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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Lipodystrophic and Lipo-Peroxidative Effects of Hydrogenated Coconut Oil Diet in Rats

A.A. Odutuga[1] and A.A. Amballi[2]
[1]Department of Biochemistry, Joseph Ayo Babalola University,
Ikeji-Arakeji, PMB 5006, Ilesa, Osun State, Nigeria
[2]Department of Chemical Pathology, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria

Abstract: The effects of dietary saturated fat on blood lipid and lipoprotein composition and lipid peroxidation were studied in rats. Male albino rats (Rattus norvegicus) were maintained either on diets with 5% fat supplements made of 5% soyabean oil or 5% coconut oil. Similarly, two other groups were fed diets that were isocaloric with the former but contained 25% fat supplements made of 25% soyabean oil or 25% coconut oil. The concentration of triacylglycerols in the plasma of the high coconut oil fed rats was three to six-times those of the others. The concentration of free cholesterol was much higher in the plasma of the coconut oil-fed rats. There were significant decreases in the plasma level of chylomirons, VLDL and HDL, but a significant increase in the LDL level of the coconut oil-fed rats. In all the tissues studied, the malondialdehyde level increased significantly in the coconut oil fed rats and most prominently in the liver and the kidney, while the reduced glutathione levels decreased significantly in the coconut oil-fed rats. It is considered that ingestion of coconut oil for a considerably long time may affect lipid metabolism and alter the structure and function of the enzymes responsible for converting the essential fatty acids to prostaglandins or their endoperoxide precursors. The increase in the malondialdehyde level and the decrease in the reduced glutathione level are indications of the lipid peroxidative effect of saturated fat diet, while the reduced HDL and high level of LDL can be major contributory factors to atherogenesis.

Key words: Essential fatty acids, saturated fats, lipoprotein, lipid peroxidation

Introduction

Dietary oils provide the much needed nutritionally essential fatty acids in the body. These nutritionally essential fatty acids, linoleic and linolenic acids are needed for normal growth and development. Tissue lipid composition is influenced by the type of fat in the diet (key *et al*., 1965; Odutuga, 1977; Steinberg, 1987). The dietary alteration of lipid composition may be of importance due to the special biochemical functions of the blood in lipid energy metabolism. Alterations of the levels of dietary EFAs will affect the stores of these fatty acids and their metabolites; and may in turn affect the availability of these fatty acids for membrane incorporation or their conversion to biologically active substances, namely, the eicosanoids. Membrane composition and permeability are altered in the extreme case of EFA-deficiency (Odutuga, 1977).

The deficiency of EFAs has been linked to high blood cholesterol level and cholesterol, in turn, has been shown to be a major constituent of the plagues that form on the inside of some blood vessels (Steinberg, 1987). In the present study, therefore, the effect of diets containing various amounts of saturated fatty acids or EFAs on the lipid and lipoprotein composition of the blood has been carried out. In addition the relationship between saturated fatty acids consumption and malondialdehyde formation in rat tissues has been studied. This investigation will enable us assess the importance of dietary manipulation of oils on mammalian tissues.

Materials and Methods

Animals and diets: Forty male albino rats (Rattus norvegicus) were divided into four groups of ten animals each. Two groups were fed similar diets with 5% fat supplement made of 5% soyabean oil (S-5) or 5% coconut oil (K-5). The other two groups were fed diets that were isocaloric with the former but containing 25% fat supplements made of 25% soyabean oil (S-25) or 25% coconut oil (K-25).

The composition of the diets is shown in Table 1. Groundnut cake was smoothly powdered and subjected to lipid extraction as previously described (Odutuga, 1982). There was no lipid left in the protein. The soyabean was adequate in EFAs while the coconut oil was in adequate in EFAs. The soyabean oil used in this study contained 58.5 and 8.1 per cent linoleic acid and linolenic acid respectively. The hydrogenated coconut oil, on the other hand, is composed mostly of fatty acids of 6 to 14 carbon chain length (89.0%), palmitic acid (7.6%), stearic acid (2.8%), oleic acid (0.4%) and traces of linoleic acid (0.2%). The diets were stored at 4°C and the rats were fed fresh food daily. The diets and water were given ad libitum. All rats were fed their respective diets daily and weighed weekly.

