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Derrangement of K^+, Na^+, Cl^- and HCO_3^- levels by Chronic Consumption of oxidized Palm Oil

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Original Research Article

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Abstract: The study was undertaken to find out the effects of chronic consumption of oxidized palm oil on renal handling of Na+, K+, Cl-, HCO3- in Wistar rats. Twenty four male wistar rats weighing 140-160 grams at the begining of the experiment were randomly divided into four groups namely: control, fresh palm oil diet fed (fed 15% w/w fresh palm oil), photoxidized palm oil diet fed (fed 15%w/w photoxidized palm oil), thermoxidized palm oil diet fed (fed 15% w/w thermoxized palm oil) groups. All four groups received water at libitum. At the end of twelve weeks, urine and blood samples were collected for the analyses of the concentration of Na+, K+, Cl-, HCO3-. Results showed that Plasma Na+ and Cl- concentrations in the TPO and PPO groups were significantly lower than the control (P<0.001) groups and FPO (P< 0.001 and 0.01 respectively)groups. Their (TPO and PPO) Na+ urine concentrations were however significantly (P<0.001) higher than the control and FPO. K+ plasma levels on the other hand, were significantly higher in the TPO (P<0.001) and PPO (P<0.05 and P< 0.01respectively) groups when compared with the control and FPO; but significantly (P<0.001 and P<0.01)lower in the urine. Plasma HCO3- concentration in the TPO group was significantly (P<0.01) lower than the control, FPO and PPO groups while that of PPO was significantly (P<0.05) lower than FPO. Urine K+ concentration of TPO was significantly(P<0.001) lower that the control and FPO while PPO levels were significantly (P<0.05) lower than the FPO only. In conclusion, chronic consumption of oxidized palm oil causes hyperkalemia, hyponatremia and hypobicarbonatemia.

Keywords:, Palm oil, Thermoxidation, Photoxidation, hyperkalemia, hyponatremia, hypobicarbonatemia.

INTRODUCTION

The kidneys play a crucial role in the regulation of electrolytes (especially Na+, K+ Cl-) in the ECF and ICF and by so doing play a major part in maintaining the averall osmotic and fluid balance in the body[1]. Bicarbonate (HCO3-) on the other hand is part of the body's most essential buffering system [1].The amount of electrolytes reabsorbed or excreted by the kidneys is dependent on the osmolarity as well as pH of the ECF and ICF which is also usually dependent on our diet and status of health [2]. The rate of reabsorption is also dependent on the glomerular filtration rate and the integrity of the transport proteins reponsible for the transport of specific ions at different portions of the nephron [3].

If the GFR is high, the rate of flow of filtrate throughout the length of the nephron is increased leaving little time for the reabsorption of solutes/ions. This will result in a higher quantity of electrolytes in the urine and vice versa [4]. The above reabsorption can only also be possible if the transport proteins lining the epithelial membrane are intact. If however, the integrity of the structural architecture of the epithelium is compromised, it will result to distortion or destruction of the transport of the afore mentioned electrolytes [5]. As a result, there will be an imbalance in the internal milleu.

There are quite afew factors that affect the integrity of the transport proteins lining the nepron namely; genetics[6], age [7], diet [8], e.t.c. However, diet will be our focus in this study.

The consumption of different food substances especially oils, affect the functionality of different tissues either positively or negatively. The consumption of oils in various forms is widespread in Africa especially in Nigeria. Palm oil is one of such oils which is consumed as cooking oil (fresh and thermoxidized) [9,10]. It is one vegetable oil which makes up about 15% of almost all black African dishes especially Nigeria.

