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Full Length Research Paper

Hydrogenation impairs the hypolipidemic and antioxidant effects of palm oil in rats

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The effect of hydrogenated palm oil on lipid profiles and on activities of selected glutathione-dependent enzymes in rats was investigated. Male albino wistar rats were randomly divided into 2 groups, fed on fresh palm oil (control) and hydrogenated palm oil (HPO) supplemented (5% by weight) diets respectively for 10 weeks. Serum cholesterol, liver lipid peroxidation and daily excretion of triacyl glycerol (TAG) and cholesterol in faeces as well as hepatic activities of glutathione transferase (GST), glutathione reductase (GSSG-Rx), glutathione peroxidase (GSH-Px) and gamma glutamyl transpeptidase (γ-GT) (also in serum) were monitored both at pre- and post-diet periods. Serum cholesterol, liver lipid peroxidation and daily excretion of TAG and cholesterol in faeces and γ-GT (serum and liver) were significantly ($p < 0.05$) higher in the HPO than in the control group. TAG values in the serum and liver and liver activities of GST, GSSG-Rx and GSH-Px were significantly ($p < 0.05$) lower in the HPO than in the control group. The data presented in this study show that consumption of dietary HPO may be associated with cardiovascular disease.

Key words: Trans fats, lipid excretion, triacylglycerol, lipid peroxidation, cardiovascular disease.

INTRODUCTION

Partial hydrogenation of vegetable oils, a process that converts vegetable oils into semisolid fat used in margarines, commercial cookings and manufacturing processes, produces trans fats. Trans fats are unsaturated fatty acids with at least one double bond in the trans configuration. Partially hydrogenated vegetable oils are attractive to the food industries because of their long shelf life, their suitability during deep-frying and their semisolidity, which can be customized to enhance the palatability of baked goods and sweets. In the United States, the average consumption of industrially produced trans fatty acids is put at 2 to 3% of total calorie consumed (Allison et al., 1999).

During the past decade, reduction in fat intake has been the main focus of national dietary recommendations to decrease the risk of cardiovascular disease (CVD) (Mensink and Katan, 1990; Lichtenstein, 1998; Sanchez-Moreno et al., 2004). Several lines of evidence, however, have indicated that certain types of fats have a more important role in determining risk of CVD than the total amount of fat in the diet (Lichtenstein, 1998). Recent evidences suggest that dietary fatty acid composition influences numerous values including the cholesterol and triacylglycerol (TAG) content of plasma lipoproteins (Oluba et al., 2008; Sanchez-Moreno et al., 2004).

The link between dietary fats and cardiovascular diseases has necessitated a growing research interest in palm oil, the second largest consumed vegetable oil in the world. Although palm oil-based diets induce a higher blood cholesterol level than the corn, soybean, safflower seed and sunflower oil, the consumption of palm oil causes the endogenous cholesterol level to drop. This phenomenon seems to arise from the presence of the tocotrienols and the peculiar isomeric position of its fatty acids. Palm oil has been used in the fresh and/or at various levels of oxidation. Oxidation is a result of processing the oil for various culinary purposes. However, a considerable amount of the commonly used palm oil is in the oxidized state, which poses potential dangers to the

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dangers to the biochemical and physiological functions of the body.

Because many of the xenobiotic metabolizing enzymes are membrane-bound, conditions of lipid peroxidation may compromise the ability of animals to detoxify potentially harmful chemicals. In this study the effect of partially hydrogenated palm oil obtained from a local bean- ball (popularly called 'akara') seller on lipid peroxidation indices and activities of some antioxidant enzymes in rat is determined.

MATERIALS AND METHODS

Chemicals

Oil source

Partially hydrogenated palm oil was obtained from a local bean-ball (popularly called akara) seller in Akungba Akoko, Nigeria, while fresh palm oil used in control diet was obtained from Okitipupa oil mill, Nigeria.

