Original Article

Comparative Contributory Modulatory Effects of Selected Saturated and Unsaturated Fatty Acids in Lipids Metabolism: An Experimental Animal Model

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ABSTRACT

Objective: Supplementation of food materials with selected micro- and macro-nutrients is a form of dietary intervention which is effective in improving well-being in human population by preventing, mitigating or reducing the risk factors of certain ailments. This study evaluated contributory effects of linoleic, oleic, palmitic and isopalmitic acids on selected lipids in serum, liver, kidney and small intestine in male Wistar rats.

Methods: The rats were given the diets for 60 days after which the animals were sacrificed. Blood and organs such as liver, kidney and small intestine were collected from the rats. Serum and tissue concentrations of total cholesterol, triglyceride, HDL-C and free cholesterol were determined spectrophotometrically while VLDL, LDL-C, atherogenic index and body weight gain were calculated.

Results: Oleic acid caused highest increase in the serum concentrations of HDL-C and linoleic acid resulted in an increase in LCAT activity. Isopalmitic acid caused highest increase in serum concentration of total cholesterol (TC) while palmitic acid caused highest increase in serum concentrations of triglyceride (TG), LDL-C and VLDL. Estimating atherogenic index by TC: HDL-C ratio showed that oleic acid demonstrated least atherogenicity. In small intestine, the levels of TC and TG were least in rats given oleic acid supplemented feed. In kidney, the levels of TC and TG were least in rats given palmitic acid. In liver, the level of TC was least in rats fed palmitic acid supplemented feed while TG was least in rats fed isopalmitic acid supplemented feed.

Conclusion: The study demonstrated that fatty acid supplemented diet may possess modulatory effect on lipid metabolism with

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atherogenic implications.

Keywords: Fatty acids, Lipids, Diet, Supplementation.

INTRODUCTION

In recent years there has been a greater demand for foods with increased levels of functional fatty acids, such as long-chain omega-3 fatty acids and conjugated linoleic acids, because of their biological roles in cells[1].For these reasons, the advisory panel of the World Review of Nutrition and Dietetics urged producers to improve the lipid profile of foods of animal origin through optimal feeding systems, as animal foods are still a major source of lipid for humans[1]. These health professionals stressed the importance of increasing the functional lipid components in meat and egg products, while reducing the levels of saturated and *trans* fatty acids and cholesterol[1].

Hyperlipidemia is a pathological condition, mostly attributed to a marked increase in plasma lipids, particularly cholesterol and triglycerides[2]. Strong association exists between hyperlipidemia and cardiovascular diseases. Cardiovascular diseases, particularly coronary artery disease, have become a growing problem, the world Health Organization predicts that by the year 2020, up to 40% of all deaths will be related to cardiovascular disease and associated hyperlipidemia[3]. Several lines of evidences have shown that the improvements in the incidence of coronary artery disease are associated with lowering hypercholesterolemia[4]. Some of the interventions prescribed for reducing hyperlipidemia include diet control and use of anti-hyperlipidemic drugs. Reports of adverse effects and inability of some patients to tolerate some of these drugs led to search for safer and probably more effective alternatives. Many epidemiological studies have indicated that diet with high plant contents can protect people from various serious diseases such as cancer, diabetes mellitus and cardiovascular disease[5]. This study therefore determined and compared the modulatory potentials of diets supplemented separately with fatty acids (linoleic, oleic, palmitic and isopalmitic acids) on serum and tissue concentrations of lipids and lipoproteins.

EXPERIMENTAL DESIGN

Animals

Healthy adult male Wistar rats of average weight 190g were used for the study. The animals were housed in wire gauge cages in a well-lighted and adequately ventilated room, under standard environmental conditions (12 h light and 12 h dark cycle). They were allowed to acclimatize while being fed with standard laboratory animal chow and water *ad libtum* for 2 weeks.

Experimental groups

The twenty five rats were divided into five groups of 5 animals each and fed basal diet (Table 1) supplemented with or without dietary fatty acid for 60 days with free access to water. Group I served as control, fed with basal, non-supplemented diet. Groups II, III, IV and V were fed with basal diet supplemented with dietary fatty acids of linoleic acid (1 g/1.2 kg diet), Oleic acid (1 g/1.2 kg diet), palmitic acid (1 g/1.2 kg diet), and Isopalmitic acid (1 g/1.2 kg diet) respectively. (See table 1.)

After 60 days, the animals were anesthetized under light ether and sacrificed after an overnight fast. Blood was collected through cardiac puncture and the liver, kidney and small intestine harvested. Serum was separated after centrifugation and the organs were frozen until used for analyses.

