Effects of Palm Oil Supplementation on Lipid Peroxidation and Glutathione Peroxidase Activity in Cholesterol – Fed Rats

O Oluba, C Onyeneke, G Ojieh, G Eidangbe, R Orole

Citation

O Oluba, C Onyeneke, G Ojieh, G Eidangbe, R Orole. *Effects of Palm Oil Supplementation on Lipid Peroxidation and Glutathione Peroxidase Activity in Cholesterol – Fed Rats*. The Internet Journal of Cardiovascular Research. 2008 Volume 6 Number 1.

Abstract

The effects of palm oil supplementation (5%) to a cholesterol-based (5%) diet on lipid peroxidation, and on glutathione peroxidase activity in rat's liver were examined. The rate of lipid peroxidation in the liver was determined in vitro by the thiobarbituric acid (TBA) test, and by conjugated diene measurement. Palm oil supplementation significantly reduced ($p<0.05$) the rate of lipid peroxidation in the liver of rats fed 5% cholesterol, compared to the rate observed in the liver of rats fed 5% cholesterol diet without palm oil. On the other hand glutathione peroxidase activity was significantly increased ($p<0.05$) in the liver of rats fed palm oil supplemented diet compared with those fed cholesterol diet without palm oil. The study suggests that palm oil feeding could be a veritable nutritional tool in the prevention of lipid-induced oxidative damage as usually observed in atheromatous plaque.

INTRODUCTION

Cardiovascular disease (CVD), is the leading cause of death in the world. Elevated concentrations of serum total cholesterol (TC), and LDL-cholesterol (LDL-C), have proved to be among the risk factors in the development of CVD ([1]). Dietary fats play an important role in influencing blood lipid concentrations, thrombotic tendency and thus the onset of CVD ([2]). It is generally believed that diets high in cholesterol increase serum TC and LDL-C and in return increases the risk of cardiovascular disease.

Cholesterol feeding has often been used to elevate serum cholesterol levels in studying the etiology of hypercholesterolemic-related metabolic disturbances such as atherosclerosis. The metabolic alterations associated with cholesterol feeding have received increasing attention in recent years. Cholesterol feeding has recently been observed to increase the activity of some enzymes involved in lipid metabolism. These enzymes include triglyceride lipase (TGL), lipoprotein lipase (LPL), and lecithin: cholesterol acyl transferase (LCAT) which together plays a crucial role in the metabolism of HDL-cholesterol ([3]). Cholesterol inclusion in the diet has also been reported to decrease the circulating concentration of insulin and plasma reduced glutathione levels ([4]).

It is conceivable that these metabolic alterations may play significant roles in the development of hypercholsterolemic-related metabolic disturbances. Feeding of cholesterol to rats results in a rapid hepatic infiltration of lipids rich in triglycerides and cholesterol. Much of this accumulated cholesterol are usually in the esterified form ([5]) and thus highly susceptible to peroxidation. The elevated levels of liver cholesterol and cholesteryl esters presumably could increase the susceptibility of the tissue to lipid peroxidation unless proper amounts of antioxidant are present in the tissues.

Palm oil represents the second largest volume of vegetable oil produced in the world. It is highly saturated and contains nearly 50% palmitic acid. Thus, Keys et al. ([6]) considered palm oil a hypercholesterolemic oil. But this extrapolation of the Keys-Anderson equation about palm oil was not based on actual experimental studies. Studies with animals and humans have indicated that palm oil is quite different from other hypercholesterolemic fats such as lard or coconut oil ([7,8,9]). Thus well-controlled studies are required to investigate the effects of palm oil and its relation to cardiovascular disease.

Although palm oil is the major vegetable oil consumed in

Nigeria, information about the relation of palm oil to health is limited. A few papers have shown that palm oil could maintain the normal growth of rats and cause a more significant reduction of serum cholesterol in rats compared with soybean oil ([10]). Therefore, it is very important to observe the effect palm oil inclusion as a supplement to a cholesterol-based diet on lipid peroxidation and on the activity of glutathione peroxidase, a physiologically important lipid peroxide-decomposing enzyme, in rats.

