ROLE OF VIRGIN COCONUT OIL ON MALE REPRODUCTIVE FUNCTIONS IN RATS ADMINISTERED L-NAME

OLUDARE, G.O.[1]*, AFOLAYAN, G.O.[2], AKANBI, K.A.[1], OKEKE, M.O.[1], SALAU, L.B.[1] AND MORAKINYO, A.O.[1]
[1]Department of Physiology, [2] Department of Pharmacology, Therapeutics and Toxicology, University of Lagos, Lagos, Nigeria

ABSTRACT

Nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) has been reported to diminish sperm functions, reduce fertility, and affect male sexual function. Both nitric oxide (NO) and essential oils are known to stimulate sperm motility. We investigated the role of virgin coconut oil (VCO) on sperm functions and biochemical parameters in rats administered L-NAME. Twenty-four male rats weighing 90–100 g were divided into four groups of 6 rats each. Group I – control, Group II – L-NAME (50 mg/kg b.w), Group III – L-NAME + 10% VCO enriched diet, and Group IV – 10% VCO enriched diet only. Rats were fed for 3 weeks, and the caudal epididymis was used for the assessment of sperm parameters. Blood samples were withdrawn to assay biochemical parameters. Sperm count and motility were significantly reduced in rats administered L-NAME and L-NAME + VCO. VCO supplementation with L-NAME increased testosterone and nitric oxide levels that were decreased by L-NAME ($p< 0.05$). The cardiac risk ratio was increased in L-NAME-administered rats. Malondialdehyde level was increased in the L-NAME group while the antioxidant enzymes reduced glutathione, superoxide dismutase and catalase activities were reduced. However, VCO supplementation reduced the effect of L-NAME on malondialdehyde and increased the antioxidant enzymes. The weights of the animals remained the same even though there was an increase in food consumption during the first week of supplementation in the VCO-supplemented rats. VCO attenuated the adverse effects of L-NAME on lipid profile, nitric oxide, antioxidant status, and testosterone in male rats.

Keywords: Antioxidant, L-NAME, Nitric oxide, Sperm, Testosterone, Virgin coconut oil
***Correspondence:** goludare@unilag.edu.ng, +2347035363115

INTRODUCTION

Infertility is a universal problem which affects about 10-15% of the global population [1]. An estimated one in seven couples globally has some difficulty with conception [2]. Males are accountable for about 20-30% of infertility cases, contributing to the causes for infertility in about 50% of cases [3]. Various causes have been attributed to the reason of infertility in males such as varicocele, infection, oxidative stress, ejaculation issues, undescended testicles, immunological effects of antibodies, gonadotropin deficiency, sexual function disorders, tumours, hormone imbalances, sperm transport defects, genetic defects, lifestyle, and many other causes which are without a clear aetiology [3].

Nitric oxide (NO) is a cell-signalling substance produced by several mammalian cells and plays a vital role in male reproduction. A decrease or excess of its production has been implicated in affecting various male reproductive functions such as sperm motility, viability, and morphology. Essential functions like acrosome reaction, hyperactivation, apoptosis, capacitation, and fusion of the sperm cells with the oocyte are critical steps NO could affect [4, 5]. Nitric oxide has the potential to get oxidized thereby producing harmful radicals known as reactive oxygen and nitrogen species (ROS and RNS). These free radicals are responsible for inducing oxidative stress and are believed to be responsible for the cause of idiopathic male infertility [6]. The plasma membrane of sperm cells is very rich in polyunsaturated fatty acids and these polyunsaturated fatty acids are highly susceptible to attack by ROS and RNS resulting in impaired membrane fluidity and mobility [7]. Thus, its physiological homeostasis is vital to male organ functions.