When consumed in its fresh form, there are many nutritional and health attributes because fresh palm oil is rich in many food nutrients. It is the richest natural source of tocotrienol which is the most potent form of the antioxidant vitamin E [11]. From its reddish orange hue, palm oil is also a rich source of beta carotene, a nutrient that is also found in sweet potatoes, carrrots and other orange foods [12]. Infact, it is the richest dietary source of provitamin A carotenoids (beta cacrotene and alpha carotene) and has 15 times more vitamin A than carrots and 300times more than tomatoes [13]. Vitamin A and E are well established antioxidants, protecting the cells and tissues from the damaging effects of free radicals; and they both play important roles in the prevention of artherosclerosis [14-16]. Unfortunately, alot of the ‘fresh palm oil’ sold in Nigerian markets is not fresh as shown by their peroxide value [17, 18, 19]. Much of the oil has been exposed to sunlight and is therefore photoxidized. Most of the time, the palm oil called “fresh” has already been “auto” or photoxidized and so the term “fresh” when refering to it is erroneous. Another form of palm oil commonly used is thermoxidized palm oil which has undergone several rounds of heating.

Typical thermoxidized palm oil has been stripped of most of its nutrients resulting in a clear oil [20]. Studies have shown that 70% of the carotenes may still be maintained after one deep fry, but after four deep fries, there may be virtually no carotenes left [21]. It also produces adverse effects on the serum and plasma lipid profile, cerebrosides and free fatty acids [10,11]. Adam *et al*. [17] showed that consumption of repeatedly heated palm oil (thermoxidized) increases lipid peroxidation and generation of free radicals/ reactive oxygen species that are deleterious to health. Other studies have also shown deleterious effects of thermoxidized palm oil on health. In 1994, Osim and others [9] showed that chronic consumption of palm oil caused damage to the lungs, liver, kidneys and also increased basal metabolic rates. Chronic consumption of thermoxidized palm oil reduced glomerular filtration rate and increased blood pressure in rats [22]. From the foregoing, it is very obvious that chronic consumption of palm oil affects the integrity of the glomerular filter to the extent that even though blood pressure was increased, the GFR, instead of increasing, was rather reduced. Is it only the glomerulus that is adversely affected by chronic consumption of palm oil? Does it affect the transport processes along the length of the nephron? Is it enough to alter the ability of the nephron to regulate electrolyte reabsorption and excretion and therefore the osmotic balance? If so, to what extent? The aim of this study is therefore to find out the effects of both photoxidized and thermoxidized palm oil on the renal handling of electrolytes.

MATERIALS AND METHODS

Experimental animals

Twenty four male Wistar albino rats were purchased from the Department of Pharmacy, University of Uyo. They weighed between 140-160grams at the beginning of the experiment. They were kept in the animal house of the Department of Physiology, University of Calabar, Calabar to acclimatize for two weeks before the experiments began. The temperature of the animal house was maintained at 26±2^{o}C. The rats were exposed to light/dark cycles of about 12/12 hours and were allowed access to clean drinking water at libitum. Before the commencement of the experiment, ethical approval was gotten from the Faculty ethical committee and regulations in accordance with the National and institutional guidelines for the protection of animal welfare were followed during the experiments.

Experimental procedure

Animals were randomly assigned to four groups of six rats each namely: Control (fed normal rat chow), fresh Palm oil diet fed group (FPO; fed 15% w/w fresh palm oil); photoxidized palm oil diet fed group (PPO; fed 15% w/w photoxidized palm oil diet) and thermoxidized palm oil diet fed group (TPO; fed 15% w/w thermoxidized palm oil diet).

