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# Altered responses of isolated aortic smooth muscle following chronic ingestion of palm oil diets in rats

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**Summary**

The responsiveness of the rat aorta after chronic consumption of 15% (wt/wt) fresh and thermally oxidized palm oil diets was studied under standard organ bath procedures. Aortic rings from the oxidized oil-fed group showed significantly ($P < 0.05$) enhanced vascular responses to noradrenaline and potassium chloride when compared with the control and fresh palm oil-fed groups. The maximum tensions were 285.10 ± 30 mg/mg tissue weight for the oxidized oil-fed group and 148.98 ± 36 mg/mg for the control in response to noradrenaline. The fresh oil-fed group produced maximum tension of 133.9 ± 20 mg/mg which was not significantly different from the control. The trend was similar with potassium chloride. The maximum tensions were 206.31 ± 25 mg/mg for the oxidized oil-fed group and 93.33 ± 13 mg/mg for the control group. The fresh oil-fed group produced maximum tension of 109.31 ± 7.8 mg/mg which was not significantly different from the control. Relaxation to acetylcholine was significantly ($P < 0.01$) attenuated in the aortic rings obtained from the oxidized palm oil-fed group when compared with the control and fresh palm oil-fed groups. The percentage maximum relaxations to acetylcholine were 28.1 ± 6.7% in the oxidized oil-fed group, 71.4 ± 6.0% in control and 78.2 ± 6.0% in the fresh oil-fed groups. The relaxation in the fresh oil-fed group was not significantly different from control. These results suggest that functional changes occur in rat blood vessels after chronic consumption of thermally oxidized palm oil.

**Résumé**

La repondance de l'aorte de rat après une consomation chronique d'un regime fait de 15% (poids/poids) d'huile de palme fraiche, et d'huile de palme oxydé thermiquement a été étudié sous les procedures normales de bain d'organes. Les bagues aortiques de animaux nourrit avec de l'huile oxydé a montré une augmentation significative ($P < 0.05$) des responses vasculaire à la noradrenaline et au chlorure de potassium, l'orsquiels sont comparé ceux des valeurs des controles, et a ceux nourrient à l'huile de palme fraiche. Les tensions maximales ont été de 285.10 ± 30 mg/mg de tissue pour les animaux nourrient a l'huile oxyde, et de 148.98 ± 36 mg/mg pour les controles en reponse à la noradrenaline. Le groupe de rats nourrit à l'huile fraiche a produit une tension maximale de 133.9 ± 20 mg/mg, qui n'est pas significativement differente de celle des contrôles. La tendance a été similaire avec le chlorure de potassium. Les tensions mazimales ont été de 206.31 ± 25 mg/mg pour le groupe nourrit à l'huile oxyde, et de 93.13 ± 13 mg/mg pour les controls. Le groupe nourrit à l'huile fraiche a produit une tennsion maximale de 109.31 ± 7.8 mg/mg, qui na pas été significativement differente de cells des controles. Ces resultats suggèrent que des changements fonctionnels surviennent dans les vaissaux sanguin de rats après une consomation chronique d'huile de paalme oxydé de mamière thermale.

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## Introduction

Functional abnormalities occur in blood vessels of hypertensives [1]. Such abnormalities include hyperreactivity and decrease compliance [2]. Recently, it has been shown that loss of the modulatory role of endothelium is involved in hyperreactivity of blood vessels [3,4].

Damage to the endothelium could result from elevated plasma cholesterol and low density lipoproteins [5]. The main source of cholesterol in the body is our dietary fats. Palm oil is one of the sources of dietary fats. It is obtained from the tropical plant, *Elaesis guineensis*. Palm oil contains both saturated and unsaturated fatty acids and can be eaten both as fresh (just after milling) or the thermally oxidized (as in frying food) form. Both forms are commonly used in domestic cuisines and food processing industries. Chronic ingestion of palm oil (whether fresh or oxidized) leads to raised cholesterol and free fatty acid levels [6,7]. In such circumstances, any resulting damage of the vascular endothelium will alter the responsiveness of blood vessels, increase the total peripheral vascular resistance, and raise the blood pressure. We have recently shown an increase in mean arterial pressure following the chronic consumption of thermally oxidized palm oil diet [8]. However, the relationship between palm oil and vascular responsiveness has not been documented.

In the light of the above, the present study was designed to examine the effect of both forms of palm oil in the diet on aortic responses to contracting and relaxing agents as a way of establishing this relationship.

## Materials and methods

### *Experimental animals*

Male Wistar rats initially weighing 60-70g and aged 70-80 days were obtained from the animal house of the Department of Physiology, University of Calabar (Calabar, Nigeria) for the study. Approval for the study was obtained from the College Ethical Committee. The rats were randomly grouped into three, comprising one control and two test groups.