MATERIALS AND METHODS

Rat feed: Basal diet of rat chow was purchased from Guinea Feeds Ltd. (Nigeria).

Chemicals: All chemicals used were of analytical grade and were products of British Drug House Chemicals Ltd, Poole, England unless otherwise stated.

Palm oil: The palm oil used for feed formulation was purchased from the Okitipupa Oil Palm Mill Ltd, Ondo State, Nigeria.

Animals and Diets: Male albino Wistar rats (n = 24) of average weight 122.3±7.7 g obtained from Nigerian Institute of Medical Research, Lagos (Nigeria), were used for the study. The animals were housed individually in stainless steel cages with raised wire floor in a room with a 12 hour light/dark cycle and 50–60 % relative humidity at a temperature of about 300C. The animals had free access to food and tap water and were treated according to the Nigerian guidelines for the care and use of laboratory animals. The rats were acclimatized to the facility for 2 weeks before the start of the experiments. They were then assigned to four groups of six animals each designated: control; palm oil only; cholesterol only; and cholesterol + palm oil and placed on their respective diet for a period of six weeks. The composition of each diet is as shown in Table 1. Before the commencement of the feeding experiment, rats were fasted overnight but allowed access to water ad libitum. Three rats from each group were sacrificed and blood and liver samples collected to determine the entry (baseline) levels of the test parameters. The rats had free access to their diet and were weighed weekly.

Lipid peroxidation: Lipid peroxidation of the liver homogenate was determined in vitro by the thiobarbituric acid (TBA) method of Wills ([11]) with little modifications. Immediately after the animals were sacrificed (by cardiac puncture), the livers were quickly removed and weighed; approximately 1 g of each of the livers was homogenized in 9 ml of 75 mM potassium phosphate buffer, pH 7.0. One-half of 1 ml of the homogenate was added to a 25 ml incubation flask containing 3.5 ml of the same buffer. After mixing, 0.5 ml of this incubation mixture was delivered to a test tube containing 2.5 ml of 8% trichloroacetic acid for TBA test ([11]). Another 0.5 ml was removed for the determination of conjugated diene levels according to the method of Placer ([12]). The remaining incubation mixture was then incubated under air at 370C for 2 hours, and the same sampling procedures were repeated. For the TBA test, 2 ml of 0.67% TBA solution was added to each tube and boiled for 15 minutes. After centrifugation, the colour intensity was determined at 532 nm. TBA-reacting compound was expressed as micrograms malonaldehyde (MA) per gram of liver. Tubes containing known levels of MA were treated similarly and used as standards. The level of conjugated diene was expressed as 1X102 nmole per gram of liver based on an extinction coefficient of 2.2X104 ([13]).

ENZYME ASSAY

The remaining liver was then centrifuged under cold (40C) at 105,000 X g for 45 minutes. The supernatant fraction was then carefully removed for enzyme assays. Glutathione peroxidase (EC 1.11.1.9) activity was determined according to the method of Little et al. ([14]) with cumene hydroperoxide as substrate.

Statistical analysis: Statistical analysis was carried out using one way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). $P<0.05$ was considered to be significant.

Figure 1

Table 1: Diet Composition (% by weight)

Feed composition	Control	Palm oil only	Cholesterol only	Cholesterol + Palm oil
Maize flour	70	65	65	60
Fish meal	10	10	10	10
Ground nut cake	20	20	20	20
Cholesterol	-	5	-	5
Palm oil	-	-	5	5

RESULTS

The results obtained in this study are presented in Table 2. Despite the differences in feed composition of the different diets fed the respective groups no significant difference ($p>0.05$) was observed in the body weight among rats in the different groups. Similarly, the average daily food

consumption was not significantly different between the groups. Liver size was significantly higher ($p>0.05$) in the group fed cholesterol diet only and cholesterol + palm oil group compared with the control group and palm oil diet only group. The highest liver size value was recorded in the group fed cholesterol diet only.