Biochemical analyses

The serum total cholesterol, triglyceride and HDL-cholesterol were estimated using commercial kits. Serum VLDL and LDL were calculated according to Friedewald's formulae: LDL = TC-HDL-VLDL where VLDL = TG/5[6].

Atherogenic index was calculated as: AI = LDL + VLDL/HDL. The HDL/total cholesterol ratio was also calculated.

LCAT activity was measured according to modified method of[7].

Lipid was extracted from the tissues according to[8] while cholesterol and triglyceride estimated in them according to the method of[9,10] respectively.

Statistical analysis

Data were analyzed using one-way ANOVA, expressed as mean ± SEM followed by *post hoc* Tukey's test. The level of significance was set at $p < 0.05$.

RESULTS

Serum lipid profile, as well as, the LCAT activity of rats fed fatty acid supplemented feeds (FASD), are depicted in Table 3. The serum cholesterol and triglyceride levels were significantly increased ($p<0.05$) in all the groups fed fatty acid supplemented feeds (FASD), except in oleic acid and palmitic acid groups where a significant reduction in total cholesterol and non-significant increase in triglyceride were observed respectively. A significant increase in HDL-C concentration and a drastic reduction in LDL-C level were observed in the animals fed oleic acid supplemented feed. Though isopalmitic acid caused an increase in HDL-C level, its supplementation led to marked increase in LDL-C, likewise, palmitic acid in its effect on LDL-C. All the fatty acid supplemented diets, except isopalmitic acid, induced significant ($p<0.05$) increase in VLDL concentrations in the rats. The same trend was repeated with LCAT activity of the rats, with the highest stimulation observed with linoleic acid supplemented diet. HDL/TC ratio was unchanged in rats fed with linoleic and oleic acids supplemented diets whereas, palmitic acid and isopalmitic acid diets lowered the ratio. The atherogenic index was lowered by isopalmitic acid supplemented diet, while it was increased by palmitic acid diet.

Table 4 represented the levels of lipids in the liver, kidney and small intestine of rats fed on FASD. Linoleic acid supplement caused a perturbation in the lipid compositions of all the tissues investigated. It caused significant increase ($p<0.05$) in cholesterol levels in the three organs and in the triglyceride contents of the kidney and the small intestine, but resulted in a significant reduction in liver triglyceride. Oleic acid supplemented diet increased total cholesterol in the liver while significantly reducing same in both the kidney and the small intestine. Triglyceride on the other hand, was reduced by the diet in the liver but elevated in the kidney. Palmitic acid diet markedly cholesterol levels in the kidney and small intestine while increasing and decreasing triglyceride contents in the small intestine and kidney of the rats respectively. Isopalmitic acid supplemented diet, on the other hand, could only increase hepatic cholesterol and triglyceride contents, while leaving the other organ unaffected. (See table 2-4.)

DISCUSSION

Although dietary fat is known to affect serum concentrations of triglyceride, total and lipoprotein cholesterol, however, all components of fatty acids – saturated, monounsaturated and polyunsaturated fatty acids do not have identical effects on serum cholesterol levels[11]. The observation of Scott, was confirmed by the findings of this study. Here, linoleic acid (PUFA) supplemented diet caused highest reduction in the serum concentrations of total cholesterol, and low density lipoprotein cholesterol. In contrast, the saturated fatty acid (Palmitic acid) supplemented diet caused sharp elevation of serum concentrations of both total cholesterol (%) and low density lipoprotein cholesterol (%). Over the years, attention has been mainly on the saturated and polyunsaturated fatty acids constituents of diet. Saturated fatty acids have been considered to raise cholesterol levels while polyunsaturated fatty acids lower them. This is in agreement with our observation. Diets enrich in polyunsaturated fatty acids are considered to be beneficial because of their hypocholesterolemic effects[12]. Numerous studies have shown that the substitution of dietary saturated fatty acids for monounsaturated (MUFA) or polyunsaturated (PUFA) reduced low density lipoprotein cholesterol concentrations in blood plasma[13].

