The groups fed a ketogenic diet, regardless of the lipid source, had significantly lower levels of serum triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol compared to control. The frequency and severity of steatosis was higher in the control group, and no differences were found in hepatic or renal markers for all diet groups. These findings suggest that coconut oil intake may be safe and useful as an alternative lipid source, even in ketogenic proportions.

Keywords: Body weight, Coconut oil, Fatty liver, Ketogenic diet, Rat

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INTRODUCTION

Coconut oil comes from the coconut palm (*Cocos nucifera*), which is abundant in the Brazilian northeast. This product is inexpensive and widely used in the cosmetic and chemical industries because of its resistance to oxidation, its low melting point, and its stable emulsion- forming property. The pharmaceutical industry is also benefiting from the anti-viral, anti-fungal, and anti-bacterial properties of coconut oil, which are particularly attribute to lauric acid. Crude coconut oil is increasingly becoming a topic of interest, given the demand for natural and safe products that retain their beneficial properties since it is not processed [**1 -2**].

Despite being a saturated fat (containing 81-85% saturated, 5-8% monounsaturated, and 1-8% polyunsaturated fatty acids), coconut oil is rich in Medium-Chain Triglycerides (MCTs) (around 85%), which lend advantageous metabolic features. MCTs are absorbed quickly because they do not require metabolism by pancreatic lipase and were transported to the liver by the hepatic portal vein, where they are quickly oxidized for energy production. This is the reverse of the process for Long-chain Triglycerides (LCTs). In coconut oil, the MCT and LCT ratio is approximately 3:1 **[3-4]**.

Presently, coconut oil is used under various conditions, including weight reduction, and glycemic and lipid control in obese people, as well as in the treatment of disorders such as Hypercholesterolemia, Diabetes, Crohn

disease, irritable bowel syndrome and as an alternative lipid source in the treatment of epilepsy, replacing LCTs in the ketogenic diet **[5-6]**. Despite its potential benefits, the consumption of coconut oil by the general population is insignificant, perhaps because of the stigma associated with serum lipid profile and increased cardiovascular risk. This is strongly related to the component fatty acids, mainly lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids. These fatty acids could theoretically contribute to elevated serum cholesterol concentration and the development of disease. The palmitic and myristic acids are generally associated with elevated serum cholesterol; however, the effects of lauric acid on serum lipids and lipoproteins are less known **[5-7]**.

The increased use of coconut oil in food, the potential therapeutic effects of its use in different groups of patients, and the controversies concerning the metabolic impact of its consumption on human health, highlight the importance of studying the use of this oil. This study aimed to evaluate the effect of sub-chronic coconut oil consumption in normal and ketogenic proportion on the growth and metabolic and histological parameters of Wistar rats.

EXPERIMENTAL SECTION

Diet and animals

The number of Ethics Research Committee from the Federal University of Alagoas is 009694/2011-52 and approved animal use. Male Wistar rats (n = 41) from the Central Animal Laboratory of the Federal University of Alagoas were weaned at 30 days of age, then weighed and housed individually under controlled conditions (20–24°C, light/dark cycle of 12 h). The animals were randomly allocated by simple draw into four different diet groups, each of which was based on the AIN-93G diet **[8]**. Control (AIN-93G diet, soybean oil, 7% lipids); NormoCoco (AIN-93G, coconut oil, 7% lipids, supplementation of essential fatty acids); KetoSoy (ketogenic diet, soybean oil, 69.79% lipids); and KetoCoco (ketogenic diet, coconut oil, 69.79% lipids).

The animals had free access to water and their relevant diet for 8 weeks and food consumption and weight gain were evaluate weekly. The margarine and soy oil used in food production were purchase locally; coconut oils were purchase at COPRA® (Maceió-AL, Brazil), and other ingredients were purchase at RHOSTER® (São Paulo-SP, Brazil). The composition of each diet was show in Table 1.