Nutrition has been found to affect sperm quality either negatively or positively [8]. Since the 1990s, the National Institute of Health has funded research on dietary interventions to manage or reduce hypertension [9]. This approach showed some promise by reducing blood pressure in individuals with high blood pressure and in those without blood pressure. [10]. Since hypertension contributes to male infertility, there is increased attention on the role of nutrition in male infertility [11]. Sperm quality is negatively affected by diet types rich in saturated fatty acids (SFA) and low in polyunsaturated fatty acids (PUFA), while diets rich in unsaturated fatty acids have been known to improve sperm quality [11]. Coconut oil is historically known for its medicinal and nutritional value. The major fatty acids in coconut oil (lauric acid, capric and caprylic acid) have been found to possess antioxidant function, suppress microbial and viral activities, and have anti-ulcerogenic effects [12, 13]. The use of coconut oil for its nutritional, medicinal, and aesthetic purposes is on

the increase generally. Nature remains a major source of new lead compounds for drug development to resolve several of man's unknown and unresolved problems [14]. This study explored the role of VCO supplementation on impaired male rats' reproductive function induced by L-NAME a NOS inhibitor.

MATERIALS AND METHODS

Experimental animals

Twenty-four male Sprague-Dawley rats weighing between 90-100g were used in this study. The rats were maintained under similar environmental conditions and were allowed access to food and water *ad libitum*. This research work was approved by the College of Medicine University of Lagos research and ethics committee (CMUL/ACUREC/06/21/865).

Experimental groups

- Group I (control group); was fed with normal rat pelletized chow.
- Group II (L-NAME); received normal rat chow and was administered L-NAME (50mg/kg b.w.) intraperitoneally daily
- Group III (L-NAME + VCO diet); was administered L-NAME and received normal rat chow enriched with 10% VCO.
- Group IV (VCO only); received normal rat chow enriched with 10% VCO

Preparation of feed sample, proximate analysis, and food consumption

To the normal rat chow, 10 mL of VCO was added to 90 g of feed and thoroughly mixed to compound the 10% VCO-enriched diet. The feed sample for the control and VCO-enriched diets were then subjected to proximate analysis and fatty acid profile analysis. Food consumption was measured daily and reported as weekly average consumption. The feed samples were weighed and administered to the rats per group. Feed left over was weighed the following day and was subtracted from the quantity of feed administered the previous day to calculate food consumption.

Fatty acid profile

Agilent 7890B Gas chromatograph (Santa Clara United States) fitted with a flame ionization detector (FID) was employed to analyze fatty acid methyl esters as described by Dodds [15].

Sample collection

Following the completion of 21 days of feeding of the rats with VCO enriched diet and L-NAME. Blood samples were collected by cardiac puncture after the rats were euthanized by cervical dislocation. The samples were centrifuged at 3000 rpm to collect the serum samples. The serum sample was stored at - 4 ºC and used for the analysis of testosterone concentration. The epididymis was isolated for sperm parameters while the testes were excised, weighed, and used for the oxidative assay in the study.

Sperm count, morphology, and motility

The sperm count was accessed using the caudal epididymis. The sperm cells were counted with the aid of a light microscope (Olympus CX 21, Beijing, China) at 400X magnification. All the sperm parameters were determined as described in a previous study [16].

Oxidative stress parameters

The concentrations and activities of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione, were carried out in the homogenized testicular samples using standard established biochemical methods previously described in another study [16]. All absorbances were read using T70/UV/VIS spectrophotometer.

Lipid profile, Testosterone assay and Nitric oxide metabolites concentration

Serum lipid profile assay was carried out using ERBA Mannheim diagnostics kits (Mannheim, Germany). The chemistry analyzer used for this assay was the fully automated Mindray BS-120 (Shenzhen, China). Calculated indices were as described by Aluko [17].

$$\text{Atherogenic index} = \text{Log}_{10} \frac{Triglycerides}{High\ density\ lipoprotein - cholesterol}$$

- $\text{Atherogenic coefficient} = \frac{Total\ cholesterol - High\ density\ lipoprotein - cholesterol}{High\ density\ lipoprotein\ cholesterol}$
- $\text{Cardiac Risk Ratio} = \frac{Total\ Cholesterol}{High\ density\ lipoprotein - cholesterol}$

Elabscience ELISA kit (Wuhan, China) was used to measure the concentration of testosterone according to the manufacturer's protocol, while nitric oxide metabolites nitrite/nitrate (stable NO metabolites) concentrations were measured in the serum samples using a colourimetric assay kit (Enzo-life Science, Switzerland)

Statistical analysis

The data generated were analysed statistically using Graph Pad Prism version 6 software. One-way analysis of variance was used to analyse the values followed by Turkey's multiple comparison post-hoc tests. The significant value was taken when $p<0.05$. The reported results in this study were presented as mean ± standard error of the mean (SEM).

