ORIGINAL RESEARCH

# Evaluation of Pituitary-Testicular Axis and Lipid Profile Levels in Male Wistar Rats Administered Extra Virgin Olive Oil Versus Oleic Acid with a Normal Diet

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## ABSTRACT

**Background:** Dietary fats impact male reproductive functions. The effect of consumption of normal rat chow with the Mediterranean main fat, extra-virgin olive oil (EVOO), and its major component, oleic acid (OA) in non-metabolic disease states on reproductive hormonal functions is however unknown.
**Objectives:** This study investigated the effect of EVOO and OA in normal diet feeding on reproductive hormones and lipid profiles.
**Methods:** Eighteen male Wistar rats were divided into 3 groups and fed as follows: Group I (Control) fed the normal rat chow. Group II (extra-virgin olive oil: EVOO) fed the normal diet plus 1 ml/kg/day EVOO. Group III (oleic acid: OA) fed the normal diet plus 1 ml/kg/day OA as previously described. After 4 weeks of the experimental period, reproductive hormones, lipid profile, glucose, and total protein levels were assessed.
**Results:** The serum luteinizing hormone (LH) level was significantly increased in both EVOO and OA groups, with no significant difference in serum testosterone (T) levels. The testicular T and serum oestradiol levels were significantly decreased in the EVOO groups but were significantly increased in the OA groups when compared to the control. There was a significant increase in the serum LDL-C in both EVOO and OA groups when compared to the control. The serum HDL-C levels were significantly decreased ($p < 0.05$) in both EVOO and OA groups when compared to control.
**Conclusion:** The administration of EVOO and OA in normal diet feeding resulted in diminished serum testosterone levels and serum HDL-C levels.

**Keywords:** Extra-virgin olive oil (EVOO); Oleic acid (OA); Lipid profile; Testosterone; Oestradiol; Prolactin

## INTRODUCTION

Diets and the male reproductive makeup including fertility, go hand in hand. Studies regarding the Mediterranean diet are linked to the health and wellbeing of humans. The reproductive benefits of this diet are however unclear. The Mediterranean diets' main source of plant-based fat is virgin olive oil (VOO) or extra-virgin olive oil (EVOO). The VOO and EVOO differ in the way they are processed[1]. Virgin olive oils are obtained from ripe olive fruits by mechanical extraction without undergoing any treatment other than

washing, decantation, centrifugation, or filtration. This keeps the properties intact. Olive oil has excellent antioxidants like polyphenols and vitamin E. Virgin olive oils have modest taste defects and a slightly higher acidity level (<2%). Extra-virgin olive oils (EVOO) on the other hand are those that have no taste defects and have a very low acidity rate (<0.8%)[1]. The VOO and EVOO ameliorate sperm dysfunctions that occur because of reproductive adverse effects observed in animals fed diets to induce metabolic diseases such as those associated with obesity, insulin-resistance[2-4] hypercholesterolemia[5] as well as following consumption of genetically modified soybean[6]. Findings on the effects of VOO and EVOO in ameliorating reproductive hormonal dysfunctions are however scanty.

The main source of fat in the Mediterranean diet is olive oil, although it contains several potent bioactive compounds. A large percentage of the olive oil component is oleic acid, a monosaturated fatty acid. Oleic acid accounts for between 55-80% of olive oil[7] and it is also a major component of other seed oils such as argan oil (44.8%)[8], sesame seed oil (40.7%)[9], *Carica papaya* oil[10], etc. In general, edible oils such as soybean oil, palm oil, and corn oil contain about 10-40% oleic acid[7]. Fruits such as avocado, and durian (raw) are also high in oleic acid. Poultry and beef contain 30-45% oleic acid. Oleic acid has been reported to have beneficial effects such as improvement of sperm integrity[11], and management of benign prostate cancer (BPH)[12]. Oleic acid has however also been reported to possess some adverse effects on reproductive functions, for instance, oleic acid was reported to have negative effects on pregnancy outcomes and foetal development[13], and male rats that were exposed *in-utero* to oleic acid developed altered sperm variables and an increase in the level of sperm DNA fragmentation and oxidative stress[14] was another reproductive adverse effect.

Olive oil appears to possess ameliorative properties while the effects of oleic acid exposure depend on the age at exposure, type of exposure, and duration to determine whether it would be beneficial or cause certain disruptions in the systems being studied. An example is a study that reported negative pregnancy outcomes and poor foetal development[13]. In that study, although oleic acid affected foetal development, the oleic acid caused a shift in offspring sex, giving preference to the male offspring in the exposed dams[13]. The ameliorative effects of oleic acid have also been reported[15].

Dietary fats impact lipid composition in the male reproductive system which contributes positively or negatively to male infertility[16,17]. The dietary fatty acid and oil composition modify the reproductive axis and fertility[18] although the mechanisms are not altogether understood. These questions could then come to mind. Apart from their ameliorative properties, could olive oil and oleic acid consumption in healthy states enhance better reproductive ability in the context of the Mediterranean diet? Which of the two, olive oil or oleic acid could be better at improving sex steroid hormones and lipid profile levels? The health benefits accredited to olive oil are specifically related to EVOO[19]. This study thus explored the effect of extra-virgin olive oil versus oleic acid consumption in normal-fed animals on reproductive hormones and lipid profile levels in pubertal male rats.