Table 1: Composition of Diets (in g/100g)

Components	Dietary Groups S-5	K-5	S-25	K-25
Groundnut cake[1]	20.00	20.00	20.00	20.00
DL-Methionine	0.30	0.30	0.30	0.30
Corn Starch	51.11	51.11	17.81	17.81
Sucrose	15.00	15.00	3.40	3.40
Cellulose	5.00	5.00	30.00	30.00
Choline chloride	0.11	0.11	0.11	0.11
Vitamins-Minerals mixture[2]	3.48	3.48	3.48	3.48
Soyabean oil	5.00	-	25.00	-
Coconut oil	-	5.00	-	25.00

[1]Groundnut cake purchased locally was milled and extracted with organic solvents as reported by Odutuga (1982). It contained approximately 61.8% crude protein, [2]The vitamins-minerals mixture used contained (g/100g diet): Vitamin A (0.068), vitamin D_3 (0.014), vitamin E (0.204), vitamin K (0.014), vitamin B_1 (0.007), vitamin B_2 (0.027), niacin (0.170), Ca D-pantothenate (0.054), vitamin B_6 (0.020), vitamin B_{12} (0.102), folic acid (0.005), biotin (0.0005), Mn (0.68), Fe (0.34), Zn (0.306), Cu (0.014), I_2 (0.011), Co (0.002) and Se (0.001)

Tissue preparations: After 14 weeks of feeding, the rats were fasted overnight and anaesthetized with light petroleum ether. Blood was removed by cardiac puncture using heparinized syringes, collected into tubes containing 3.8% trisodium citrate (blood/citrate, 9:1,v/v) and immediately centrifuged at low speed to separate plasma from the blood cells (Folch *et al.*, 1951). After sacrificing the animals, the brain, liver, lungs and kidneys were excised, washed with cold saline solution and stored at-4°C until analyzed. Total lipids were extracted from the plasma as described by Folch *et al.* (1951).

Analysis of lipids and lipoproteins: Lipid fractions and individual lipids were obtained from the total lipid extract and analyzed as previously described (Amenta, 1964; Renkonen *et al.*, 1972; Prout *et al.*, 1973; Odutuga, 1982). Plasma triacylglycerols, total VLDL and HDL were measured as previously described (Odamaki *et al.*, 1999; Odutuga *et al.*, 2007).

Determination of tissue peroxidation: The reduced glutathione was determined by the method of Beutler and Kelly (1963). The technique involves protein precipitation by metaphosphoric acid and spectrophotometric assay at 412nm of the yellow derivative obtained by the reaction of the supernatant with 5-5[1]-dithiobis-2-nitrobenzoic acid. The extent of lipid peroxidation in tissues was assessed by measuring the level of malondialdehyde (MDA) in the various tissues (Wills, 1985).

Analyses of variance were carried out to determine the statistical significance of the results.

Results and Discussion

The results of this study indicate that the four dietary regimes supported the growth of the rats. Compared with other diets, the S-5 diet, however, supported growth most. Both S-5 and S-25 diets contained adequate amounts of EFAs for the normal development of rats which require approximately 40mg of EFAs per day (Alfin-Slater and Aftergood, 1968). The coconut oil diets, on the other hand, contained a marginal amount of linoleic acid (<1.0 calories %); the appearance of rats reared on this diet was similar to those of the soyabean oil-fed rats but the growth was retarded (Table 2). Animals maintained on the S-25 diet consumed one another's furs. This is probably due to the fact that the furs were oil-soaked and the soyabean oil was very palatable to the animals. It was the consumption of the furs that led to their low final body weight and consequently low weight gain per day as compared to rats fed the S-5 diets. Compared to the rats fed the S-5 diets, the final body weight of those fed the K-5 and K-25 diets were significantly reduced to 74.0 and 71.6 percent respectively ($p<0.001$).