RESULTS

Animal weight and food consumption

Figure 1 shows the animal weights. The rats' weights were also not different from the control from week one through to week four. Food consumption was also not different during acclimatization among the groups (Figure 2). In the first week of enriched diet feeding, food consumption was significantly increased in the L-NAME + VCO and the VCO groups compared to the food consumption level in the control and the L-NAME groups ($p < 0.05$).

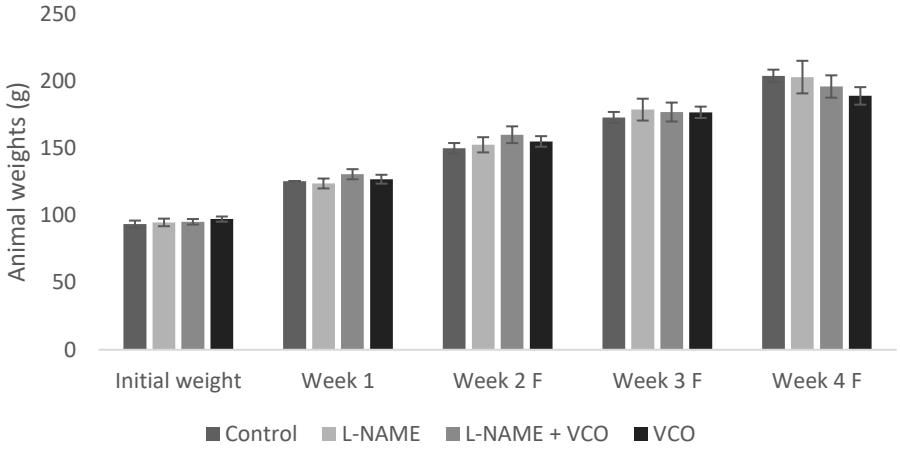

Figure 1: Animal weights male rats administered VCO enriched diet and L-NAME. F= feeding with a specially prepared diet

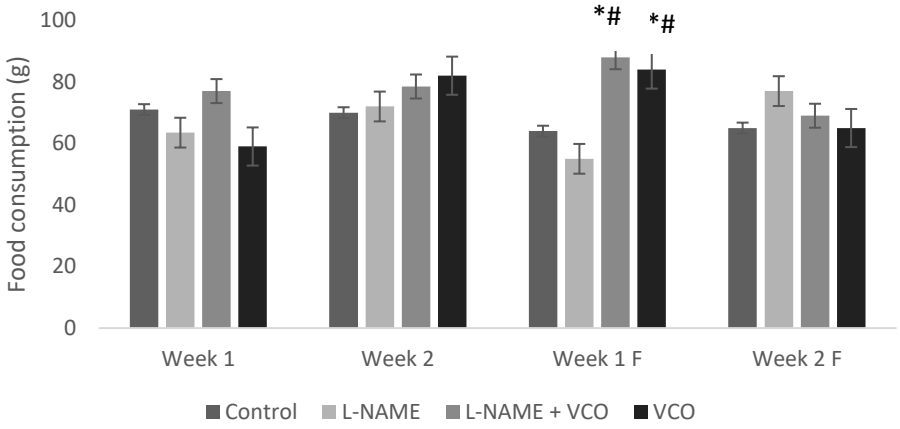

Figure 2: Food consumption pattern of male rats administered L-NAME and VCO enriched diet
*connotes statistical difference from Control, # indicates statistical difference with the L-NAME group, F= feeding with a specially prepared diet

Proximate analysis and fatty acid profile

Table 1 shows the proximate analysis results of the feed samples. The table showed that the VCO diet had about 5% more protein content than the control diet. The fat content was also about 4.6% higher than that of the control diet. However, the VCO diet had less moisture content compared to the control diet by about 5.3%. The crude fibre was also lesser in the VCO diet compared to the control diet by about 3.2%. As expected, the energy content of the VCO-enriched diet was higher (3730.27 kcal/kg) than that of the control diet (3010.43 kcal/kg).