Fifteen litres of fresh palm oil was purchased directly from the palm oil mill at Ugep in Yakurr Local Government Area, Cross River State and immediately stored in a dark container. the palm oil was hereafter divided into three parts of 5lires each. One part was left fresh and the other two parts were thermoxidized and photoxidized using the methods of previous workers Owu *et al*. [23] Syed *et al*. [24] and also stored in dark containers to prevent further oxidation. Palm oil diets were formulated as previously reported by Osim *et al.* [9] and Owu *et al* [23]. Briefly, eighty five grams of rat chow was mixed with fifteen grams of fresh, thermoxidized or photoxidized palm oil which is the usual composition of a typical Black African diet. The peroxidation numbers of the TPO and PPO were 5.16 and 3.48 respectively. The peroxide values were determined using the standard AOCS methods [25]. The animals were fed their separate diets for 12 weeks. Each animal was allowed free access to water and was kept in a separate metabolism cage throughout the duration of the experiment. At the end of 12 weeks, the animals were subjected to the following: They were anaesthetized intraperitoneally with a mixture of 1 percent (w/v) alpha chloralose and 25% (w/v) urethane in normal saline at a dose of 5ml/kg body weight, a tracheostomy was performed to gaurantee free breathing. The right femoral vein was cannulated for infusion of normal saline. This was done by connecting the cannula to an infusion pump [11] plus, Havard apparatus, Holliston MA, USA) which pumped in normal saline at a rate of 0.06ml/min [26, 27]. The left femoral artery was cannulated with the use of an intra-arterial cannula (Portex limited, Hythe Kent England). This cannula was connected to a blood pressure transducer (P23D Statham Hart Rey Puerto Rico) which was connected to a grass polygraph (Model 7D; Grass instruments Co. Quincy Mass. U.S.A.) for the monitoring of blood pressure.

Collection of blood and Urine samples

Through a small lower abdominal incision, the urinary bladder was cannulated with a short self-retaining catheter (pp100, polythene tubing). The urethra was ligated to avoid voiding of urine. After the equilibration period (a period within which three 20 min urine collections yielded constant or the same volume) of 60 mins, urine samples were collected in pre-weighed vials for another 60 min period. The urine samples were thereafter stored in a freezer until when required. Terminal blood samples were collected from the left femoral artery into heparinized tubes and blood plasma was immediately separated by centrifugation (3000 g for 10 min). The plasma so separated was put into Eppendorf tubes and stored in a freezer until when required for analysis.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard error of mean (SEM). The results were analysed using graph Pad prism software version 5 (GraphPad Software, SanDiego, CA). One-way analysis of variance (ANOVA) was used to compare means followed by a post hoc Bonferroni test where P values <0.05 were considered significant.

RESULTS

Table 1 shows Comparison of the mean concentration of Na+, Cl-, K+ and HCO3- in the plasma of control, FPO, PPO and TPO groups.

The results in table 1 show that there was no signifiant difference in the mean concentration of Na^+,Cl^-, K^+ and HCO_3^- between the control and FPO groups.

However, when the mean plasma concentration of Na^+ in the PPO, and TPO groups were compared with the control and FPO groups, there was a significant (P<0.001) decrease (table 1). The mean plasma chloride concentration of the PPO and TPO groups followed thesame trend as that of sodium (table 1). The Na^+ concentration in the plasma of the TPO group was also significantly (P< 0.05) lower than that of PPO. However, Chloride concentration in the TPO was not significantly lower than that of PPO.

On the other hand, the mean plasma concentration of K+ in the PPO and TPO groups were significantly (P<0.05; P<0.001 respectively) higher than that of the control (table1). When compared with the FPO, Plasma K^+ was significantly (P<0.05; P<0.001respectively) higher in the PPO and TPO groups. The mean plasma K+ in the TPO group was significantly (P<0.001) higher than that of PPO.

The mean concentration of HCO_3^- in the PPO group was not significantly different from that of the control but was significantly (P<0.05) lower than the FPO group. When the mean plasma concentration of HCO_3^- in the TPO group was compared with the control , FPO and PPO groups, there was a significant (P<0.01) decrease (table 1).