The results of the lipid analyses (Table 2) indicate that triacylglycerols (TAGs) constituted 26.6, 26.6, 25.8 and 53.0 percent of the total neutral lipids in the plasma of the S-5, K-5, S-25 and K-25 dietary groups respectively. Compared to the other dietary groups, rats maintained on the K-25 dietary group had their plasma TAGs elevated three to six-fold. This may not be unconnected with the fact that the animals were consuming a high amount of saturated (i.e EFA-deficient) fat in their diet.

The results also indicate that plasma free cholesterol constituted approximately 29.0% of the total neutral lipid in all the dietary groups. Compared to the S-5 dietary group, the concentration of cholesterol, however, was higher in the plasma of the K-5 dietary group by a factor of 1.6. Similarly, cholesterol concentration was higher in the plasma of the K-25 dietary group groups by a factor of 1.47 when compared to the S-25 dietary group. These figures are statistically significant ($p<0.001$). Esterified cholesterol also showed the same pattern as cholesterol.

The results of the lipoprotein analysis are shown in Table 3. Compared to the rats fed the S-5 dietary group, the concentration of chylomicrons was reduced in the plasma of the K-5 dietary group by a factor of 0.51. Similarly, chylomicrons was reduced in the plasma of the K-25 dietary group by a factor of 0.20 when compared to the S-25 dietary group. Similar trends were obtained for VLDL and HDL of the plasma of the K-5 and K-25 dietary groups when compared to the S-5 and S-25 dietary groups respectively.

LDL levels, however, were significantly ($p<0.05$) increased in the plasma of the K-5 and K-25 dietary groups when compared to the S-5 and S-25 dietary groups respectively. Animals fed the S-25 diet also exhibited considerable reduction in their plasma chylomicrons, VLDL and HDL concentrations when compared with the S-5 dietary group. This is surprising in view of the fact that the animals were fed soyabean oil,

Table 2: Body Weights (g) and Plasma Lipids (mg/100ml) of Soyabean and Coconut Oils-fed Rats

Dietary Groups	Intial body wt. (g)	Final body wt. (g)	Wt. Gain per day (g)	Cholesterol (mg/100 ml)	Cholesterol ester (mg/100 ml)	Triacylglycerol (mg/100 ml)	Total neutral lipids (mg/100 ml)	Total phospholipids (mg/100 ml)
S-5	49.33±4.71	70.33±5.28	0.60±0.06	27.1±0.40	30.9±0.31	24.8±1.30	93.3±6.80	154.9±22.41
K-5	46.00±5.71	52.00±7.25	0.16±0.05	42.1±0.02	48.0±0.42	38.5±2.10	145.0±8.32	116.1±14.62
S-25	47.04±2.21	59.00±6.10	0.34±0.05	54.5+0.10	61.6±1.21	48.0±3.41	186.4±7.45	307.8±21.32
K-25	45.00±3.27	50.33±4.58	0.15±0.09	80.2±0.62	88.4±4.0	145.0±6.72	273.5±8.20	230.4±18.22

The results are the mean values for ten rats in each group±SD

Table 3: Plasma lipoproteins (mg dL^{-1})

Lipoproteins	S-5	S-25	K-5	K-25
Chylomicrons	165.62±7.11	121.36±10.81	80.81±9.31	96.77±8.48
VLDL	39.10±4.04	32.41±3.42	9.48±0.82	11.41±6.30
LDL	37.10±4.22	85.80±8.92	87.52±11.38	106.30±5.67
HDL	114.37±6.42	91.30±4.7	71.82±830	69.31±11.25

The results are the mean values for ten rats in each group±SEM. Significant difference ($p<0.05$)

Table 4: Reduced Glutathione Levels in Rat Tissues (mg/100g Wet Tissue)