Table 1: Feed sample proximate analysis for control and 10% VCO-enriched diet

	Carbohydrate %	Protein %	Crude fat %	Moisture %	ASH %	Crude fibre %	Energy kcal/kg
Control	43.545	15.405	7.29	16.8	4.53	12.43	3010.43
VCO-Enriched	42.15	20.215	11.995	11.575	4.8	9.265	3730.27

The results of the analyzed fatty acid profile of the feed sample are shown in Table 2. Twenty-eight fatty acid methyl esters were targeted in the oil samples of the control and VCO-enriched diet. Seventeen were detected in the control diet while 11 were not. In the VCO-enriched diet, twenty-two were detected in the oil sample while six were not detected. Those commonly detected had the same range in the control and the VCO-enriched diet except for palmitic acid which had about 3.95 mg/L more content than that of the control (11.00 mg/L). Decanoic acid, dodecanoic acid (Lauric acid), methyl myristate, pentadecanoic acid and nervonic acid methyl esters were the 5 fatty acids methyl esters detected in the oil of the VCO enriched diet but not in the control diet.

Table 2: Fatty acid methyl ester of feed sample

Compounds	Control diet	VCO enriched diet
Decanoic acid, methyl ester	N.D.	5.43 mg/L
Undecanoic acid, methyl ester	N.D.	N.D.
Dodecanoic acid, methyl ester	N.D.	6.05 mg/L
Tridecanoic acid, methyl ester	N.D.	N.D.
Methyl myristoleate	2.85 mg/L	3.02 mg/L
Methyl myristate	N.D.	2.98 mg/L
Pentadecanoic acid, methyl ester	N.D.	2.89 mg/L
Palmitoleic acid, methyl ester	4.03 mg/L	4.06 mg/L
Palmitic acid, methyl ester	11.00 mg/L	14.95 mg/L
cis-10-Heptadecenoic acid	2.71 mg/L	3.10 mg/L
gamma-Linolenic acid	2.77 mg/L	2.69 mg/L
9,12-Octadecadienoic acid	3.51 mg/L	3.58 mg/L
Oleic acid Methyl ester	8.92 mg/L	8.77 mg/L
Elaidic acid, methyl ester	5.15 mg/L	4.68 mg/L
Methyl stearate	7.13 mg/L	7.17 mg/L
Arachidonic acid, methyl ester	3.14 mg/L	3.16 mg/L
5,8,11,14,17-Eicosapentaenoic acid	3.66 mg/L	3.69 mg/L
8,11,14-Eicosatrienoic acid,	4.06 mg/L	3.07 mg/L
cis-11,14-Eicosadienoic acid	3.58 mg/L	3.61 mg/L
11-Eicosenoic acid,	3.49 mg/L	3.22 mg/L
Arachidic acid	N.D.	N.D.
Heneicosanoic acid,	N.D.	N.D.
Methyl 4,7,10,13,16,19-Docosahexaenoic acid	4.51 mg/L	4.03 mg/L
13-Docosenoic acid, methyl ester	3.35 mg/L	3.27 mg/L
Behenic acid, methyl ester	N.D.	N.D.
Tricosanoic acid, methyl ester	N.D.	N.D.
Lignoceric acid, methyl ester	3.46 mg/L	4.06 mg/L

Nervonic acid, methyl ester	N.D.	7.51 mg/L

N.D. = not detected

Sperm function, testosterone, and nitric oxide concentrations

The result of sperm analysis is presented in Table 3. The table shows that sperm count and progressive motility were significantly reduced in L-NAME-administered rats and the L-NAME + VCO-administered rats compared with the control values ($p < 0.05$). The VCO-only group showed a significant increase in sperm count and sperm motility compared with the L-NAME group. No significant difference was found in the group's normal morphology of the sperms. Figure 3 shows the testosterone concentration. The testosterone level was reduced in the L-NAME group ($p < 0.05$). VCO-enriched diet plus L-NAME increased testosterone concentration significantly when compared with testosterone concentration in the L-NAME group. A similar pattern of results was found in the nitric oxide levels in the rats that received L-NAME and VCO (Figure 4).