Liver homogenates from rats fed cholesterol diet only and those fed cholesterol + palm oil diet had significantly higher ($p<0.05$) rate of lipid peroxidation based both on TBA tests and on conjugated diene measurements than samples from rats fed palm oil diet only and control diet. The rate of lipid peroxidation was observed to be highest in the liver samples from rats fed cholesterol diet without palm oil.

Liver glutathione peroxidase activity was significantly lower ($p<0.05$) in both rats fed cholesterol diet only and cholesterol + palm oil diet compared with the control rats and rats fed palm oil only diet. The enzyme activity was observed to be significantly lower ($p<0.05$) in rats fed cholesterol only diet compared with those fed cholesterol + palm oil diet.

Figure 2

Table 2: Lipid peroxidation and glutathione peroxidase activity (at baseline and post diet intervention) in rats fed cholesterol and palm oil diet

	Control	Palm oil only	Cholesterol only	Cholesterol + palm oil
Initial body weight (g)	122.0± 6.3[a]	122.6±5.0[a]	121.8±3.8[a]	122.1±10.0[a]
Final body weight (g)	358.0±48.5[a]	360.5±34.2[a]	349.9±27.0[a]	355.4±43.3[a]
Average daily food intake (g)	15.8±1.2[a]	15.6±1.4[a]	15.5±1.0[a]	15.6±1.0[a]
Initial liver weight (g/100g body weight)	3.2±0.3[a]	3.3±0.1[a]	3.1±0.2[a]	3.3±0.2[a]
Final liver weight (g/100g body weight)	3.8±0.1[a]	3.8±0.2[a]	5.2±0.3[b]	4.1±0.3[ab]
Lipid peroxidation				
Baseline TBA values[1]: 0 hour	8.2±1.5[a]	8.4±1.3a	8.5±1.0[a]	8.2±1.3[a]
2 hours	12.7±1.2a	12.6±1.2[a]	12.9±1.3[a]	12.6±1.5[a]
Net change	4.5±0.6[a]	4.2±0.3[a]	4.4±0.9[a]	4.4±0.3[a]
Post diet TBA values[1]: 0 hour	16.6±4.3[a]	17.0±7.0[a]	33.7±5.0[b]	22.6±2.3c
2 hours	85.2±10.4[a]	88.3±5.6a	373.0±20.1[b]	249.2±14.5[c]
Net change	68.6±17.0[a]	74.3±11.6[a]	339.3±28.1[b]	226.6±30.6[c]
Baseline conjugated diene[2]: 0 hour	2.5±0.2[a]	2.3±0.1[a]	2.3±0.3[a]	2.4±0.1[a]
2 hours	2.8±0.1[a]	2.7±0.3[a]	2.8±0.3[a]	2.8±0.1[a]
Post diet conjugated diene[2]: 0 hour	7.2±1.0[a]	7.8±1.3[a]	34.8±7.9[b]	21.1±3.3[c]
2 hours	10.5±1.6[a]	11.0±2.3[a]	63.3±12.0[b]	56.5±7.8[c]
Glutathione peroxidase activity[3]				
Baseline	1.3±0.2[a]	1.3±0.1[a]	1.1±0.1[a]	1.3±0.1[a]
Post diet	6.1±1.3[a]	5.9±1.0[a]	3.0±1.1[b]	4.8±1.0[c]

Values are means ± SEM of triplicate determinations. Values in the same row carrying different superscripts are significant.

Note: [1] µg malonaldehyde/g liver, [2] 1 X 10^3 nmoles/g liver, µmoles NADPH consumed or formed/g liver/min.

DISCUSSION

In the present study, the concentration of TBA-reacting compounds, and the level of conjugated diene in the incubation of liver homogenates were assumed to represent the peroxidative activity of the livers. The observed effects of palm oil as a supplement to a cholesterol based-diet on lipid peroxidation, and on glutathione peroxidase activities in rat's liver were based on results expressed on a per gram liver basis.