An important observation in this study is that all fatty acid supplemented diets except that of linoleic acid raised serum cholesterol concentration when compared with group given basal diet (control). The increase is more pronounced in groups given palmitic and isopalmitic acids supplemented diets. This suggested that linoleic acid contained in the diet reduced the cholesterol concentration, hence exhibiting anti-hypercholesterolemic property. The polyunsaturated fatty acid of n-6 series has beneficial effects on plasma lipids. PUFAs of the n-6 series have been reported to be more effective in reducing cholesterol[14]. Linoleic acid is an essential fatty acid ingested at the rate of approximately 10g per day, and is the most abundant fatty acid in western diets. It is present in many vegetable oils and is the precursor of arachidonic acid. Some beneficial effects of PUFAs, especially linoleic acid, are related to the reduction of blood pressure[15], platelet aggregability and blood viscosity[16,17]. The health significance of linoleic acid is very important, hence, its deficiencies have been linked to decreased memory and mental abilities, tingling sensation of the nerves, poor vision, increased tendency to form blood clots, diminished immune function and increased LDC levels, hypertension etc. In addition, oleic acid had been reported to lower heart attack risk and atherosclerosis.

The antiatherogenic potential benefit of unsaturated fatty acids in this study was demonstrated by lowered free cholesterol and reduced atherogenic index observed in groups given linoleic and oleic acids supplemented diets respectively, (table 3). Linoleic acid caused most reduction in the serum concentration of free cholesterol. The rate of disappearance of serum free cholesterol has been used as an index of activity of lecithin cholesterol acyl transferase, an enzyme which plays important role in reverse cholesterol transport. Lecithin: cholesterol acyl transferase catalyses conversion of free cholesterol (which is more dangerous) to a neutral esterified cholesterol. This is very protective as it is antiatherogenic in nature. Sustained excessive serum concentration of total cholesterol (hypercholesterolemia) has been linked with different lipid based disorders such as coronary heart diseases (CHD). Considerable cardiovascular disease researches focus on the cardioprotective effects of fish oils and of individual n-3 PUFA[18,19]. Studies have shown that regular consumption of (n-3) PUFA can protect against the debilitating effects of myocardial ischemia[20]. Because PUFA such as linoleic acid cannot be synthesized in the human body, they must be obtained through diet; hence, they are termed essential fatty acids. Their major sources include fish oils (eicosapentaenoic acid and docosahezaenoic acid) and some plant oils such as canola etc.

In this study, consumption of linoleic acid (PUFA) and oleic acid (MUFA) resulted in elevated serum concentration of HDL-C. This may further support the observed increased activity of lecithin: cholesterol acyl transferase (LCAT). Conversion of free cholesterol to esterified cholesterol occurs in HDL particle and this is catalysed by LCAT and this leads to matured HDL. HDL is very important in the cholesterol transport, a process in which extra hepatic or peripheral cholesterol is transported to the liver for excretion in form of bile acids or in unchanged form in bile, thus preventing excessive accumulation of free cholesterol. Numerous intervention studies have shown that substitution of dietary saturated fatty acid by poly unsaturated fatty acids (PUFA) in diet has hypocholesterolemic effects, decrease the levels of total cholesterol, LDL-C and increase HDL-C. Studies also reported MUFA causing elevated total cholesterol and LDL-C[21].

In this study consumption of palmitic acid (saturated fatty acid) supplemented diet caused elevated serum concentrations of triglyceride, total cholesterol and LDL-C in the rats. One of the risk factors for coronary heart disease is elevated total studies cholesterol, low density lipoprotein-cholesterol and triglyceride. Epidemiological studies have shown that diets rich in saturated fatty acids seem to increase the risk of coronary heart disease by increasing cholesterol and LDL-C[22]. Coronary heart disease has become unarguably the major cause of death in the developed countries and is one of the leading causes of disease burden in developing countries as well. Data from this study showed that linoleic and oleic acids caused elevated amounts of hepatic total cholesterol. Similar findings were reported by[23].

The serum lipid lowering effect of linoleic acid may be associated with series of possiblities which may include increase in whole body cholesterol[24], turnover due to increased biliary excretion or repartitioning of whole body cholesterol as evidenced by increased hepatic and kidney cholesterol concentrations in all the linoleic acid supplemented groups compared with other groups (Table 4); and increased catabolism of fatty acids in tissues as evidenced by significantly reduced triglyceride concentration in liver of rats fed with diet supplemented with linoleic acids (table 7).

CONCLUSION

The study demonstrated that fatty acid supplemented diet may possess modulatory effect on lipid metabolism with atherogenic implications.

ACKNOWLEDGEMENT

We acknowledge the support of colleagues at the Departments of Chemical Pathology and Biochemistry, Ladoke Akintola University of Technology, Nigeria.

Conflict of interest

There is no conflict of interest and the study was self funded.