Table 3: Sperm function of rats administered virgin coconut oil and L-NAME

	Control	**L-NAME**	**L-NAME + VCO**	**VCO**
Sperm count (sperm/million 10^6)	72.03 ± 2.34	49.33 ± 3.5*	58.78 ± 2.41*	64.75 ± 2.64#
Sperm progressive motility (%)	77.83 ± 2.69	48.16 ± 6.19	61.33 ± 2.41*	76.16 ± 2.35#
Sperm normal morphology (%)	80.16 ± 2.57	76.33 ± 2.88	80.50 ± 3.05	76.16 ± 4.34

*Connotes statistical difference from control

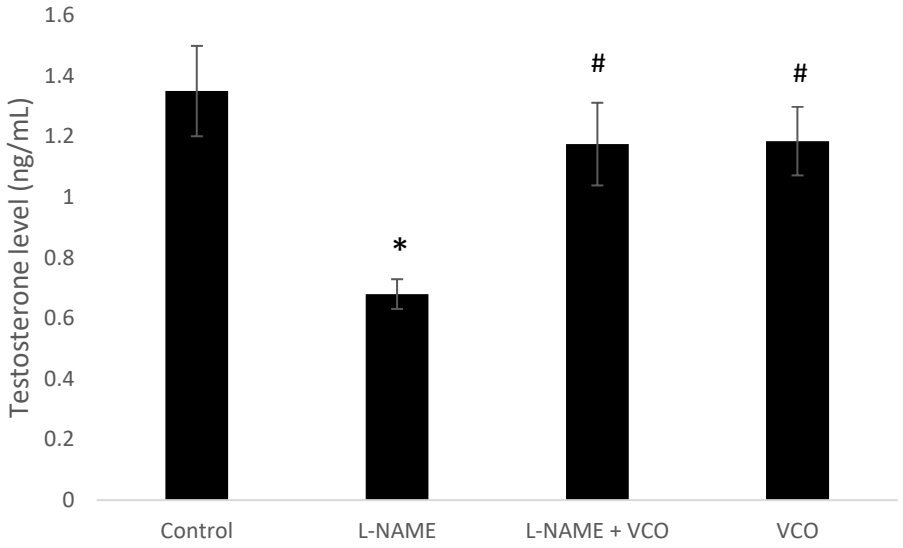

Figure 3: Serum testosterone level of male rats administered L-NAME and VCO
*Connotes statistical difference from control, # indicates statistical difference with the L-NAME group,

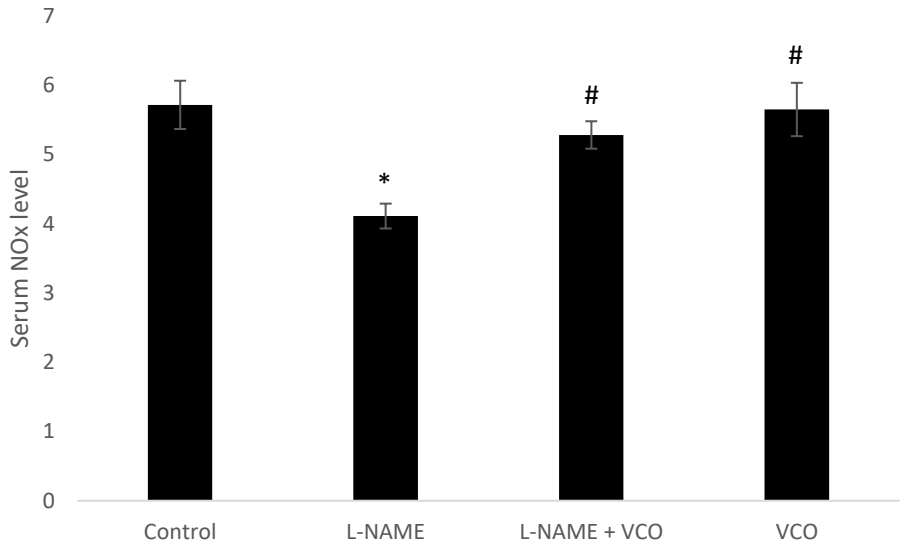

Figure 4: Nitric oxide metabolite level of male rats administered L-NAME and VCO
*Connotes statistical difference from control, # indicates statistical difference with the L-NAME group,