Animals and diets

Male albino wistar rats (n = 40) of average weight 64.3 ± 2.1 g obtained from the National 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 h light/dark cycle and 50 - 60% relative humidity at a temperature of about 30°C. The experimental diets were prepared according to the recommendations of the American Institute of nutrition and contained (% by weight) casein 20, fat 10, vitamin mixture (AIN-76) 1.0, mineral mixture (AIN-76) 3.5, choline bitartrate (BDH, England) 0.2, DL-methionine (Sigma, England), 0.3 corn starch 15 and sucrose to 100%. In addition, fresh palm oil (5%) and hydrogenated palm oil (5%) were added and mixed with control and test diet respectively. The animals had free access to food and tap water and were treated according to the International 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 two groups (n = 20) designated as control and test and placed on their respective diet for a period of 10 weeks. Before the commencement of the feeding experiment, rats were fasted overnight but allowed access to water *ad libitum*. 3 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.

Serum preparation

10 rats from each group were sacrificed before the commencement of the diet experiment and the remaining 10 were sacrificed at the end of the feeding trial. The animals were sacrificed by cervical dislocation. The blood was collected by cardiac puncture and pooled together into plain tubes and serum was separated by centrifugation (3000 g at 4°C for 10 min).

Cytosolic and microsomal fractions

Liver cytosolic and microsomal fractions were prepared by the method of Speir and Wattenberg (1975). Briefly, rat liver was immediately removed upon sacrifice, washed in ice cold 1.15% $^{w}/_{v}$ KCl solution, blotted, weighed, cut into small pieces in 1.15% w/v KCl at a volume of 3 ml of KCl per gram liver and homogenized for 5 min using a Potterelvegin homogenizer. The homogenate was centrifuged at 10,000 g at 4°C for 20 min in a Sorvall RC-5B superspeed centrifuge. The supernatant was pipetted into a clean centrifuge tube and centrifuged further at 105,000 g at 4°C in a Beckman L5-50B ultracentrifuge for 45 min. The pellets obtained represent the microsomal fractions and was used for γ-GT assay. The cytosol was used for GST, GSH-Px and GSSG-Rx activities.

Estimation of lipid peroxidation products

Lipid peroxidation was estimated in liver by assessing malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) methods of Varshney and Kale (1990). The reaction mixture contained 0.4 ml of microsomal sample mixed with 0.15 M Tris KCl buffer, 0.5 ml of 30% TCA and 0.5 ml of 52 mM TBA. The mixture was placed in a water bath for 45 min at 80°C, cooled in ice and centrifuged at room temperature for 10 min at 3000 rpm. The absorbance of the clear supernatant was measured spectrophotometrically against reference blank of distilled water at 532 nm.

Lipid assays

Total cholesterol concentration was determined by the method of Roeschlau et al. (1974). In this method cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Triacylglycerol (TAG) concentration was obtained according to the method described by Tiez (1990). The triacylglycerols are determined after enzymatic hydrolysis by lipases. The indicator is quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Enzyme assay

Glutathione peroxidase (GSH-Px) activity was measured according to the method described by Rotruck et al. (1973). The assay mixture (0.8 ml, pH 7.0) which contained 0.2 mM NADPH, 1 mM NaN3, 1 mM GSH and 1 EU/ml GSSG-reductase and 0.1 ml of cytosol. The reaction was started by the addition of 0.1 ml 15 mM cumene hydroperoxide as substrate. Glutathione transferase (GST) activity was determined spectrophotometrically at 37°C according to the method of Habig et al. (1974). The assay mixture consisted of 0.1 M phosphate bufer (pH 7.5), 1 mM GSH, 1 mM CDNB and cytosol in a final volume of 1.0 ml. Glutathione reductase (GSSG-Rx) activity was measure by the method of Racker (1955) with slight modifications. The assay mixture consisted of 0.1 M phosphate buffer (pH 7.6), 0.1 mM NADPH, 0.5 mM EDTA, 1 mM GSSG and cytosol in a final volume of 1.0 ml. The enzyme activity quantitated at 29°C by measuring the disappearance of NADPH at 340 nm. Specific activity for GSSG-Rx was defined as the oxidation of 1 µmol of NADPH per min per mg protein. Microsomal γ-GT activity was determined according to the method of Szaz (1976). γ-GT activity was measured by monitoring the increase in absorbance at 405 nm of p-nitroaniline formed by the reaction between L-gamma-glutamyl-p-nitroanilide and glycylglycine. Protein estimation was by Lowry et al. (1951) method. In this method, copper ion (blue) is made to react with the peptide bond of protein to give a purple coloured complex, the intensity of which is measured spectrophotometrically at 540 nm.