The first test group was fed on a fresh palm oil diet (first test diet) while the second test group was fed on thermally oxidized palm oil (second test diet). The control group was fed on commercial rat feed obtained from Livestock Feed, Lagos, Nigeria. The animals were housed in stainless steel cages at a room temperature of 27 ± 2°C and were quarantined for a 2-week period prior to the introduction of test diets. All animals had free access to food and water for 18 weeks.

### *Source and formulation of palm oil diets*

Twelve litres of fresh palm oil were purchased from a local market in Calabar, Nigeria. It was certified fresh by virtue of its low oxidation number of 5.5 obtained using the method described by Rossell [9]. The fresh palm oil was divided into two equal parts. The first part was administered in the fresh form while the other part was thermally oxidized as described previously [7]. Briefly, the fresh palm oil was heated at 150°C in a stainless steel pot intermittently five times with each round lasting 20 minutes. The oil was allowed to cool for 5 hours after each round to give the thermally oxidized palm oil

oxidation number of 15.1. The two test diets were formulated by mixing 15% wt/wt of each oil with the commercial rat feed.

*Preparation of tissue*

After the feeding period, rats were killed by cervical dislocation and the thoracic aortas quickly dissected, freed from connective tissue, and put into Krebs-Ringer bicarbonate buffer of the following composition (mM/L) NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0 and glucose, 11.1 of pH 7.4. The aorta was cut into rings of about 3 mm long. Disruption of the endothelium and unnecessary stretching was avoided during vessel preparation. Each aortic ring with intact endothelium was suspended between two rectangular stainless steel wires in a 10 ml tissue bath containing Krebs-Ringer solution at a temperature of 37°C. The bath was continuously bubbled with 95% $0_2$ and 5% $CO_2$ gas mixture. One of the suspending wires was hooked to a solid base in the bath while the other was connected to a force displacement transducer (Washington Type D S/N 1108) which was in turn connected to a physiograph (Washington 400 MD/2) for recording isometric tension. All vascular rings were equilibrated for 90 minutes under a resting tension of one gram prior to the start of each experiment. The rings were challenged with 30mM potassium chloride during the equilibration period to establish tissue viability and the bathing fluid was changed at intervals of 15 minutes.

*Concentration-response tests to potassium cholride (KCL) and noradrenaline (NA):*

After the equilibration period, concentration-response tests to KCl and NA were conducted separately by cummulative addition of the agonist into the fluid bathing the tissues; the higher concentration was added only when the effect of the previous one had reached a plateau. These tests were carried out on aortic rings from both the control and the two test groups. Tension developed in response to each agonist concentration was expressed as mg tension per mg weight of wet tissue. In addition, the data was transformed to percentage of maximum tension developed by the control rings in response to each agonist.

*Functional assessment of the endothelium*

Endothelial function was assessed by dose-response tests to acetylcholine (ACh) in aortic rings precontracted with $10^{-7}$ NA. At the contraction plateau, cummulative concentration-response curves for Ach ($10^{-10}$1 - $10^{-5}$ M) were generated. A higher concentration of Ach was added only after the effect of the previous one had been established. The drops in tensions in responses to Ach were measured and expressed as percentages of the original tension produced by NA.

*Daily food intake*

During the last 4 weeks of the study, rats were placed in separate cages in order to measure daily food intake. The food contents were measured before and after 24 hour feeding periods. The food intake was expressed as energy intake per day.

*Drug and chemicals*

Noradrenaline (+-) arterenol hydrochloride), acetylcholine chloride, and potassium chloride were obtained from Sigma Chemical Co (Poole, England). All other chemicals were purchased from May and Baker (Dagenham, England). All drugs were dissolved in deionized distilled water and were prepared fresh prior to the start of experiment.

*Statistical evaluation*

All data are expressed as the mean ± standard error of mean (SEM). The $EC_{50}$ of KCl and the $pD_2$(-log$EC_{50}$) of NA and ACh for the different aortic preparations were calculated graphically from the concentration-response curves as measures of the tissue sensitivities. One way analysis of variance was used for multiple comparisons. Statistically significant F values were tested as described by Ericker [11]. A $P$ value of less than 0.05 was considered to indicate significance.

## Results

*Contractile responses to noradrenaline*

Noradrenaline produced concentration-dependent contraction of the aortic rings from both control and experimental rats. Significant ($P < 0.05$) enhancement in the contractile responses were obtained in the entire range of NA concentration used only in aorta from rats fed on oxidized oil when compared with control and fresh oil-fed groups (Fig. 1). The maximum tensions obtained at the concentration of 1.6 x $10^{-5}$mol/L of NA were 285.1 ± 3.0 mg/mg for the oxidized oil-fed group and 148 ± 36 mg/mg for the control. The fresh oil fed group produced maximum tension of 133.9 ± 20 mg/mg which was not significantly different from the control.

