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Original Research Article

Chronic Consumption of Oxidized Palm Oil Diets Increase Renal NA^+/K^+ATPASE and K^+/H^+ATPASE Activities, Reduce SGLUT2 and Increase Aldosterone Levels in Wistar Rats

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Abstract

This study aimed at finding out whether chronic consumption of oxidized palm oil affects the expression and/or activity of the renal transport proteins involved in the transportation of the electrolytes and glucose. 20 male Wistar rats weighing 120-140gms at the beginning of the experiment were randomly divided into four groups namely: control group, fresh palm oil diet-fed group (FPO); photoxidized palm oil diet-fed group (PPO), and Thermoxidized palm oil diet-fed group (TPO). The control group received normal rat chow while the oil-fed groups received 15% of the respective palm oil diet regimen in addition to tap water for 90 days. After 90 days, the animals were sacrificed, and blood samples collected while the kidneys were excised for biochemical analyses. Results showed that aldosterone levels in the PPO and TPO were significantly ($P<0.01$ and $P<0.001$ respectively) higher than that of control with the levels in the TPO being significantly ($P<0.001$) higher than PPO. Na^+/K^+ATPase and H^+/K^+ATPase activities were significantly ($P<0.01$; $P<0.001$, $P<0.001$) higher in FPO, PPO and TPO compared with control; with the Na^+/K^+ATPase activities in TPO being significantly ($P<0.001$ and $P<0.05$) than FPO and PPO respectively; while K^+/H^+ATPase activities in TPO were significantly ($P< 0.001$; $P<0.001$) higher than FPO and PPO respectively. SGLUT2 concentration in PPO was significantly($P<0.001$) lower than control and FPO; and significantly ($P<0.001$) lower in TPO when compared with control and PPO and FPO. Therefore, chronic consumption of photo-and thermoxidized palm oil diets increases Na^+/K^+ATPase, and H^+/K^+ATPase activities, aldosterone levels but lowers SGLUT2 levels in Wistar rats.

Keywords: Chronic consumption, palm oil, electrolytes, tropical plant cultivated, SGLUT2.

INTRODUCTION

African oil palm (*Elaeis guineensis*) is a popularly known tropical plant cultivated mostly to produce palm oil. It is the world's highest yielding and least expensive vegetable oil, making it the preferred cooking oil for millions of people globally and a source of biodiesel (Vijay, *et al*, 2016). It is the most important tropical vegetable oil globally when measured in terms of both production and its importance to trade, accounting for one-third of vegetable oil production in 2020 (Ritchie, 2021). Palm oil has a balanced percentage of fatty acids, which comprise 50% of saturated fatty acids, 40% monosaturated 10% polyunsaturated fatty

acids (Ayeleso and Oguntibeju, 2012). This balanced composition of fatty acid allows red palm oil to stay at a semi-solid state and is more resistant to lipid oxidation than other vegetable oils that contain high monounsaturated acids (Tan *et al.*, 2021). Palm oil is rich in minor components, including carotenoids (precursor of vitamin, tocopherols and tocotrienols vitamin E), sterols, phospholipids, squalene, aliphatic hydrocarbons and aliphatic alcohols (Goh, 1985).

Palm oil has been a vital resource in the majority of Nigerians diet. Unfortunately, it is mostly consumed in various states of oxidation due to some dangerous culinary process such as repeated heating (Ebong *et al.*, 1999). Due to this repeated heating especially during frying of bean cakes and other food, there occurs oxidative changes in the physical appearance and chemical nature of the oil. Some of the chemical reactions that occur during the frying of oils are hydrolysis, oxidation, and polymerization (Falade and Oboh, 2015; Li, *et al.*, 2016). The hydrolytic and oxidative reactions that occurred during frying of foods with oil result in the increment of free fatty acids, reactive oxygen species (ROS), and trans fatty acids (Almeida, *et al.*, 2019). These foods can induce organ failure and histopathology changes on different organs like the heart, intestinal mucosa, liver, and kidney when consumed (Alaam, *et al.*, 2012; Boniface and Ejimofor, 2014). Also, different chemical mechanisms such as autoxidation and photosensitized oxidation occur during storage and are responsible for the oxidation of edible oils. Autoxidation is a reaction between unsaturated fatty acids, (regardless of whether they are in their free state or esterified as a triglyceride molecule) and oxygen. These reactions originate hydroperoxides, which are rapidly decomposed to aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Choe & Min, 2006; Adetola *et al*., 2016). Changes in oil quality during inappropriate storage conditions are still a major issue from the health perspective (Almeida, *et al.*, 2018). Also, most of the oils erroneously referred to as fresh palm oil is photoxidized (Beshel and Beshel, 2019). Chronic consumption of these oils is known to cause derangement of electrolyte balance (Beshel, *et al.*, 2018, Beshel and Beshel, 2019); renal glycosuria and distortion of renal function indices (Beshel *et al.*, 2019); reduction in GFR and increase in blood pressure (Beshel, *et al*., 2014).