We used the feed efficiency coefficient (FEC) to estimate the overall quality of the diets and their influence on the animals' growth. FEC was calculated using equation 2.1:

$$FEC = \frac{weight\ gained\ (g)}{intake\ amount\ (g)} \times 100 \qquad [2.1]$$

Table 1. Composition of experimental diets

	Groups[a]			
Constituents (g/kg)	Control	NormoCoco	KetoSoy	KetoCoco
Corn starch	495.9	495.9	0	0
Dextrinized corn starch	132.0	132.0	0	0
Casein	200	200	200	200
Cellulose	50	50	50	50
Mineral mix AIN-93 G	35	35	35	35
Vitaminic mix AIN-93	10	10	10	10
L-cysteine	3	3	3	3
L-methionine	1.6	1.6	1.6	1.6
Choline Bitartrate	2.5	2.5	2.5	2.5
t-Butylhydroquinone (mg)	14	14	139.58	139.58
Soybean oil	70	0	297.9	70
Coconut oil	0	70	0	227.9
Margarine	0	0	400	400
Supplement EFAb (mg)	0	18	0	0

[a]Composition based on AIN-93G diet (REEVES et al, 1997); [b]Essential fatty acids.

Biochemical and histological analysis

At the end of the eight-week trial period, the animals were fasted for 12 h and anesthetized by using 100 mg/kg of ketamine and 15 mg/kg of xylazine, both delivered intraperitoneally; blood collection was performed via cardiac puncture. The collected blood was centrifuged (3500 × g for 20 min) and analyzed spectrophotometrically, employing specific laboratory kits for the determination of: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT) total protein,

albumin, urea, creatinine, uric acid, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and glucose.

Following blood collection, the animals were sacrifice by removing the heart, liver, intestine, spleen, stomach, brain, and left kidney. After fixation in 10% formaldehyde, the organs were cleave, and sections were processed, embedded in paraffin, and dyed by the hematoxylin-eosin method. The kidney was slice lengthwise, while the liver, medium third of the duodenum and jejunum, and proximal third of the ileum were sliced transversally. Possible macroscopic alterations of the organs when found were noted and histological alterations were evaluated by the Pathology Department of the Federal University of Alagoas.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD), while the discrete variables are expressed as medians, followed by the full range. Dichotomous variables are expressed as relative and absolute frequency.

All continuous variables were tested for normality (Lilliefors test) and homoscedasticity (Levene test). Those variables meeting these assumptions were subjected to analysis of variance (ANOVA), with post-hoc Tukey honest significant difference (HSD) test. Any variables that did not meet these assumptions were subjected to the Kruskal-Wallis test with a post hoc Dunn test. This test was also used for the analysis of discrete variables. The difference in the frequencies of histological events between groups was tested by using the chi-square test, with analysis of the adjusted residuals to identify which groups harbored those differences. In all analyzes, an alpha value equal to 5% (P = 0.05) was used to reject the null hypothesis. The analyses were conducted with the aid of the SPSS v17.0 (IBM statistics, Chicago-IL) software.

RESULTS AND DISCUSSION

In this study, we observed that animals following a ketogenic diet, regardless of their lipid source, gained less weight than animals fed with the control diet, which is consistent with the other report **[9]**. All data for energy intake, food intake, FEC, and final weight of the trial groups has been show in the Table 2. The between-group differences in weight gain began in the second week of the experiment. The ketogenic groups showed no significant between-group differences, but had lower weight gain when compared to the control group. The ketogenic groups consumed an equal amount of food, which was less than the consumption of the control and NormoCoco groups, who also did not differ in food intake. The same behavior was found when measuring FEC. When examining caloric intake, we found a significant difference between the control and KetoCoco groups, but the NormoCoco and KetoSoy groups did not significantly differ from the control and the KetoCoco groups. When weight gain was corrected by the energy intake, only the KetoSoy group showed significantly lower weight gain than the control group. This was unexpected, as MCTs found in the KetoCoco diet should have induced higher ketosis and led to less weight gain in this group. The ability to prevent or limit weight gain is a characteristic to the ketogenic diet **[10]**. Although the reasons for this have been not fully elucidated, it is thought that increased satiety, which reduces voluntary food intake and thus causes energy restriction, as well as the anorexic effect of ketone bodies may be responsible **[11]**. Studies examining coconut oil-based ketogenic diets are limited. Some studies show that the weight gain was similar between groups fed with ketogenic and soybean oil diets after 14 days **[12]**. Other types of MCT use have been reported and evaluated the use of triheptanoin for seven weeks found that the groups consuming ketogenic diets based on soybean oil or triheptanoin did not show differential weight gain; in fact, only the group fed with the ketogenic diet and triheptanoin differed in this parameter from the control group. **[9-13]**