The results generated in this study showed that the inclusion of palm oil as a supplement to a cholesterol-based diet markedly reduced lipid peroxidation in the liver, as opposed to the high rate of lipid peroxidation in the livers of rats fed cholesterol diet only (Table 2). These results suggest that

factors related to cholesterol feeding such as accumulation of cholesterol or other lipids can influence the requirement of antioxidant in the liver. Anelevated liver cholesterol content has been demonstrated to be associated with increase rate of liver cholesterol degradation. Because lipid peroxidation has been suggested as a normal process in cholesterol catabolism ([15,16]), the increased rate of cholesterol degradation as a result of increased dietary intake may increase the rate of hepatic lipid peroxidation.

The potential ability of palm oil in being able to suppress the attendant rate of lipid peroxidation observed in this study is of interest since the pathogenesis of many diseases including CVD can involve free radical-mediated lipid peroxidation in biological membranes. Palm oil with a rich content of monounsaturated fatty acid and antioxidant vitamin (vitamin E), has been demonstrated to reduce oxidative stress-induced hypertension in normal rats ([17]). In a related study conducted by Tosaki et al.([18]) , the level of oxidatively modified protein during reperfusion in hearts of rats fed palm oil supplemented diet was significantly reduced compared with that of control rats fed without palm oil. Tosaki et al.(18) , equally observed that the burst in generation of oxygen free radicals in the course of reperfusion was fully prevented in the hearts from palm oil-fed animals. Furthermore, Serbinova et al.([19]) , reported that palm oil vitamin E (consisting of a mixture of 45% tocopherols and 55% tocotrienols) completely suppressed LDH leakage from ischaemic hearts, prevented decrease in adenosine triphosphate (ATP) and creatine phosphate levels, and also inhibited the formation of endogenous lipid peroxidation products.

Another interesting revelation from our results is that palm oil supplementation was observed to significantly elevate the activity of glutathione peroxidase in cholesterol-fed rats. We observed, in accord with other data ([20]) a pronounced decrease in the activity of glutathione peroxide in rats fed cholesterol diet only. However, palm oil was able to produce a significant increase in the enzyme activity despite the 5% cholesterol content of the diet. Glutathione peroxidase is an enzyme involved in the detoxification of lipid peroxides (20). A reduction in the activity of this enzyme would decrease the protection of the tissue against lipid peroxidation. Cholesterol accumulation is known to occur in the aorta or other arteries of hypercholesterolemic animal or human subjects leading to the formation of lipid-rich plaque and atheroma ([21]). Since the aorta and other arteries are known to contain a very low level of glutathione peroxidase ([22]), the elevated rate of lipid peroxidation in this tissue would seem to be especially undesirable and may be one of the mechanisms responsible for the development of atherosclerosis in this tissue.

In conclusion, the protective role of palm oil against cholesterol-induced lipid peroxidation in the liver, and in replenishing hepatic level of glutathione peroxidase as revealed in this study is more than mere academic interest as palm oil could be a veritable nutrition tool in the fight against CVD.