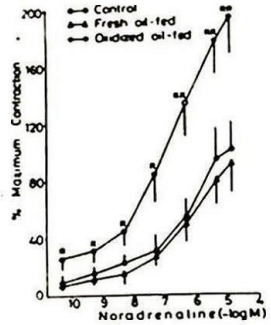


Fig. 1: Concentration response curves to noradrenaline in control (•), fresh oil fed (Δ) and oxidized oil-fed (○) groups. Each point represents the mean of results from 7 – 12 aortic rings (seven to eleven animals) with the mean ± SEM indicated by a vertical line. *$P <$ 0.05 and **$P < 0.01$ statistical difference.

Like the contractility, a significant increase ($P < 0.05$) in sensitivity to NA was noted in blood vessels from the oxidized oil-fed group. However, no significant difference was found in $pD_2$ between the fresh oil group and the control (Table).

Table 1: $pD_2$ values of NA and Ach agonists in control and rats fed on palm oil diets

| Group | Noraldrenaline | Aceylcholine |
|---|---|---|
| Control | 6.45 ± 0.24 | 7.81 ±0.40 |
| Fresh oil-fed | 6.45 ± 0.21 | 8.39 ± 0.35 |
| Oxidized oil-fed | 7.10 ± 0.21* | 7.43 ± 0 32 |

Each value represents the mean ± SEM *P<0.05 (statistical difference compared with control and fresh oil-fed groups). n≥

*Contractile responses to KCl*

Concentration dependent contractions of tissues from both control and experimental groups were also produced by KCl. The contractile responses were significantly ($P < 0.05$) - 0.01) enhanced in the whole range of concentration used only in the group fed on the oxidized palm oil diet when compared with the control or fresh oil fed group (Fig 2) The maximum tensions were 206.31 ± 25 mg/mg for the oxidized-fed group and 93.33 ±*13 mg/mg for control. The maximum tension was 109.31 ± 7.8 mg/mg for the fresh oil-fed group, which was comparable with the control.

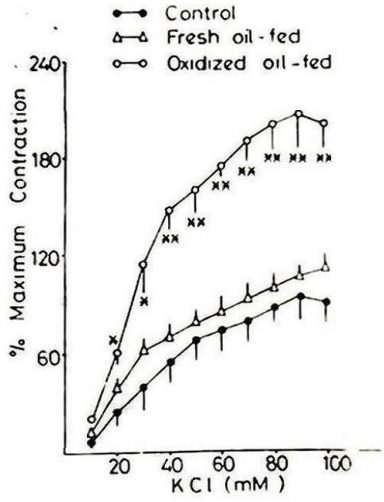


**Fig. 2:** Concentration-response curves to potassium chloride in control (•), fresh oil-fed (Δ) and oxidized oil-fed (○) groups. Each point represents the mean of results from 7 - 11 aortic rings (seven to ten animals) with the mean ± SEM indicated by a vertical line. $*P < 0.05$ and $**P < 0.01$, statistical difference compared with control and fresh oil-fed groups.

The sensitivities of the aortas to KCl were also influenced by dietary regimen. The mean $EC_{50}$ values were 24.0 ± 4.0 mM/L for the oxidized oil fed group, 21.5 ± 6.1 mM/L for the fresh oil fed group, and 40.0 ± 3.7 mM/L for the control. The $EC_{50}$ values of the two test groups were significantly lower ($P < 0.05$) than that of control.

*Relaxation-responses to ACh*

Figure 3 represents relaxation responses to ACh of aortic rings from control as well as dietary oil supplemented groups. It is evident that the control as well as the fresh palm oil-fed group exhibited a marked relaxation in response to ACh. The mean relaxation was 71.4 ± 6.0% in the control and 78.0 ± 6.0% in the fresh oil-fed group. There was no significant difference in relaxation responses between the two groups. The relaxation responses in aortic rings from rats fed on oxidized palm oil was significantly ($P < 0.05$ - 0.01) inhibited when compared with the control and fresh oil-fed groups. The mean maximum relaxation was 28.1 ± 6.7% of NA precontraction. The sensitivity of the aortic rings to ACh was not affected by any form of dietary treatment when compared with the control (Table).