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Table 1. Nutritional composition of the basal diet

Dietary composition	Percentage (%)
Maize	1.5
Soya	4
Wheat	1
Bone	0.5
Shell	0.5
Feed concentrate	1
Salt	0.03
Lysine	0.02
Methionine	0.02
Groundnut cake	5

Table 2. Food consumption data and intake of fatty acid

Fatty Acid Supplement	Food consumption (g/rat/day)	Mean daily intake of fatty acid supplement (mg/kg BW/day)[1]	Total intake of Supplement (g/kg BW)[2]
Control	23.54 (112.63)[3]	0	0
Linoleic acid	23.20 (111.01)	92.51	5.55
Oleic acid	22.66 (110.54)	92.12	5.53
Palmitic acid	22.86 (111.51)	92.93	5.58
Isopalmitic acid	23.09 (109.43)	91.19	5.47

[1]Values were calculated by multiplying the food consumption (g/kg BW/day) by the dietary level of fatty acid supplement (g/kg diet).
[2]Values are sums of fatty acid supplement consumed during the experimental period calculated by multiplying the mean daily intake of supplement (g/kg BW/day) by 60 (days).
[3]Values in brackets are food consumed calculated as g/kg BW/day.

Table 3. Effect of fatty acid supplements on serum lipid profile and LCAT activity in rats

Fatty Acid Supplement	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-cholesterol (mg/dl)	HDL/total cholesterol ratio	LDL-cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)	AI	LCAT activity
Control	58.4 ± 4.3[a]	39.6 ± 2.1[a]	37.4 ± 3.7[a]	0.64	13.1 ± 3.5[a]	7.9 ± 0.4[a]	1.56	0.60 ± 0.04[a]
Linoleic acid	48.4 ± 3.5[b]	52.2 ± 6.4[b]	33.2 ± 1.6[a]	0.69	4.7 ± 1.2[c]	10.4 ± 1.3[a]	1.46	6.10 ± 0.22[b]
Oleic acid	79.2 ± 8.0[c]	67.4 ± 5.9[c]	54.8 ± 7.4[b]	0.69	10.9 ± 3.5[b]	13.5 ± 1.2[b]	1.45	5.40 ± 0.34[b]
Palmitic acid	84.8 ± 6.9[c]	67.8 ± 13.7[c]	39.6 ± 4.1[a]	0.47	31.6 ± 4.7[d]	13.6 ±2.7[b]	2.14	1.90 ± 0.01[c]
Isopalmitic acid	86.4 ± 9.9[c]	42.6 ± 7.0[a]	50.8 ± 15.6[b]	0.59	27.1 ± 6.8[d]	8.5 ± 0.6[a]	1.70	0.30 ± 0.01[d]

Results expressed as mean ± SEM (n = 5). The significant difference between the groups was analyzed by one-way analysis of variance (ANOVA). Mean values with different superscript roman letters are significantly different ($P < 0.05$) as determined by Tukey's test.

Table 4. Effect of fatty acid supplements on tissue lipids in rats

	Liver		Kidney		Small intestine	
Fatty Acid Supplement	Total cholesterol (mg/g)	Triglyceride (mg/g)	Total cholesterol (mg/g)	Triglyceride (mg/g)	Total cholesterol (mg/g)	Triglyceride (mg/g)
Control	6.78 ± 1.55[a]	9.14 ± 1.67[a]	3.15 ± 0.64[a]	4.97 ± 1.27[a]	3.73 ± 0.95[a]	12.07 ± 1.24[a]
Linoleic acid	11.30 ± 1.42[b]	7.42 ± 1.01[b]	4.58 ± 0.53[b]	7.65 ± 1.02[b]	4.14 ± 0.76[b]	15.36 ± 0.76[b]
Oleic acid	14.42 ± 2.68[b]	7.98 ± 0.97[b]	4.69 ± 0.49[b]	7.56 ± 1.04[b]	1.94 ± 0.60[c]	11.98 ± 0.60[a]
Palmitic acid	6.68 ± 1.45[a]	8.06 ± 1.07[a]	2.62 ± 0.23[c]	3.22 ± 0.91[c]	2.25 ± 0.70[d]	14.18 ± 0.70[b]
Isopalmitic acid	18.06 ± 4.01[c]	17.64 ± 2.77[c]	3.15 ± 0.82[a]	4.54 ± 1.16[a]	3.32 ± 1.87[a]	12.68 ± 1.87[a]

Results expressed as mean ± SEM (n = 5). The significant difference between the groups was analyzed by one-way analysis of variance (ANOVA). Mean values with different superscript roman letters are significantly different ($P < 0.05$) as determined by Tukey's test.