Table-1: Comparison of the mean concentration of Na^+, Cl^-, K^+ and HCO_3^- in the plasma of control, FPO, PPO and TPO groups

Parameter	control	FPO	PPO	TPO
Na^+	142.34±1.56	140.34±1.30^{ns}	135.60± 1.25^{***,r}	129.83±0.32^{***,a,b}
Cl^-	110.05± 1.21	140.34±1.30^{ns}	135.60± 1.25^{***,r}	87.23±1.32^{***a,s}
K^+	3.23±0.31	102.13± 1.21^{NS}	90.11±3.72^{***,c}	9.10±0.11^{***,a,z}
HCO_3^-	24.67±0.47	3.42±0.56^{NS}	23.83±0.32^{NS,r}	22.17±0.32^{**,d,b}

;**;=** P<0.05; P<0.01; P<0.001 vs control

r; d; c;a = not significant, P<0.05; P<0.01; P<0.001 vs FPO

s;b;z = not significant; P<0.05; P< 0.01 vs PPO

The results in table 2 show the comparison of the mean concentration of Na^+, Cl^-, K^+, and HCO_3^- in the urine of the control, FPO, PPO and TPO groups. The mean concentration of all the electrolytes in the urine of the FPO group were not significantly different from that of control.

The mean concentrations of Na^+ and Cl^- in the urine of the PPO and TPO groups were significantly (P<0.001) higher than that of control and FPO groups. The mean Urine Na^+ and Cl^- concentrations of TPO were also significantly (P<0.001) higher than PPO.

On the other hand, the mean urine concentration of K^+ in the PPO and TPO groups were significantly (P<0.01; P<0.001 respectively) lower than that of the control group. Mean K+ concentration in the urine of PPO and TPO were also significantly (P< 0.01;P< 0.001respectively) lower than that of FPO. When TPO and PPO were compared, there was also a significant (P< 0.001) reduction in TPO urine K^+ concentration.

Bicarbonate levels in the urine of TPO group were significantly (P< 0.001) higher than control and FPO groups but there was no sigmificant difference when compared with the PPO group. Urinary bicarbonate levels in the PPO and control groups were not significantly different, but that of FPO was significantly (P<0.05) higher than that of PPO.

Table 2: comparison of the mean concentration of Na+, Cl-, K+, and HCO3- in the urine of control, FPO, PPO and TPO groups

Parameter	control	FPO	PPO	TPO
Na+	28.17±1.37	27.24±0.15^{NS}	34.33±1.05***^{,a}	46.17±0.67***,^{a,z}
Cl-	20.33±0.26	20.51±0.35^{NS}	35.17±1.12***^{,a}	47.33±0.37***^{,a,z}
K+	35.00±1.15	34.25±1.22^{NS}	28.16±0.49**^{,c}	16.17±1.21***^{,a,z}
HCO3-	13.33±0.49	14.63±0.25^{NS}	10.28±0.49^{NS,r}	10.50±0.22***^{,a,s}

Key: NS=not significant vs control
;**;=** P<0.05; P<0.01; P<0.001 vs control
r; d; c;a = not significant, P<0.05; P<0.01; P<0.001 vs FPO
s;b;z = not significant; P<0.05; P< 0.01 vs PPO

DISCUSSION

The results from this study showed significant differences in the mean plasma sodium, chloride, potassium and bicarbonate concentrations of the photoxidized (PPO) and thermoxidized palm oil (TPO) diet-fed groups of rats. The control and fresh palm oil (FPO) diet fed values of all the plasma electrolytes obtained in this study were similar to those obtained by other investigators [28]. This confirms that the methods used in obtaining the electrolytes were standard, and that consumption of fresh palm oil is not harmful.

Plasma sodium and chloride levels in particular were significantly (P<0.001) lowered in the two oxidized (photoxidized and thermoxidized) palm oil groups. The sodium level in the PPO and TPO fed group (133.33±1.05 and 129.83±0.32mEq/L), were lower than 135mEq/L, the animals could be said to be moderately hyponatremic [29, 30]. Hyponatremia could result from a number of factors. It could be Pseudohyponatremia, which is secondary to hyperlipidemia [31, 32] or hyperproteinemia [33]. It could also be translocational; as a result of other highly osmotically active solutes, eg mannitol, glucose etc in serum (34) or true, associated witha reduction in serum osmolality [35, 36]. It could also be classified as hypovolemic (due to mineralocorticoid deficiency) with urine concentrations of sodium above 20mEq/L [37].