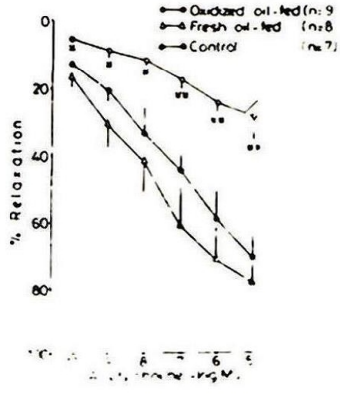


**Fig. 3:** Relaxation responses to acetylcholine in control (•), fresh oil fed (Δ) and oxidized oil-fed (○) groups in vessels precontracted with noradrenaline. Each point represents mean ± SEM of result obtained from 77 - 9 aortic rings (seven to nine animals). $*P < 0.05$, $**P < 0.01$ compared with control and fresh oil-fed groups.

*Daily food intake*

The rats fed on oxidized palm oil consumed an average of 8.10 ± 0.15 g of feed per day equivalent to 169.3 Kcal/day. Energy intake of 18% was derived from fat. Rats fed on the fresh palm oil diet ingested 8.53 ± 0.30 g feed per day. Energy intake of this amount was 178.3 Kcal/day out of which 18% was contributed by fat. The daily food consumption by rats fed on the control diet was 11.01 ± 0.30 g. The energy intake was equivalent to 253.0 Kcal/day and 3.5% of this value was derived from fat. The mean food or energy intake of rats in the control was significantly ($P < 0.05$) higher than in the two test groups. However, there was no significant difference between the food or energy intake of rats in the two test groups.

## Discussion

The functional changes that occur in the aorta after chronic consumption of fresh and oxidized palm oil diets were recorded. Responses to noradrenaline and KCl were enhanced in aortic rings from rats fed oxidized oil, but not in those fed fresh oil. This means that oxidized oil, which shows a significant qualitative difference from fresh oil, altered the contractile mechanism of the aortic smooth muscle.

The present design was not made to isolate the particular active ingredient in the oil that may have been responsible for the observed responses. However, it is known that following oxidation of palm oil, the concentration of unsaturated fatty acids decreases while that of saturated fatty acids increases [12]. We have also confirmed from our previous work that, based on iodine values, oxidized palm oil has significantly less unsaturated fatty acids than the fresh oil [8]. This is because during thermal oxidation, unsaturated fatty acids are converted to saturated fatty acids and free radicals (hydroperoxides and cyclic hydroxyl esters) [13]. On consumption, this modification could raise the plasma levels of cholesterol and low density lipoprotein and thus alter the vascular smooth muscle function [5,6]. Another possible reason for our observation is altered endothelial function. Oxidized palm oil contains hydroperoxides and hydroxyl esters which could accummulate in blood vessels [14] and cause endothelial damage [15]. Such endothelial damage would enhance vascular smooth muscle responses to noradrenaline [3,4]. This may have been the case in our present preparation.

A further confirmation of decreased endothelial function in our preparation is provided by the results with acetylcholine. The responses to acetylcholine were attenuated in aortic rings obtained from rats fed on oxidized palm oil. Physiologically, the functional integrity of the endothelium is assessed with acetylcholine [10,16], because the relaxation responses to acetylcholine are mediaated by an endothelium dependent relaxant factor (EDRF), which has been confirmed to be nitric oxide [16,17] and are curtailed by endothelial damage [18]. The present observation of attenuated responses to acetylcholine is, therefore, consistent with our earlier speculation of possible endothelial damage following chronic ingestion of oxidized palm oil in the diet.

The contractile responses to KCl were also enhanced in rings obtained from the oxidized oil-fed group but not in rings from the fresh oil-fed group. This would not be attributed to altered endothelial function since KCl-induced contractions are not endothelium dependent [3]. KCl depolarizes smooth muscle cell membrane and opens specific calcium channels (the potential sensitive calcium channels) to allow for increase in the sarcoplasmic calcium to cause contraction [19]. The present results will therefore suggest some alteration in this mechanism in favour of enhanced responses induced by chronic ingestion of oxidized palm oil. Enhanced vascular responsiveness and to sensitivity circulating agents may lead

to an increase in peripheral resistance which may be one of the factors responsible for the elevated mean arterial pressure in rats fed on the thermally oxidized palm oil diet in our previous study [8].

It might be argued that the observed differences in responses may be due to high dietary fat supplementation and food intake. Our results have shown that the vascular responses to various agonists in the fresh oil-fed group (which received high dietary fat) were similar to the control. Also, there were significant differences in vascular responses between the two test groups which received similar high dietary fat supplements and daily food intakes. These observations eliminate the possible effect of a high fat diet or food consumption.

In conclusion, functional changes occurred in both the endothelium and smooth muscles of the aorta following chronic ingestion of thermally oxidized palm oil, a development that could be attributed to the qualitative changes in the oil.

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