Oxidative stress indices

Table 4 shows the oxidative stress indices of the rats administered VCO and L-NAME. The MDA levels were significantly increased in the rats administered L-NAME ($p < 0.05$). MDA levels were reduced in the groups in which their diets were supplemented with VCO when compared with L-NAME. Reduced glutathione, as well as SOD and CAT levels, were depleted in the L-NAME group but the group whose diet was enriched with VCO and administered L-NAME had the values of the antioxidants restored significantly ($p < 0.05$).

Table 4: Lipid peroxidation and antioxidant levels of male rats fed with VCO diet and administered L-NAME

	Control	L-NAME	L-NAME + VCO	VCO
Malondialdehyde (MDA) level (µmol/ml)	2.21 ± 0.18	5.71 ± 0.44*	3.07 ± 0.12#	2.41 ± 0.12#
Reduced Glutathione (GSH) level (µmol/ml)	66.18 ± 6.32	46.74 ± 3.72*	68.32 ± 4.32#	61.23 ± 2.79
Superoxide dismutase activity (SOD) µmol/ml/min/mg prot	1.69 ± 0.13	1.16 ± 0.07*	1.52 ± 0.07#	1.60 ± 0.09#
Catalase activity (µmol/ml/min/mg prot)	5.83 ± 0.27	3.28 ± 0.18*	4.25 ± 0.50*#	5.59 ± 0.27#

*Connotes statistical difference from Control, # indicates statistical difference with the L-NAME group

Lipid profile and Atherogenic indices

Table 5 shows the lipid profile of the rats. Serum triglyceride levels were significantly increased in the L-NAME alone, L-NAME + VCO rats, and VCO alone fed rats when compared to the control ($P < 0.05$). HDL-cholesterol was also reduced in the L-NAME group but VCO-enriched diet supplementation increased HDL-cholesterol levels when compared with the L-NAME group ($P < 0.05$). The atherogenic index was increased in all the test groups compared with the control this could be because of increased triglyceride levels in these groups (Table 5). However, our results showed that the atherogenic coefficient and cardiac risk ratio were significantly increased in the L-NAME group but not in the VCO-enriched diet-supplemented groups ($p< 0.05$).

Table 5: Lipid profile and Atherogenic indices of the male rats

	Control	L-NAME	L-NAME + VCO	VCO
Total cholesterol (mmol/L)	1.66 ± 0.11	1.71 ± 0.11	1.98 ± 0.05	1.85 ± 0.09
Triglycerides (mmol/L)	0.41 ± 0.06	0.73 ± 0.08*	0.63 ± 0.03*	0.62 ± 0.03*
HDL-Cholesterol (mmol/L)	0.86 ± 0.01	0.62 ± 0.06*	0.83 ± 0.03#	0.83 ± 0.03#
LDL-Cholesterol (mmol/L)	0.71 + 0.09	0.94 ± 0.12	1.02 ± 0.06	0.89 ± 0.08
VLDL (mmol/L)	0.08 ± 0.013	0.14 ± 0.017	0.12 ± 0.007	0.12 ± 0.007
Atherogenic index	-0.34 ± 0.06	0.06 ± 0.08*	-0.12 ± 0.02*	-0.12 ± 0.02*
Atherogenic coefficient	0.91 ± 0.10	1.92 ± 0.18*	1.40 ± 0.12#	1.23 ± 0.09#
Cardiac risk ratio	1.91 ± 0.10	2.92 ± 0.38*	2.40 ± 0.12#	2.23 ± 0.09#

*connotes statistical difference from Control, # indicates statistical difference with the L-NAME group