Table 2. Food intake, energy intake, FEC and weight of rats

	Experimental Groups			
Variables[a]	control (n = 11)	NormoCoco (n = 11)	KetoSoy (n = 10)	KetoCoco (n = 9)
Energy intake(kcal)	4408.27 ±193.8	4148.9 ±349.2	4346.1±302.3	3999.9 ±339.8[b]
Food Intake (g)	1130.3 ± 49.7	1063.8 ± 89.5	629.8±43.7[b,c]	579.6 ± 49.2[b]
Feed Efficiency	4.9 ± 0.6	5.3 ± 0.9	3.6 ± 0.6[b,c]	3.4 ± 0.7[b,c]
Coefficient				
Initial weight (g)	116.1 ± 32.1	115.1 ± 32.7	118.1 ± 35.6	111.1 ±31.0
Final weight (g)	346.4 ± 29.2	322.4 ± 37.5	294.5 ± 27.3[b]	287.9 ± 30.3[b]
Weight gain (g)	230.4 ± 33.2	207.3 ± 52.9	176.5 ± 30.4[b]	176.8 ± 42.3[b]
Weight gain (g/100	5.21 ± 0.6	4.9 ± 0.9	4.0 ± 0.7 [b]	4.39 ± 0.8
kcal)				

[a]Variables subjected to ANOVA with Tukey HSD test (P <0.05).
[b]Denotes a significant difference from the control group
[c]Denotes a significant difference from the NormoCoco group.

Some studies have evaluated the influence of coconut oil in lowering weight and anthropometric parameters in overweight people and showed a significant reduction in body mass index (BMI) and waist circumference in overweight women who received a daily dose of 30 mL ofcoconut oil for 12 weeks. In another study examining overweight people, a reduction in waist circumference was observed only in males during the six weeks of the experiment. The observed waist circumference reduction was attributed to the medium-chain fatty acid (MCFA) content of coconut oil, which is poorly stored in adipose tissue **[4,14].**

Table 3 summarizes the biochemical profile of the animals. The ketogenic groups showed significantly lower levels of triglycerides, total cholesterol, and LDL and VLDL when compared to control group, regardless of their lipid source. The NormoCoco group showed intermediate results regarding their total cholesterol and LDL and VLDL levels, and did not exhibit any significant difference in the latter compared to the other diet groups. No difference were found in any of the other parameters. Due to the stigma associated with the consumption of saturated fat and its influence on lipid profile, tests conducted on laboratory animals have sought to assess the influence of coconut oil consumption on these parameters. A study investigated the influence of coconut oil in mice, and found that the group fed with naturally extracted virgin coconut oil had significantly lower serum cholesterol, LDL, and triglycerides than the control and refined coconut oil-fed groups. Another study showed that the individuals subjects to consume of coconut oil had higher HDL concentrations compared to soybean oil group, suggesting that the use of coconut oil may be beneficial, possibly due to the rapid oxidation of MCFA, particularly lauric acid **[14-19]**. In the present study, we observed that the protective effect on these biochemical parameters was primarily related to the proportion of oil in the diet, and not specifically to the lipid source and there was no significant change in triglyceride and LDL + VLDL levels between the NormoCoco, KetoCoco, and control groups.