Osim *et al.* [38] had shown that chronic consumption of thermally oxidized palm oil increases LDL levels in the extracellular fluid. Also, in our study, (ongoing; unpublished), chronic consumption of thermoxidized palm oil resulted in hyperprotienemia. In the present study, urine sodium levels were 46mEq/L (above 20mEq/L). Putting all of the afore mentioned facts together, the hyponatremia in this study may have been pseudo as well as hypovolemic. It is also possible that photoxidized and thermoxidized palm oil diets suppressed the secretion of aldosterone since the function of the hormone is the reabsorption of sodium ions from the renal tubules to increase sodium concentration [1,39]. Unfortunately, plasma hormone levels could not be measured owing to technical reasons.

Chloride levels are known to go parallel with sodium levels, for wherever sodium goes, chloride follows to balance the electrochemical gradent, for these are the major extracellular fluid electrolytes [39]. This may explain why the chloride levels are in positive correlation with sodium levels.

Potassium was significantly(P<0.05 and P<0.01 respectively) higher in the photoxidized and thermoxidized palm oil fed groups. Potassium is the major intracellular cation. Under normal circumstances, it is secreted into the urine at the distal convoluted tubule of the nephron in exchange for sodium under the influence of aldosterone [40]. Therefore, the urine concentration of potassium is usually higher than the plasma concentrations [40].

In this study however, the plasma concentration of the PPO and TPO diet fed animals (5.42±0.60 and 8.10±0.32) were significantly higher than that of control and FPO diet fed animals. Hyperkalemia can also be pseudo [40]; due to cell shift [41], Impaired renal excretion [42] which can be caused by decreased renal delivery of sodium [43]; decreased mineralocorticoid activity [44] or a distal tubular defect [40, 45]. In the present study, it may have been as a result of impaired renal excretion. This is because in a previous study [22] we showed that chronic consumption of photo and thermoxidized palm oil reduced GFR. Reduced GFR slows tubular movement of fluid giving enough time for reabsorption hence reducing the sodium delivered to the distal tubule [42]. The picture in our study is also typical of hemolytic anemia where there is usually hyperkalemia but low potassium urine levels [46]. This may also have been the case here because chronic consumption of TPO leads to hemolytic anaemia [47]. Hyperkalemia with decresed potassium excretion is also seen in acute or chronic kidney disease [48]. Thermoxidization generates reactive oxygen species which are destructive to tissues including the kidneys and adrenal glands [38]. It is possible therefore that the culprit here may also have been a diseased kidney.

The bicarbonate levels in the pllasma and urine of the TPO group was significantly lower than control, FPO and PPO values while that of PPO was significantly kower than FPO only. This picture is typical of a hyponatremic state as reported by DeCaux *et al*. [49] who showed that alkalosis was induced by a hyponatremia. As has been shown in the present study, PPO and TPO caused hyponatremia. This study is therefore inline with the study of DeCaux *et al*. [49].

The study also went ahead to show that low plasma bicarbonate was a simple laboratory test done to distinguish between adrenocortical induced hyponatremia and SIADH induced hyponatremia. In another article, Zahedi [50] stated that the adrenocortical deficiency leads to the accumulation of hydrogen ions in circulation; and that the bicarbonate buffering system acts to reduce this acidity by neutralising it and this therefore leads to low bicaarbnate levels in plasma.The low bicarbonate ion levels in our study may therefore have been as a result of its consumption by hydrogen ions which may have increased in the plasma as a result of TPO and PPO diets.

CONCLUSION

We conclude that chronic consumption of oxidized palm oil causes a derrangement in the kidneys ability to handle elcttrolytes.

RECOMMENDATIONS

Measurement of pH, aldosterone and ADH levels were outside the scope of this study. We recommend further studies with the above parameters incuded to clarify the results we got.

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