Renal transport proteins such as Na^+/K^+ ATPase, H^+/K^+ATPase, SGLUT2, play vital roles in the functioning of the kidneys, which are vital organs responsible for filtering waste products from the blood and regulating fluid and electrolyte balance in the body. These proteins are involved in the transport of various substances across the renal tubules, facilitating processes such as reabsorption of essential nutrients and ions, secretion of waste products, and maintenance of acid-base balance (Drozdzik *et al*., 2021).

The Na^+/K^+ pump is an electrogenic transmembrane ATPase first discovered in 1957 and situated in the outer plasma membrane of the cells, on the cytosolic side. (Pivovarov, 2018). The ATPase pump helps to maintain osmotic equilibrium and membrane potential by regulating sodium and potassium movement in cells. This pump maintains the gradient of a higher concentration of sodium extracellularly and a higher level of potassium intracellularly. It plays a crucial role on other physiological processes, such as maintenance of filtering waste products in the nephrons (kidneys), sperm motility, and production of the neuronal action potential (Clausen *et al.*, 2017).

The kidneys have a high level of expression of the Na^+/K^+-ATPase. The renal proximal tubule mediates over 50% of the filtered Na^+ reabsorption (Curthoys and Moe, 2014). The transport of Na^+ out of the tubules is done most often by sodium hydrogen exchangers and sodium glucose transporters 1 and 2. It must also be noted that the Na^+/K^+ATPases are responsible for the extrusion of Na^+. This sodium gradient is necessary for the kidney to filter waste products in the blood, reabsorb amino acids, reabsorb glucose, regulate electrolyte levels in the blood, and to maintain pH (el Mernissi, 1991).

The H^+/K^+-ATPases on the other hand use the energy of ATP hydrolysis to pump hydrogen (H^+) (Ahn and Kone. 1995) and potassium (K^+) ions against their concentration gradients. These enzymes are classified as P-type ATPases because they form a high-energy phosphorylated intermediate during the catalytic cycle. Two renal H^+/K^+ATPases have been identified that contain the catalytic sub-units HK^+1 or HK^+2 (Crowson and Shull, 1992). Both forms acidify the tubular fluid and reabsorb K^+ in the kidney (Gumz, *et al.*, 2010). Both isoforms are found primarily in the collecting duct (Ahn, *et al.*, 1996; Bastani, 1995. Aldosterone administration and metabolic acidosis stimulate the activity of H^+/K^+ ATPase in all collecting duct segments, whereas hypokalemia has only a limited effect on H^+/K^+ATPase activity. On the other hand, hypokalemia, as well as metabolic acidosis, stimulates H^+/K^+ ATPase activity in the collecting duct segments, whereas aldosterone administration alone plays a minor role in the regulation of this enzyme (Garg, 1991).

The sodium–glucose transporters (SGLUT) are a family of transporters from the SLC5 gene family that couple sodium to the transport of sugars most often glucose, galactose, and mannose and, in addition, myoinositol and short-chain fatty acids (Szablewski, 2017). The SGLUTs transport glucose against a concentration gradient, using the electrochemical force of the sodium gradient to accomplish this (Sun *et al.*, 2023). SGULTs work in either proximal tubule cells or intestinal cells transporting glucose into the requisite cell at the luminal surface followed by GLUT-mediated transport of glucose into the bloodstream at the

basolateral membrane (Hermansen and Mortensen, 2007). In the kidney, SGLUT2, and to a lesser extent SGLUT1, account for more than 90% and nearly 3%, respectively, of glucose reabsorption from the glomerular ultrafiltrate.

