Biokemistri Vol. 7, No. 2, December, 1997
Printed in Nigeria

0795-8080/97 $3.00 + 0.00
Klobex Academic Publishers

BKR 9757/7206

EFFECTS OF DIETARY SOYABEAN AND COCONUT OILS ON LIPID COMPOSITION OF THE BLOOD AND AORTA OF RATS

Adewale A. Odutuga, Oyelola B. Oloyede and Adedayo T. Folayan

Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

(Received September 22, 1997)

ABSTRACT: Male albino rats 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 diet 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 diet fed rats. Eicosatrienoic acid (C20:3) constituted one of the major fatty acids in the plasma and the aorta of the coconut oil diet 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. It is also considered to be a contributory factor to atherogenesis.

Key Words: Essential fatty acids; Coconut oil; Soyabean oil; Triacylglycerols; Cholesterol; Eicosanoids.

INTRODUCTION

Essential fatty acids (EFAs) are needed for normal growth and development. Tissue lipid composition is influenced by the type of fat in the diet (1,2). 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 prostaglandins and their metabolites. Membrane composition and permeability are altered in the extreme case of EFA deficiency (3).

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 (2). In the present study, therefore, the effect of diets containing various amounts of saturated fatty acids or EFAs on the lipid and fatty acid composition of the blood and aorta has been carried out. This will enable us assess the importance of dietary manipulation on them.

MATERIALS AND METHODS

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 sullpements 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 (4). There was no lipid left in the protein. The soyabean oil was adequate in EFAs while the coconut oil was inadequate 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^{o}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. After 5 weeks of feeding, the rats were fasted overnight and anaesthetised 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 (5). Aortas were also excised, washed with cold saline solution and stored at -4^{o}C until analysed. Total lipids were extracted from the plasma and the aorta as described by Folch *et al*. (5).

Lipid fractions and individual lipids were obtained from the total lipid extract and analysed as previously described (5-9). Fatty acids were obtained from the lipids, methylated and separated on a gas-liquid chromatograph as described earlier (9,10). 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 (11). 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 diet, the final body weight of those fed the K-5 and K-25 diets were significantly reduced to 74.0 and 71.6 per cent respectively ($P < 0.001$).

The results of the lipid analyses (Table 2) indicate that triacylglycerols (TAG) constituted 26.6, 26.6, 25.8 and 53.0 per cent 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 by a factor of 1.47 when compared to the S-25 dietary group.

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 (4). 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).

These figures are statistically significant ($P < 0.001$). Esterified cholesterol also showed the same pattern as cholesterol.

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 hydrolysed 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 (12) 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 (13) 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 suppresion of synthesis of apolipoproteins of LDL. This will raise LDL levels in plasma (14).

Table 2: Body weights (g) and plasma lipids (mg/100 ml) of soyabean and coconut oils-fed rats.

Dietary Groups	Initial body wt. (g)	Final body wt. (g)	Wt. gain per day (g)	Cholesterol (mg/100 ml)	Cholesterol ester (mg/100 ml)	Triacyl-glycerol (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: Fatty acid composition of lipids of rat plasma.

Fatty acid	S-5			K-5		
	Total lipid	CE	TPL	Total lipid	CE	TPL
14:0	1.2	0.6	2.1	1.3	-	4.1
16:0	16.0	17.0	23.1	23.3	6.0	15.4
18:0	5.6	1.4	21.3	9.4	0.8	26.9
Total saturated	22.8	19.0	46.5	34.0	6.8	46.4
16:1 (n-7)	1.6	9.8	1.0	11.1	15.1	1.9
18:1 (n-9)	19.2	30.5	13.7	33.9	33.5	23.1
18:2 (n-6)	36.4	15.3	16.3	2.8	3.4	1.9
20:3 (n-9)	0.4	-	1.9	17.6	40.3	25.9
20:4 (n-6)	15.6	22.1	12.8	0.6	0.9	0.8
22:5 (n-6)	0.6	0.6	2.1	-	-	-
Total (n-6) PUFAs > C18	16.2	22.7	14.9	0.6	0.9	0.8
18:3 (n-3)	2.2	1.0	2.8			
20:5 (n-3)	0.6	1.1	0.9	-	-	-
22:6 (n-3)	0.5	0.6	2.0			
Total (n-3) PUFAs > C18	1.1	1.7	2.9	-	-	-

The values are expressed as the percentage by weight of total fatty acids and are the means of five analyses in each case variation ± 2.5% or less of the means value. Fatty acids constituting less than 0.2% are omitted.