Tissues	S-5	S-25	K-5	K-25
Brain	78.32±5.22	68.48±6.01	41.22±4.52	38.41±8.33
Liver	91.11±8.21	40.31±4.36	25.40±3.00	20.86±2.77
Lungs	63.47±7.22	42.00±1.48	22.16±1.48	14.62±3.82
Kidney	70.38±12.82	58.10±6.32	38.30±6.22	20.35±3.45

The results are the mean values for ten rats in each group ± SEM. Significant difference ($p<0.05$)

Table 5: Malondialdehyde Levels in Rat Tissues (mg/100g of wet tissue)

Tissue	S-5	S-25	K-5	K-25
Brain	278±48.80	578±30.71	1284±61.37	1478±41.70
Liver	724±17.78	988±41.22	2616±89.52	3190±92.61
Lungs	510±12.39	482±11.60	1509±38.43	1578±38.40
Kidney	289±11.48	684±10.33	1588±21.81	1942±51.92

The results are the mean values for ten rats in each group±SEM. Significant difference ($p<0.05$)

which is the control oil. It may be, that excess oil was probably affecting the rate of synthesis and secretion of the lipoproteins.

What constitutes danger in the lipoproteins is the cholesterol content. Both cholesterol and its ester have been implicated in atherogenesis. Dietary cholesterol is first incorporated into chylomicrons which is composed mainly of triacylglycerols. The latter are hydrolyzed in the endothelial cells by lipoprotein lipase, but the cholesterol component remains with chylomicron remnants, which when released into circulation are rapidly cleared by the liver. Zilversmit (1973) has suggested that chylomicron cholesterol could be atherogenic; during lipolysis of triacylglycerols, some of the cholesterol contained in the chylomicron may be released and make its way into the sub-endothelial region of the arterial wall and thereby contribute to the development of atherosclerosis.

Hepatic cholesterol may be incorporated into Very Low Density Lipoproteins (VLDL) which are secreted into plasma. Results of the present study have shown that the plasma of rats fed the K-5 and K-25 diets had higher concentrations of cholesterol. This will indicate that saturated fats may cause increased secretion of cholesterol-rich lipoproteins. Steinberg and Olefsky (1987) have shown that after lipolysis, VLDL becomes remnants which are either taken up by the liver or degraded to Low Density Lipoproteins (LDL) which are the major cholesterol-carrying lipoproteins of plasma. It is possible that the elevated plasma cholesterol concentration of K-5 and K-25 fed rats observed in this study may be a result of raised hepatic concentrations of cholesterol leading to suppression of synthesis of apolipoproteins of LDL. This will raise LDL levels in plasma (Grundy, 1987).

The lipo-peroxidative effect of saturated fat was monitored by assessing the tissues' levels of reduced glutathione (Table 4) and (Table 5). In all the tissues investigated, there was significant reduction in the level of reduced glutathione ($p<0.05$) in saturated fat-fed animals. It is suggested that when rats were fed saturated fats, prostaglandins necessary for body physiological functions are not being adequately synthesized. This may lead to impaired enzyme function and affect the metabolism of glutathione.

The significant increase ($p<0.05$) in the concentration of MDA observed in the tissues of animals fed hydrogenated coconut oil diet is an indication of increased lipid peroxidation in those tissues. Lipid peroxidation is assessed by maximal rate of MDA formation (Gaudin, 1991; Cheild *et al.*, 1999). The deposition of MDA in tissues have various significance. In the brain, for instance, elevated MDA level has been proposed to be one of the major mechanisms of

secondary damage in traumatic brain injury (Weighand *et al*., 1999). MDA level in liver may also be used to investigate the oxidative damage of protein and lipoproteins which is a possible pathogenic mechanism for liver injury (Kojic *et al*., 1998).

This study has shown that consumption of hydrogenated coconut oil for a long time causes accumulation of low density lipoproteins in the blood. This may result in arteriosclerosis and myocardial infarction. It also causes the accumulation of free radicals, an important factor in pathology of most diseases.

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