The relationship between aldosterone and renal transport proteins, particularly the Na^+/K^+ATPase and SGLUT2), is essential for understanding the regulation of electrolyte balance and fluid homeostasis in the kidney. Aldosterone functions in the regulation of sodium and potassium balance in the body. One of its primary actions in the kidney is to increase the activity of the Na^+/K^+ATPase pump. Aldosterone binds to mineralocorticoid receptors (MRs) located in the distal convoluted tubule (DCT) and the cortical collecting duct (CCD) of the nephron, leading to the transcriptional upregulation of Na^+/K^+ATPase pump expression and synthesis (Lifton, *et al.*, 2001).

In our previous studies (Beshel *et al.*, 2018, Beshel and Beshel, 2019), derangements in the electrolyte levels as well as renal glycosuria even though glucose levels were normal were reported in rats fed with oxidized palm oil diets. Aldosterone is known to regulate the balance of these electrolytes in the body. It is also known to affect the activities of the transport proteins (Na^+/K^+ATPase, H^+/K^+ATPase and SGLUT 2) responsible for regulating electrolytes and glucose in the kidneys. The aim of this study is therefore to investigate the role played by renal transport proteins namely: Na+/K+ATPase, H+/K+ATPase, SGLUT and aldosterone on electrolyte balance and renal glycosuria in rats fed on different forms of oxidized palm oil diets.

MATERIALS AND METHODS

Animals

A total of twenty (20) healthy adult male Wistar rats weighting between 140-160g were used in this study. The animals were kept at a room temperature of 29 ± 20C temperature, and a relative humidity of 40-55%, and had free access to water and normal rat chow. They were allowed to acclimatize for two weeks before the commencement of the experiments. All the animal experiments were done in accordance with the guidelines of the Faculty of Basic Medical Sciences Ethical Committee on the use of laboratory animals (approval no. 296PHY3724).

Acquisition and preparation of different forms of palm oil

We purchased palm oil directly from the palm oil mill at Odukpani Palm Oil Mill in Obudu Local Government Area of Cross River State, Nigeria and immediately stored inside a black container. It was kept in a cool dry room and not exposed to sunlight or heat. The palm oil was divided into three portions. The first palm oil portion was kept fresh and kept in a dark container. The photo-oxidized palm oil was prepared by exposing the second portion of fresh palm oil to sun light for 5 hours daily for 15 days to mimic what happens in the open market. This is according to Beshel *et al.*, (2014), with slight modification. The thermo-oxidized palm oil was prepared by exposing the third portion of fresh palm oil to five rounds of heating for 10 minutes each. After each round of heating, the palm oil was made to cool down before reheating at 190°C. This was to mimic what is used in frying akara, yam, etc.

Formulation of Palm Oil diets

The palm oil (fresh palm oil, photo-oxidised and thermo-oxidised palm oil) diets were formulated as previously described by Beshel *et al.*, (2018). Briefly, 15g of fresh, photoxidised and thermoxidized palm oil were mixed with 85g of rat chow to make 15% fresh, photoxidised and thermoxidised palm oil diets. This is the average percentage palm oil composition of a typical palm oil diet (Osim, *et al.*, 1994).

Experimental Protocol

Animals were housed in separate labelled metabolic cages. They were randomly divided into four (4) groups, each containing five (5) animals. Group 1 served as the control group. Group 2 were fed with 15% fresh palm oil (FPO) diet. Group 3 were fed with 15% photo-oxidized palm oil (PPO) diet. Group 4 were fed with 15% thermo-oxidized palm oil (TPO) for 90 days.

Collection of Samples

Tissue Samples

The animals anaesthetised with 60mg/kg pentobarbital and sacrificed. Blood samples of the animals were collected via cardiac puncture using 5ml syringes attached to 21G needlesi A middle abdominal incision was made anteriorly, and the kidneys were excised. The kidneys were trimmed of connective tissues and rinsed with normal saline to remove excess blood.