Table 4: Fatty acid composition of lipids of rat plasma.

Fatty acid	S-25			K-25		
	Total lipid	CE	TPL	Total lipid	CE	TPL
14:0	1.0	-	3.0	2.1	-	2.0
16:0	13.0	15.2	15.6	24.6	8.3	28.8
16:1	2.4	12.3	2.4	2.1	10.6	10.2
18:0	5.0	16.1	12.3	5.4	7.7	6.1
Total saturated	21.4	43.6	33.3	34.2	26.6	47.1
16:1 (n-7)	2.2	2.1	1.6	1.0	0.8	0.8
18:1 (n-9)	14.2	32.7	11.2	32.2	32.7	24.0
18:2 (n-6)	37.9	9.8	18.2	-	-	-
20:3 (n-9)	1.4	0.9	1.3	32.2	39.9	28.1
20:4 (n-6)	18.4	10.9	19.3	-	-	-
22:5 (n-6)	0.9	-	-	-	-	-
Total (n-6) PUFAs > C18	19.3	-	-	-	-	-
18:3 (n-3)	1.3	-	2.2	-	-	-
20:5 (n-3)	0.9	0.2	1.2	-	-	-
22:6 (n-3)	1.4	0.7	1.2	-	-	-
Total (n-3) PUFAs > C18	2.3	0.9	2.4	-	-	-

The values are expressed as the percentage by weight of total fatty acids and are the means of five analyses in each case variation ± 2.5% or less of the means value. Fatty acids constituting less than 0.2% are omitted.

Table 5: Fatty acid composition of lipids of rat aorta.

Fatty acid	S-5	S-25	K-5	K-25
14:0	-	-	-	-
16:0 (n-7)	13.7	10.0	20.7	18.3
18:0	24.8	25.3	24.3	19.8
Total saturated	38.5	35.3	45.0	38.1
16:1 (n-7)	1.4	1.0	27.8	31.6
18:1 (n-9)	18.3	17.4	-	-
18:2 (n-6)	3.5	4.2	-	-
20:3 (n-9)	4.4	3.8	24.6	29.9
20:4 (n-6)	21.4	26.2	-	-
22:5 (n-6)	3.7	3.9		
Total (n-6) PUFAs > C18	25.1	30.1	-	-
18:3 (n-3)	2.9	2.1		
20:5 (n-3)	4.1	3.9		
22:6 (n-3)	1.4	2.2		
Total (n-3) PUFAs > C18	5.5	6.1		

The values are expressed as the percentage by weight of total fatty acids and are the means of five analyses in each case variation ± 2.5% or less of the means value. Fatty acids constituting less than 0.2% are omitted.

Palmitic, oleic, linoleic and arachidonic acids are the major fatty acids in the plasma lipids of S-5 dietary rats. Stearic acid is also a major component of the phospholipids (Table 4). In the plasma of the K-5 fed rats palmitic, palmitoleic, oleic and eicosatrienoic acid of n-9 series constitute the major fatty acids of the total lipid and the total phospholipid; palmitoleic acid, however, is not a major fatty acid in phospholipids. The major fatty acids in the plasma cholesterol esters of this group of animals are palmitoleic, oleic and eicosatrienoic acid; the latter constituting about 40% of the total fatty acids. This high percentage of eicosatrienoic acid observed in the present study is surprising in view of the fact that oleic acid constituted only 0.4% of the total fatty acid of the K-5 dietary lipid. This fatty acids also constitutes a major percentage of the total fatty acids of aortic lipids of K-5 oil-fed rats (Table 5). It is speculated, therefore, that this fatty acid is probably being specifically incorporated into the lipids (especially cholesterol esters) of rats fed the coconut (or saturated fat) diet.