Table 3. Serum biochemical markers of the animals

Parameter[a]	Experimental Groups			
	Control (n = 11)	NormoCoco (n = 11)	KetoSoy (n = 10)	KetoCoco (n = 9)
Glucose (mg/dL)	120.1 ± 32.2	117.9 ± 23.5	111.4 ± 23.7	119.0 ± 23.2
Triglycerides (mg/dL)[b]	104.6 ± 46.1	98.2 ± 34.1	49.6 ± 18.6[c,d]	44.0 ± 18.3[c,d]
Total Cholesterol (mg/dL)	66.1 ± 12.8	64.9 ± 8.5	48.5 ± 13.7[c,d]	47.1 ± 6.6[c,d]
HDL (mg/dL)	32.8 ± 12.3	33.73 ± 9	26.2 ± 6.2	25.3 ± 5.4
LDL+VLDL (mg/dL)	34.4 ± 10.4	31.2 ± 6.3	22.2 ± 9.4[c]	21.7 ± 7.1[c]
Uric acid (mg/dL)	1.9 ± 1.7	2 ± 1.7	2 ± 1.8	1.7 ± 1.6
Urea (mg/dL)	75.1 ± 83.7	59.2 ± 62.1	69.4 ± 75.9	64.4 ± 83.5
Albumin (g/dL)	2.4 ± 1	2.5 ±0.8	2.8 ± 1.1	2.2 ± 0.75
Total protein (g/dL)	5.5 ± 0.8	5.8 ± 1.3	5.5 ±1	5.8 ± 1.4
Globulins (g/dL)	3.1 ± 1.7	3.3 ± 1.5	2.6 ± 1.6	3.6 ± 1.7
Creatinine (mg/dL)	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
ALP (mg/dL)	192.2 ± 72.5	173.1 ± 66.8	266.0 ± 90.6	235.4 ± 67.7
ALT (U/L)	118.1 ± 34.1	115.4 ± 27.3	134.4 ± 40.4	125.15 ± 4
AST (U/L)	193.6 ± 33.3	205.3 ± 35.6	208. 3 ± 32.1	197.5 ± 35.9

[a]Unless indicated, all parameters used the ANOVA with post-hoc Tukey HSD; [b]Kruskal-Wallis test with post hoc Dunn test; [c]Significant difference compared to control group; [d]Significant difference compared to NormoCoco group.

Table 4 has been show the lipid infiltration in each group, with macro and microvesicular aspects. The frequency of fatty liver was significantly higher in the control group than was expected, and the control group showed the highest grade of steatosis compared to the others groups.

Table 4. Degree and frequency of steatosis

Parameter[a]	Experimental Groups			
	Control	NormoCoco	KetoSoy	KetoCoco
	(n = 11)	(n = 11)	(n = 9)	(n = 9)
Steatosis degree[b]	3 [2–4]	1 [0–3][d]	0 [0–2][d]	0 [0–1][d]
Steatosis frequency[c]	11 (100%)[e]	8 (72.7%)	4 (44.4%)	4 (44.4%)

[a]Data presented as Median [Minimum Value–Maximum value], or as absolute and relative frequency. [b]Kruskal-Wallis with Dunn test; [c]Chi-square test; [d]Significant difference from control; [e]Significantly higher than expected

The only histological abnormality detected was hepatic lipid infiltration with macro and microvesicular characteristics that showed a higher than expected frequency only in the control group. In addition, there was a significant difference between the control group and the other groups in the severity of the disease, suggesting that the replacement of soybean oil for coconut oil, even in non-ketogenic proportions, ameliorates this liver disorder. This may be due to the high polyphenol and antioxidant content of coconut oil, which may be protecting the liver of the animals (Liau et al., 2011) **[20]**. The use of the AIN-93G reference diet has been associated with cases of fatty liver, suggesting that there are some issues in its composition. This diet has already seen modifications to its

methionine and choline content, as well as to its concentration of sucrose to meet the nutritional demands of animals and prevent hepatic lipid accumulation **[13].**

CONCLUSION

Despite all these measures, a larger number of cases and greater severity of steatosis in this study were observed in the control group, fed AIN-93G. Based on these considerations, any toxic effect of dietary coconut oil under the conditions of this study may be discarded as the consumption of coconut oil had highly favorable results for the various selected indicators, compared to the control and ketogenic groups. Coconut oil may be an alternative source of MCTs.

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