Kidney Biochemicals Analysis

The kidney tissues were rinsed using ice-cold 0.1M phosphate buffer and blotted with filter paper before weighing on electrical weighing scale to determine the weight. The kidney tissues were cut diced into smaller pieces in 10 volumes of ice-cold homogenizing buffer (0.1M phosphate buffer, pH 7.4and homogenized using a Tefflon homogenizer. The resulting homogenates were centrifuged using cold centrifuge at 4°C at 10,000g for 15minutes. The supernatants were collected and used for tissue biochemical estimations.

Determination of Kidney Na^+/K^+ ATPase Activity

Kidney tissue Na^+/K^+ ATPase activities was determined following the method of Bewaji *et al.*, (1985) as modified by Salami *et al.*, (2021). A reaction mixture containing an aliquot of 200Mm NaCl/40Mm KCl/60Mm tris buffer (Ph 7.4), 80Mm MgCl2.6H20, 20Mm EGTA and enzyme source was incubated for 30

minutes at 370C. Therefore, 8Mm ATP was added and then incubated for 30 minutes at same temperature. 5% SDS was then added to stop the reaction and centrifuge at 3000rpm for 5 minutes at 40C. Reagent mixture (H2S04- Ammonium molybdate-Ascorbate) was added and allowed to stand at room temperature for 20 minutes for colour development, after which the absorbances were read at 725nm. The absorbances obtained were then extrapolated from the standard phosphate curve to obtain concentration of inorganic phosphate. Na^+/K^+ATPase was expressed as µmole P/mg Prot./hr.

Determination of Kidney H^+/K^+ ATPase Activity

Kidney tissue H^+/K^+ATPase activities was determined following the method of Bewaji *et al*., (1985) as modified in Salami *et al*., (2021). A reaction mixture containing an aliquot of 200Mm NaCl/40Mm KCl/60Mm tris buffer (Ph 7.4), 20Mm EGTA and enzyme source was incubated for 30 minutes at 370C. Thereafter, 8mM ATP was added and then incubated for 30 minutes at same temperature. 5% SDS was then added to stop the reaction and centrifuge at 3000rpm for 5 minutes at 40°C. Reagent mixture (H_2S0_4- Ammonium molybdate-Ascobate) was added and allowed to stand at room temperature for 20 minutes for colour development, after which the absorbances were read at 725nm. The absorbances obtained were then extrapolated from the standard phosphate curve to obtain concentration of inorganic phosphate. H^+/K^+ ATPase was expressed as µmole P/mg Prot./hr.

Assay of GLUT2 by immunohistochemistry

Immunohistochemistry staining study was carried out using (SLC5A2; Cat no. E-AB-93255) monoclonal antibodies in immunoperoxidase techniques as described by Hsu *et al*., (1981) with modifications (Oyagbemi *et al*., 2017; 2018). Briefly, to determine the expression of SLC5A2 protein expressions in the kidney tissues, fixed tissues were embedded in paraffin and 5µm thickness of it were sectioned on charged slides. These were subsequently deparaffinized in xylene and rehydrated with varying grades of alcohol (100% to 70%). Antigen retrieval was carried out by immersing the slides in citrate buffer at 95-100^{0}C for 25 minutes with subsequent peroxidase quenching in 3% H_2O_2/methanol solution. The sections were blocked in goat serum followed by a 2 hours incubation at 40C in the SLC5A2 primary antibodies. Detection of bound antibody was carried out using biotinylated (goat anti-rabbit, 2.0 µg/mL) secondary antibody and subsequently, streptavidin peroxidase (HRP-streptavidin) according to manufacturer's protocol (Elascience Biotechnology Inc., Houston, Teas). Reaction product was enhanced with DAB for 1-3 minutes and counter-stained with high-definition hematoxylin (Enzo, New York). The sections were subsequently dehydrated in ethanol, cleared in xylene. The slides were covered with coverslips and sealed with resinous solution. The immunoreactive positive expressions of SLC5A2 anti-rabbit intensive regions were viewed starting from low magnification on each slice then with 400× magnifications using a photo microscope (Olympus) and a digital camera (Toupcam, Touptek Photonics, Zhejiang, China). The immunoreactivity was quantified with ImageJ software version 1.51.

Statistical Analysis

GraphPad prism version 8.0.2(263) was used for the analysis of data**.** ANOVA was used for analysis of Data followed by Turkeys adhoc test. The results were expressed as mean ±SEM.