The fatty acid composition of the aortic lipids of rats fed the S-5 oil-fed rats contained a cosiderable amout (21.4 - 26.2%) of arachidonic acid; and fatty acids of the n-3 series. These fatty acids are lacking in or at the most present in trace amounts on the aortic and plasma lipids of K-5 oil-fed rats. The results of this study has demonstrated that rats fed the coconut oil diet had altered lipid and fatty acid composition. This altered lipid and fatty acid composition is considered to be a direct result of feeding saturated fat (as depicted by the coconut oil). The consumption of this fat has therefore affected lipid metabolism and altered the structure and function of enzymes

responsible for converting the EFAs to prostaglandins or their endoperoxide precursors (4).

The altered lipid composition of the aorta when rats were fed the saturated fat is considered to be related to the process of formation of the atherosclerotic lesion. It has been reported that lipids are modified in the atherosclerotic plague (15). Therefore, in rats, saturated fats consumed for a prolonged period of time is considered to be a contributory factor for atherogenesis.

ACKNOWLEDGEMENTS: We wish to thank the University of Ilorin for the Senate Research Grant to carry out this work. We express gratitude to Mr. S. A. Adediran and Mr. G. I. Odama for their help in carrying out some analyses.

REFERENCES

1. Key, A.; Anderson, J. T. and Grande, F. (1965) Serum cholesterol response to changes in the diet. II. The effect of cholesterol in the diet. Metabolism 14, 759.
2. Steinberg, D. (1987) Current Theories of the Pathogenesis of Atherosclerosis. In: Contemporary Issues in Endocrinology and Metabolism: Hypercholesterolemia and Atherosclerosis - Pathogenesis and Prevention. D. Steinberg and J. H. Olefsky eds. Churchill Livinstone, New York.
3. Odutuga, A. A. (1979) Long-term deficiency of essential fatty acids in rats and its effect on brain recovery. Clin. Exp. Pharm. Physiol. 6, 361 - 366.
4. Odutuga, A. A. (1982) Effects of low-zinc status and essential fatty acid deficiency on growth and lipid composition of rat brain. Clin. and Exp. Pharm. 9, 213 - 221.
5. Folch, J.; Lees, M. and Sloane-Stanley, G. H. (1951) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497 - 509.
6. Amenta, J. S. (1964) A rapid chemical method for quantification of lipids separated by thin-layer chromatography. J. Lipid Res. 5, 270 - 272.
7. Renkonen, O.; Gammberg, C. G.; Simons, K. and Kaariamen, L. (1972) The lipids of the plasma membranes and endoplasmic reticulum from cultured baby hamster kidney cells (BH 21). Biochim. Biophys. Acta 225, 66 - 78.
8. Prout, R.E.S.; Odutuga, A. A. and Tring, F. C. (1973) Lipid analysis of rat enamel and dentine. Arch. Oral. Biol. 18, 373 - 380.
9. Odutuga, A. A. (1977) Recovery of brain from deficiency of essential fatty acids in rats. Biochim. Biophys. Acta 487, 1 - 9.
10. Odutuga, A. A. and Prout, R.E.S. (1973) Fatty acid composition of neutral lipids and phospholipids of enamel and dentine from rat incisors and molars. Arch. Oral Biol. 19, 689 - 697.
11. Alfin-Slater, R. B. and Aftergood, L. (1968) Essential fatty acids re-investigated. Physiol. Rev. 48, 758 - 784.
12. Zilversmit, D. B. (1973) A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. Circ. Res. 33, 633.
13. Steinberg, D. and Olefsky, J. H. (1987) In: Contemporary Issues in Endocrinology and Metabolism - Hypercholesterolemia and atherosclerosis (Pathogenesis and Prevention). D. Steinberg and J. M. Olefsky, eds. Churchill Livingstone, New York.
14. Grundy, S. M. (1987) Dietary Treatment of Hyperlipidemia. In: Contemporary Issues in Endocrinology and Metabolism - Hypercholesterolemia and atherosclerosis (Pathogenesis and Prevention), pp. 169 - 193. D. Steinberg and J. M. Olefsky, eds. Churchill Livingstone, New York.
15. Palinski, W. (1993) Lipoprotein oxidation: Mechanisms and implication for atherogenesis. New horizons in coronary heart disease. Science Press, London.