References

1. Stamler J, Wentworth D, Neaton J. Is the fellowship between serum cholesterol and risk of death from coronary heart disease continuous and graded? J m Med Assoc 1986; 256: 2823-2828.
2. Hetzel BS, Charnock JS, Dwyer T, Maclennan PL. Fall in coronary heart disease mortality in USA and Australia due to sudden death: evidence for the role of polyunsaturated fat. J. Clin. Epidemiol 1989; 42: 855-893.
3. Caiazza D, Jahangiri A, Rader DJ, Marchadier D, Rye KA. Apolipoproteins regulate the kinetics of endothelia lipase-mediated hydrolysis of phospholipids in reconstituted high density lipoproteins. Biochemistry 2004; 43: 11898-905.
4. Tikekar P, Chakrabarti CH. Effect of high cholesterol diet on liver enzymes and blood and liver glutathione. Indian J. Nutr. Diet 1973; 10: 182-186.
5. Onyeneke EC, Oluba OM, Adeyemi O, Eriyamremu GE, Ojeaburu SI, Adebisi KE, Adeyemi 0. Effects of soy protein on serum lipid profile and some lipid metabolizing enzymes in cholesterol fed rats. Afr. J. Biotechnol 2007; 6(19): 2267-2273.
6. Keys A, Anderson JT, Grande F. Serum cholesterol responses to change in diet. IV: Particular saturated fatty acids in the diet. Metabolism 1965; 14: 776-787.
7. Hornstra G, Sundram K. The influence of dietary palm oil on cardiovascular disease risk factors: a human study. Abstracts, PORIM International Palm Oil Conference. 9-14 September, 1991, Kuala Lumpur, Malaysia. 154-155.
8. Ng TKW, Hassan K, Lim JB, Lye MS, Ishak R. Nonhypercholesterolemic effects of palm-oil diet in Malaysian volunteers. Am. J.Clin. Nutr 1991; 53: 1015s-1020s.
9. Sundram K, Khor KT, Ong ASH. Effect of dietary palm oil and its fractions on rat plasma and high density lipoprotein lipids. Lipids 1990; 25: 187-193.
10. Scholtz SC, Pieters M, Oosthuizen W, Jerling JC, Bosman MJ, Vorster HH. The effect of red palm olein and refined palm olein on lipids and haemostatic factors in hyperfibrinogenaemic subjects. Thromb Res 2004; 113: 13-25.
11. Wills ED. Mechanisms of lipid peroxide formation in animal tissues. Biochem J 1966; 99: 667-676.
12. Placer ZA. Lipid peroxidation in vivo. In: Nutrition Proceedings of the Eighth International Congress (Masek), Osancova K, Cuthbertson D P, eds., 1970; 100-105.
13. Scott A (1964): Conjugated chromophores. In: Interpretation of the Ultraviolet Spectra of Natural Products, 1964; 45, Pergamon Press, Oxford.

14. Little C, Olinescu R, Reid KG, O'Brien PJ. Properties and regulation of glutathione peroxidase. J. Biol. Chem 1970; 245: 3632-3636.
15. Mitton JR, Scholan NA, Boyd GS. The oxidation of cholesterol in rat liver sub-cellular particles. The cholesterol 7-hydroxylase enzyme system. Eur. J. Biochem 1971; 20: 569-579.
16. Johansson G. Oxidation of cholesterol, 3-hydroxy-5-pregnen-20-one and 3-hydroxy-5-androsten-17-one by rat liver microsomes. Eur. J. Biochem 1971; 21: 68-79.
17. Edem DO. Palm oil: biochemical, physiological, and toxicological aspects: a review. Plant Food Num. Nutr 2002; 57:319-341.
18. Tosaki A, Blasig I, Pali T, Ebert B. Heart protection and radical trapping by DMPO during reperfusion in isolated working rat hearts. Free Radical Biol Med 1990; 8:363-72.
19. Serbinova E, Khwaja S, Catudioc J. et al. Palm oil vitamin E protects against ischaemia/reperfusion injury in the isolated perfused Langendorff heart. Nutr Res. 1992; 12 (suppl. 1): S203-S215.
20. Little C. Steroid hydroperoxides as substrate for glutathione peroxidase. Biochim. Biophys. Acta 1972; 284: 375-381.
21. Onyeneke EC, Ononogbu IC. The effect of gari diet on the pathological changes of the aorta, liver and the kidneys of rats. J. Clin. Biochem. Nutr 1989; 7: 91-100.
22. Smith AG, Harland WA, Brooks CJW. Glutathione peroxidase in human and animal aorta. Steroids Lipids Res 1973;4:122-128.

Author Information

Olarewaju M Oluba, MSc.
Department of Biochemistry, University of Benin

Chukwu E. Onyeneke, Ph.D.
Department of Biochemistry, University of Benin

Godwin C. Ojieh, MBBS, MSc
Department of Medical Biochemistry, College of Medicine, Ambrose All University

George O. Eidangbe, MBBS
Department of Medical Biochemistry, College of Medicine, Ambrose All University

Regina T. Orole
Department of Biochemistry, Adekunle Ajasin University