RESULTS AND DISCUSSION

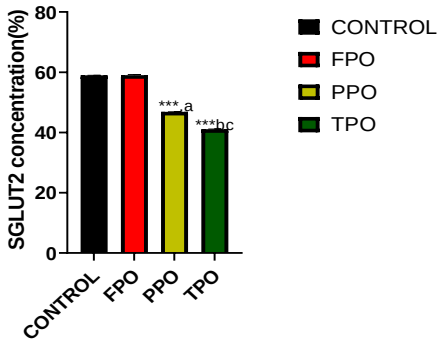

Fig 1: The mean±SEM percentage concentration of SGLUT2 in control, FPO, PPO and TPO

Fig 1 Shows the mean±SEM percentage concentration of SGLUT2 in all the groups namely: control (58.93±0.35%); FPO (59.0±0.26%); PPO (46.83±0.1%); and TPO (41.06±0.93%).

There was no significant difference in the percentage concentration of SGLUT2 between the control and FPO. However, there was a significant (P<0.001) decrease in SGLUT2 expression in PPO and TPO compared with the control. Also, the percentage concentration of SGLUT2 in TPO was significantly lower than PPO.

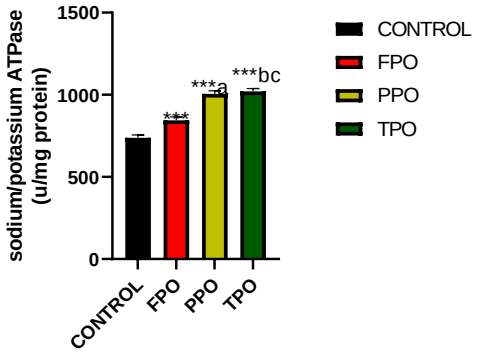

Fig 2: **Graph showing the mean ± SEM expression/activity of sodium/ potassium ATPase in the control, FPO, PPO and TPO groups**

Fig 2 shows the comparison the comparison of the activity of Na^+/K^+ATPase between the control and test groups. All test groups with values (u/mg protein): 844.9±20.18; 1006±18.94; 1021±17.20 respectively FPO, PPO, TPO; had a significantly ($p < 0.05$) higher Na^+/K^+-ATPase activities when compared to the control (737.5±18.02). PPO and TPO sodium/potassium ATPase activity was also significantly ($P \leq 0.05$) higher than control, while that of TPO was significantly ($P<0.001$) higher than PPO.

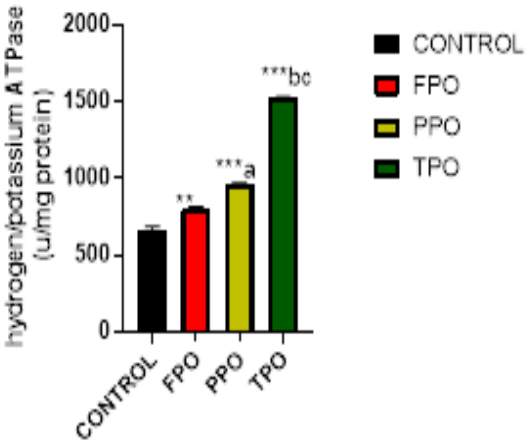

Fig 3: Graph showing the activity/expression of H^+/K^+ATPase in the kidneys of control FPO (PPO and) animals

Fig 3 shows the activity/expression of H^+/K^+ATPase in the kidneys of control (665.1±18.70u/mg protein), FPO (793.0±25.17[u/mg protein]), PPO (958.1±12.85[u/mg protein]), and TPO (1521±9.436[u/mg protein) animals. The activity in all the test groups were significantly ($P<0.001$) higher than that of the control. TPO and PPO animals' H^+/K^+ATPase activity were also significantly ($P<0.001$) higher than that of FPO animals, while the H^+/K^+ATPase activity in TPO was significantly ($P<0.05$) that of PPO group.