Statistical analysis

Data are mean ± SEM of triplicate determinations. Statis-

Table 1. Body and liver weight, average daily food intake and faecal weights of HPO- fed rats

	Control	Test
Initial body weight (g)	63.8 ± 9.4	65.1 ± 6.3
Final body weight (g)	191.0 ± 11.7	187.6 ± 14.2*
Average daily food intake (g)	13.7 ± 3.1	13.5 ± 1.2
Average daily faecal weight (g)	3.1 ± 0.5	5.2 ± 1.1*
Liver weight (g/100 g body weight)	4.2 ± 0.3	6.8 ± 0.5*

*Significantly different from control ($p < 0.05$).

Table 2. Effect of HPO on faecal triacylglycerol and cholesterol concentrations (µmol/g dry matter).

Triacylglycerol		
	Control	Test
Initial	0.44 ± 0.1	0.48 ± 0.1
Final	0.51 ± 0.2	34.8 ± 3.5*
Cholesterol		
Initial	1.2 ± 0.6	1.2 ± 0.3
Final	1.6 ± 0.4	49.5 ± 7.4*

*Significantly different from control ($p < 0.05$).

Table 3. Effect of HPO on serum and liver lipid concentrations.

Triacylglycerol				
	Serum (mg/dL)		Liver (mg/g liver)	
	Control	Test	Control	Test
Initial	0.31 ± 0.1	0.30 ± 0.1	0.23 ± 0.1	0.23 ± 0.1
Final	0.96 ± 0.3	0.51 ± 0.1*	0.62 ± 0.1	2.20 ± 0.1*
Cholesterol				
	Serum (mg/ dL)		Liver (mg/ g liver)	
	Control	Test	Control	Test
Initial	1.16 ± 0.1	1.12 ± 0.1	0.96 ± 0.1	0.93 ± 0.1
Final	3.70 ± 0.3	2.09 ± 0.1*	1.83 ± 0.4	2.81 ±0.2*

*Significantly different from control ($p < 0.05$).

Statistical analysis was by student t-test at $p < 0.05$ using SPSS 10.0.

RESULTS

As observed in this study, there were no significant differences ($p > 0.05$) in initial body weights between control and HPO-fed rats. However, HPO feeding led to a significant decrease ($p < 0.05$) in body weight compared with control as observed at the end of the feeding experiment (Table 1). The average daily food intake was not significantly different ($p < 0.05$) between the HPO group and

Table 4. Effect of HPO on liver lipid peroxidation (MDA mM/cm).

	Control	Test
Initial	57.1 ± 8.4	55.8 ± 14.2
Final	63.9 ± 13.6	121.6 ± 11.9*

Significantly different from control ($p<0.05$).
MDA = Malondialdehyde

group and control ($p > 0.05$). However, significant increases were observed in the average daily faecal weights and liver weights in the HPO-fed rats compared with the control ($p < 0.05$) (Table 1). Differences in initial faecal TAG and cholesterol levels between control and HPO rats were not statistically significant ($p > 0.05$). At the end of the feeding experiment, HPO feeding significantly increase these parameters (Table 2).