DISCUSSION

The proximate analysis revealed that the major difference in the food content is the crude fat, moisture, and crude fibre. Medium-chain fatty acids comprise about two-thirds of coconut oil [18]. They include caproic acid, caprylic acid, capric acid and lauric acid of which the most abundant in coconut oil is lauric acid. The medium-chain triglycerides (MCTs) formed from the medium-chain fatty acids have a different metabolism when compared to the long-chain triglycerides (LCTs). They are rapidly broken down in the liver and are absorbed into the body, unlike the long-chain fatty acids. Thus, they can produce an instant energy source. This in addition to the percentage fat content could account for the increased energy content in the VCO-enriched diet and a potential mechanism by which medium-chain fatty acids modulate cellular signaling and regulate key circulating metabolites and hormones. MCTs have gained increased attention for their ability to increase fat burning [19]. Our results report no difference in body weight change during the study. MCTs ingestion has been shown to increase fat oxidation during physical activity compared with LCTs which increase fat deposit due to their different way of metabolism [19].

Sperm parameters most significant for accessing male factor infertility by the World Health Organization (WHO) include sperm count, sperm

motility and sperm morphology [20]. About 90% of male infertility difficulties are connected to sperm count decreases [21]. This study revealed that L-NAME-administered rats had a lower sperm count and sperm motility. This is in line with previous reports which showed that L-NAME reduced sperm count, motility, and testosterone in rats [22]. Although VCO enriched diet plus L-NAME did not fully restore sperm count and motility, our results on the restoration of testosterone and NO levels in this group showed that VCO could restore NOS activity and might therefore restore the reduced sperm count and motility. Sperm quality is affected negatively by increased consumption of saturated fats and trans fatty acids [23]. Although VCO contains saturated fats, the different metabolism that accompany the major components of VCO (medium-chain fatty acids) could account for its protective function on sperm cells [18].

Increased levels of ROS can indirectly affect fertility by disrupting the crosstalk of the hormones responsible for reproduction in the hypothalamus, pituitary gland, and gonads [24]. Although some reproductive processes such as fertilization, capacitation and acrosome reaction require low levels of ROS to occur, high ROS have a harmful effect on sperm DNA which ultimately affects sperm function [7]. In men, increased cytokine production induced by oxidative stress affects sperm motility [25]. Results from our study showed that L-NAME disrupted oxidative balance in the testicular environment. VCO is rich in polyphenols which are potent antioxidants, this antioxidant capacity might explain the mopping up of ROS generated by L-NAME as observed in this study. The interference of ROS with hormonal release disrupts essential reproductive functions. Thus, the VCO-enriched diet's increased testosterone and NO production, probably via its antioxidant function, could be a crucial mechanistic pathway for the restoration of sperm function disrupted by L-NAME.

Increased HDL-cholesterol in the VCO-enriched diets could account for this cardioprotective function. Abnormal lipid metabolism directly influences sperm function [26]. High-fat diets that disrupt lipid profiles have been found to affect the morphology and function of the Leydig cells which subsequently downregulates steroidogenic proteins required for hormone production [27]. Also, lower testosterone levels have been associated with metabolic syndrome which is accompanied by dyslipidemia in mice with a knockout of the aromatase enzyme [28]. Nitric oxide is important for the maintenance of endothelial functions. When nitric oxide synthase (NOS) is inhibited, the development and progress of atherosclerosis are accelerated in experimental models [17, 29]. The atherogenic indices which are derived from measured lipid profile parameters are strong predictors of metabolic disturbances [17]. Our results in the L-NAME group on the atherogenic coefficient and cardiac risk ratios agree with the result of the study carried out by the research group of Aluko [17].

CONCLUSION

VCO improved testosterone and nitric oxide levels. This may be due to the antioxidant property of VCO in mopping up excessive ROS which plays a negative role in male fertility. The restoration of oxidative balance and steroidogenic function by VCO shows great promise for the eventual restoration of the reduced spermatogenic functions by L-NAME. Thus, the VCO-enriched diet has the potential capacity to attenuate the adverse effects of L-NAME on male reproductive function in rats.

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