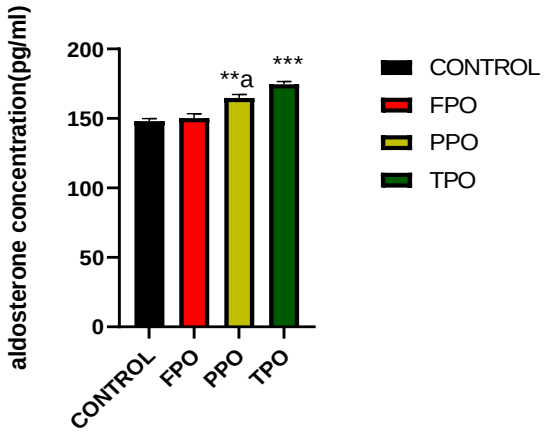

Fig 4: Shows the mean±SEM concentration of aldosterone in the control, TPO PPO and TPO animals.

Fig 4 shows the mean±SEM concentration of aldosterone in the control, TPO PPO and TPO animals. Aldosterone concentrations in all test groups were significantly ($P<0.001$) higher than that of control (148.0± 1.96). The levels in the TPO (174.6±2.0pg/ml) were also significantly higher than that of FPO (150±3.263pg/ml) and PPO (169±2.628pg/ml) groups.

Table 1: The comparison of the mean ± SEM concentrations of Na+, K+, and glucose in the serum and urine of control, FPO, PPO and TPO groups

PARAMETER	CONTROL	FPO	PPO	TPO
Na+ in serum (meq/L)	136.6±0.232	$135.2±0.321^{*}$	$128.65±0.244^{***a}$	$125.32±0.20^{***bc}$
Na+ in urine (meq/L)	97.8±1.20	$99.6±1.32^{ns}$	$103±1.02^{**d}$	$120.18±1.21^{***bc}$
K+ in blood (meq/L)	3.320±0.02	3.46±0.012*	$5.78±0.014^{***a}$	$6.23±.052^{***bc}$
K+ in urine (meq/L)	15.24±0.14	$15.53±0.22^{ns}$	$19.33±0.15^{***a}$	$19.85±0.18^{***bf}$
Glucose in blood (mg/L)	121±1.58	$120±2.32^{ns}$	$121.32±2.42^{ns}$	$121.36±2.81^{ns}$
Glucose in urine (mg/dL)	0.11±0.02	$0.12±0.01^{ns}$	$3.0±0.07^{***a}$	$5.0±0.15^{***bc}$

ns; *; **; *** = not significant; P<0.05; P<0.01, P<0.001 vs control
d; b = not significant; P<0.001 vs FPO
f; c = not significant, P<0.001) vs PPO

Table 1 shows the comparison of mean ± SEM of Na^+, K^+, and glucose concentration in the serum and urine of control, FPO, PPO and TPO animals. There was a significant ($P<0.05$, $P<0.001$, $P<0.001$) decrease in the concentration of sodium in the FPO, PPO and TPO respectively when compared to the control group. There was also a significant ($P<0.001$) decrease in PPO compared with FPO and ($P<0.001$) between TPO and PPO. When the urine Na^+ concentrations were compared, there was no significant difference between the control and FPO, but there was a significant ($P<0.01$; $P<0.001$) increase in Na^+ concentration when PPO and FPO were respectively compared with control.

The potassium concentration in the serum of FPO, PPO and TPO were significantly ($P<0.05$; $P<0.01$, $P<0.001$ respectively) higher than that of control animals, with TPO being significantly ($P<0.001$, $P<0.001$) higher than FPO and PPO, respectively. When urine K^+ concentrations were compared, there was no

significant difference between the control and FPO but there was a significant (P<0.001) difference when PPO and TPO were respectively compared with control, when TPO was compared with FPO (P<0.001), but no significant difference between TPO and PPO.

There was no significant difference in the serum glucose concentration when all the groups were compared with each other and no significant difference too when the urine glucose concentration of the FPO animals were compared with the controls. However, there was a significant (P<0.001; P<0.001) increase in urine glucose concentration when PPO and TPO were respectively compared with control. There was a significant (P<0.001) increase in urine glucose concentration of PPO compared with FPO and a significant (P<0.001, P<0.001) increase too when TPO was compared with FPO and PPO respectively.