TAG and cholesterol baseline values were not statistically different between the control and HPO groups. However, at the end of the feeding experiment, HPO treated rats showed significant decrease in serum TAG and cholesterol levels and significant increase in liver TAG and cholesterol concentration compared with control (Table 3).

As shown in Table 4, liver lipid peroxidation rates were significantly higher ($p < 0.05$) in the HPO-fed rats relative to the control at the end of the feeding trial.

Table 5 shows that the HPO treated group had significantly reduced liver GST, GSSG-Rx and GSH-Px activities compared with control ($p < 0.05$). Serum and liver γ-GT levels were however significantly higher in the HPO rats compared with control.

DISCUSSION

This study is of crucial importance because most research studies on trans fats available in literature have focused on industrially produced trans fat with little or no attention on locally generated trans fat which is of more concern in a developed economy like Nigeria. The National Agency for Food, Drug Administration and Control (NAFDAC), the organization saddled with the responsebility of regulating the activities of industrial food manufacturers in Nigeria has been concerned with the industrial sector without much effort to regulate the activities of retailed outlets producing and selling consumable products. In Nigeria, bean cake popularly known as ‘akara’ in south western Nigeria is consumed by all and sundry given its availability and affordability. The akara industry provides a means of livelihood to most local women with little or no education. Given the economic situation in Nigeria, a typical akara producer uses the same oil for nothing less than three day to maximize profit. The continual usage of oil for deep-frying could thermally hydrogenate the oil.

To the best of our knowledge, there is scarce informa-

Table 5. Effect of HPO on glutathione-dependent enzyme activities.

	Control	Test
GST (µmol GSH-CNDB/min/mg protein)	1.56 ± 0.2	0.91 ± 0.1*
GSSG-Rx (µmol NADPH/min/mg protein)	0.75 ± 0.1	0.29 ± 0.1*
GSH-Px (mol NADPH/ min/ mg protein)	2.30 ± 0.1	1.00 ± 0.1*
LIVER γ-GT (U/g protein)	3.86 ± 0.5	7.50 ± 2.1*
SERUM γ-GT (U/L)	23.5 ± 8.4	154.2 ± 57.1*

*Significantly different from control ($p<0.05$).

Table 6. Fatty acid composition of fresh and hydrogenated palm oil used in diet composition as determined using gas chromatography (Hewlett Packard, model 5750)

Dietary oil	Fatty Acids (% by weight)						
	10:0	12:0	14:0	16:0	18:0	18:1	18:2
Fresh palm oil	2.1	1.8	1.5	42.6	4.6	37.5	9.9
Hydrogenated palm oil	2.0	1.6	2.2	58.1	22.3	10.3	3.5

mation in the literature directly assessing values in the blood and tissues of hydrogenated palm oil in rats. The increased faecal excretion of TAG and cholesterol levels in the test group is suggestive of incomplete bioavailability of the hydrogenated palm oil diet compared with the control diet. This is in accord with the results of two studies that suggested that high hydrogenated soybean oil feed is poorly absorbed (Kamei et al., 1995; Kaplan and Greenwood, 1998). The studies explained that diet high in long-chain saturated fatty acids might be poorly absorbed in rats. Evidence also suggests that stearic acid and tristearin are poorly digested and absorbed in rats (Mensink and Katan, 1990; Wilson et al., 2001). Because hydrogenated palm oil is composed of stearic acid (Table 6), a large component of this fat must be made up of tristearin. Due to excretion of TAG in faeces, TAG levels in serum were markedly lower in the hydrogenated palm oil fed group than in the control group.