The present study shows that SGLUTs expression in the PPO and TPO groups was significantly lower than the control and FPO groups while the expression/activities of renal Na^+/K^+ATPase and H^+/K^+ATPase were significantly higher in FPO, PPO and TPO compared with control. The aldosterone concentration in PPO and TPO groups was significantly higher than control and FPO groups. When the levels of Na^+ and K^+ in serum were compared, there was a significant decrease in sodium, but a significant increase in potassium when the FPO, PPO, and TPO groups were compared with control. There was no significant difference in glucose concentration in serum when all the groups were compared with each other but there was a significant increase in the glucose concentration in the urine of PPO and TPO compared with control and FPO.

In our previous studies (Beshel, *et al.*, 2018, Beshel and Beshel, 2019 it was observed that the plasma levels of Na^+ dropped, while that of K^+ increased in circulation. In this study, aldosterone levels were very high. The most potent stimulus of aldosterone release is increased potassium concentration in circulation (Lote, 2007). The high concentration of potassium seen in this study have been because of chronic consumption of oxidized palm oil. Previous studies have shown that oxidized palm oil generates reactive oxygen species which when chronically consumed, can result in oxidative stress which is known to cause generation of reactive oxygen species (ROS) (Amama *et al.*, 2022); destruction of tissues and cell lysis (Mesembe, *et al.*, 2004). The chronic consumption of PPO- and TPO- diets may have led to cell lysis which may have resulted in the high potassium levels seen in our previous studies High aldosterone levels should have reduced the levels of potassium ions in circulation. Unfortunately, they remained high. There are other factors that may lead to an increase in K^+ in serum. Because potassium is the most potent stimulus for aldosterone release, it is not surprising that its concentration remained high in PPO and TPO compared to control and FPO groups.

Aldosterone upregulates the renal ATPases, Na+/K+ATPase being the most affected (Errey *et al.*, 2001). In this study, the activity of Na^+/K^+ATPase and H^+/K^+ATPase was higher in the test groups with the Na^+/K^+ATPase increasing much more than H^+/K^+ATPase in the TPO-fed animals. This is in line with previous studies which have shown that aldosterone increases both expression and activity of the two transport proteins even though its effect on Na^+/K^+ ATPase is much more than it acts on H^+/K^+ATPases (Errey, *et al.*, 2001). The increased activity of Na^+/K^+ATPase seen in this study may have helped to reduce the decrease in sodium levels especially when we consider the fact that the pump sends out three sodium ions in exchange for two potassium ions.

H^+/K^+ATPase is expressed constitutively along the length of the collecting duct and is responsible for H^+ secretion and K^+ reabsorption under normal conditions and may be stimulated with acid-base perturbations and/or K^+ depletion (Silver and Soleimani, 1999). The increased H^+/K^+ATPase seen in the oxidized palm oil-fed animals in this study may have been triggered by acidosis rather than K^+ depletion as there was an abundance of potassium ions in circulation. Increased activity of this pump too may also have contributed to the increased K^+ levels seeing as they lead to reabsorption of K^+ and excretion of H^+.

The SGLUT2 levels in this study was significantly reduced in the FPO and TPO test animals. In our previous study (Beshel, *et al.*, 2019) there was renal glycosuria even when there were normal blood glucose levels. In this study, we had the same results. This study has also shown that the expression of SGLUT2 was significantly reduced in the PPO and TPO groups. This means they may either have been destroyed or the oxidant content of then oxidized palm oil may have acted as an inhibitor therefore increasing not only the concentration of Na^+ in the urine of the PPO and TPO animals, but also the glucose concentration. The oxidized palm oil diet may have affected its expression of SGLUT such that expression of it overwhelmed the indirectly positive effect of aldosterone on its activity. The low percentage expression clearly explains the appearance of glucose in urine despite normal glucose levels in blood.

We were however not able to explain in what way the chronic consumption of oxidized palm oil caused an increase in the expression of renal Na^+/K^+ATPases and H^+/K^+ATPases, but reduced that of SGLUT2 despite high aldosterone levels.

CONCLUSION

We therefore conclude that the changes in the expression of renal Na^+/K^+ATPases and H^+/K^+ATPases

but reduction in SGLUT2 levels are responsible for the derangement in electrolyte levels seen in this study and previous ones, and renal glycosuria.

Also, despite the high levels of aldosterone in circulation, potassium levels remained high. This means chronic consumption of oxidized palm oil causes hyperaldosteronism.

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