Another major concern with hydrogenated palm oil is its high content of saturated fatty acids, which is often linked to high cholesterol levels. However, most hydrogenated palm oil contains stearic acid, which has been shown to be less cholesterolemic and atherogenic (Lichtenstein, 1998; Kamei et al., 1995; Lichtenstein et al., 1993). Nevertheless, it is possible that the absorbed portion of hydrogenated palm oil may contain more cholesterolemic fatty acids, such as palmitate. In this study, a marked reduction in serum and liver concentrations of TAG was observed in response to hydrogenated palm oil feeding. This is consistent with the fact that liver stores of TAG are mobilized rapidly when TAG absorption is reduced, they become depleted in parallel with the circulating pool (Wilson et al., 2001; Bracco, 1994; Sakono et al., 1997). The reduced serum and liver TAG levels in the test group may reflect the effect of hydrogenated palm oil on reducing the absorption of fatty acids.

In this study, lipid peroxidation rate as well as glutathione-dependent enzymes activities were used as indices of oxidative status in rats. Our findings revealed that the rates of hepatic lipid peroxidation were markedly higher in hydrogenated palm oil fed groups than in the control. In addition, results from this study show that hydrogenated palm oil feeding is associated with increased activities of serum and liver γ-GT. Accumulating experimental evidence suggests an important role for γ-GT in extracellular catabolism of glutathione, the principal thiol antioxidant in humans. γ-GT has been demonstrated to adsorb into circulating LDL-cholesterol and to be capable of catalyzing its oxidation (Paolicchi et al., 2006). It is expressed in the atheromatous core of coronary plaques, where it colocalizes with oxidized LDL and foam cells Paolicchi et al., 2004). The increased γ-GT activity observed in rats fed HPO diet in this study correlate inversely with antioxidant status of the animals as reflected in the levels of liver lipid peroxidation. Furthermore, a recent study by Puah et al. (2007) on the effect of physical refining on palm vitamin E showed that bleaching reduced the tocopherol and tocotrienol (the major antioxidants) content of palm oil significantly. Tocotrienol has been shown to exhibit cholesterol lowering effect (Traber et al., 1997) and also to inhibit cancer growth (Sundram et al., 1989). It could thus be argued that subjecting palm oil to hydrogenation treatment reduces its vitamin E content (especially the cholesterol lowering tocotrienol) which is responsible for its antioxidant effect. This could account for the increased rate of lipid peroxidation observed in HPO rats as against the control rats.

Furthermore, findings from this study show that HPO feeding decreases the activities of GST, GSH-Px and GSSG-Rx. The increased high rate of hepatic lipid peroxidation observed in the HPO group may also be associa-

associated with an increase production of reactive oxygen species (ROS) and free radicals. This assumption is further reinforced with the elevated activity of γ-GT in the serum of the HPO-fed rats. γ-GT is known to leak from injured tissue into the serum. Oluba et al. (2008) have demonstrated an increased serum γ-GT following a cholesterol feeding. The initiation stage of lipid peroxidation is a free radical reaction with lipid hydroperoxide product - products of the reaction between lipid peroxyl radical and oxygen - being the first stable intermediate. These ROS may be actively scavenged by GSH, resulting in the formation of oxidized form of GSH (that is, GSSG). The resultant GSSG is rapidly converted back to GSH by GSSG-Rx. We observed a decrease in GSSG-Rx activity in the HPO-fed animals relative to the control animals. It is expected that hepatic levels of GSH in the HPO-fed animals would be greatly reduced as a result of the increased rate of lipid peroxidation observed in the group. The observed decreases in GSH-Px and GST activities in the HPO-fed rats may be linked with the supposed decreased in liver GSH levels. Furthermore, since the animals fed HPO diet manifested higher rate of hepatic lipid peroxidation, one would suggest that the resultant decreases in GSH-Px and GST activities in the HPO rats might result from the elevated concentrations of oxygen radicals, hydrogen peroxide and lipid peroxides. Contrary to reported protective potential of fresh palm oil against oxidative damage to tissue macromolecules as observed by Tosaki et al. (1990) and Edem (2002), the data generated in this study showed that hydrogenated palm oil (HPO) is pro-atherogenic and thus is associated with cardiovascular health. It is thus recommended that regulatory authorities should take vigorous step to create awareness especially in the rural areas on the health implications of bleached or oxidized palm oil.

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