## MATERIALS AND METHODS

### Fatty acid and oil

Extra virgin olive oil from Andalusia Spain was purchased in a local store while oleic acid was purchased from Merck, Germany.

### Animal care

Three groups of male pubertal Wistar rats (170-200g, n=6) were housed at the animal facility of the Zoology Department, University of Ibadan. While they were acclimatized for two weeks, the animals were allowed free access to water and a standard normal diet *ad libitum.* They were kept in plastic cages at room temperature of between 27-30$^0$C and photoperiodicity of 12h light -

12h dark. The experimental procedure followed the National Academy of Sciences Guide for the Care and Use of Laboratory Animals[20].

**Feed formulation**

The normal rat chow was purchased from Premier Feed PLC Ibadan, Nigeria. The feed formulation and percentage of each component are described in Table 1.0 below.

**Table 1: Normal feed formulation for rats from Premier Feeds Ibadan**

| Ingredient | Amount (Kg) | Amount expressed in percentage (%) |
|---|---|---|
| Maize | 2.75 | 17.67 |
| Wheat offal | 0.25 | 1.61 |
| Fish meal | 0.50 | 3.21 |
| Groundnut cake | 2.75 | 17.67 |
| Palm kernel cake | 2.5 | 16.06 |
| Bone meal | 0.25 | 1.61 |
| Methionine | 0.125 | 0.80 |
| Lysine | 0.125 | 0.80 |
| Common salt | 0.003 | 0.20 |
| Vitamin premix | 0.003 | 0.20 |

**Experimental design**

Group I (control) was fed with standard normal diet. Group II (extra-virgin olive oil: EVOO) was fed the standard normal diet plus 1 ml/kg/day EVOO. Group III (oleic acid: OA) was fed the standard normal diet plus 1 ml/kg/day of OA. The feeding lasted for 4 weeks. At the end of the feeding period, all animals were euthanized by cervical dislocation following an overnight fast. Blood was collected by the retro-orbital sinus puncture into plain bottles, allowed to clot, and centrifuged at 8000 g for 10 minutes to obtain serum for hormonal assays and lipid analysis. The testes were removed and frozen in dry ice. To obtain the soluble fraction, tissue samples were homogenized in 10 volumes of 10 mM HCl-Tris buffer (pH = 7.4) and ultracentrifuged at 100,000× g for 30 min (4 °C). The resulting supernatants were used for the experiment.

**Biochemical Assessment**

***Reproductive hormonal measurement***

The serum and testicular homogenate levels of testosterone, and serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), oestradiol, and prolactin were determined using an enzyme-linked immunoassay (ELISA) and according to the manufacturer's instruction. The ELISA kits were manufactured by Monobind Inc., California (USA), with batch number EIA-6K2A2

***Triglycerides and Cholesterol Assay***

The plasma total cholesterol and triglycerides were determined by the colorimetric method using a commercial kit (Sigma, St. Louis, MO, USA, 352-50). HDL cholesterol was precipitated with phosphotungstic acid and quantified using the same method. LDL cholesterol fractions were calculated using Friedewald's formula[18].

***Glucose and Total protein***

The concentration of glucose level was measured using the Iran Pars Azmoon kit exploring the glucose oxidase method by way of the auto-analysis device[21]. The total protein content was measured by the Biuret method as described by Zaia and colleagues[22].

***Statistical analysis***

Data were expressed as Mean ± SEM. The significant differences between the mean values of the groups were determined by one-way analysis of variance (ANOVA) using GraphPad Prism 5.0 software. Differences were considered significant at $p < 0.05$.

**RESULTS**

**Reproductive hormone measurement**

The serum luteinizing hormone (LH) level was significantly increased in the EVOO and OA groups when compared with the control group (Table 2). The FSH level was however significantly increased in the OA group only when compared with the control. The serum testosterone level was not significant in both groups when compared with the control.

The serum prolactin level was significantly increased in the EVOO group while it was significantly decreased in the OA level when compared with the control. The serum oestradiol level on the other hand was significantly decreased in the EVOO group while it was significantly increased in the OA group when compared with the control (Table 2).

The testicular testosterone was significantly increased in the OA group when compared with the control (Figure 1).

**Table 2- Serum hormonal variables of rats administered EVOO and OA for four weeks**

| Hormonal variables | Control | EVOO | OA |
|---|---|---|---|
| LH (IU/L) | 0.57 ± 0.10 | 1.44 ± 0.27* | 0.68 ± 0.25* |
| FSH (IU/L) | 0.30 ± 0.05 | 0.55 ± 0.06 | 1.44 ± 0.25* |
| Prolactin (mIU/L) | 4.32 ± 0.71 | 4.46 ± 0.39* | 1.56 ± 0.12* |
| Oestradiol (pg/ml) | 8.06 ± 1.25 | 6.40 ± 0.63* | 26.70 ± 2.14* |
| Testosterone (nmol/L) | 2.75 ± 0.76 | 3.62 ± 1.38 | 3.36 ± 1.21 |

Values are expressed as mean ± SEM, n=6 per group. *p< 0.05 was significant when compared with control.

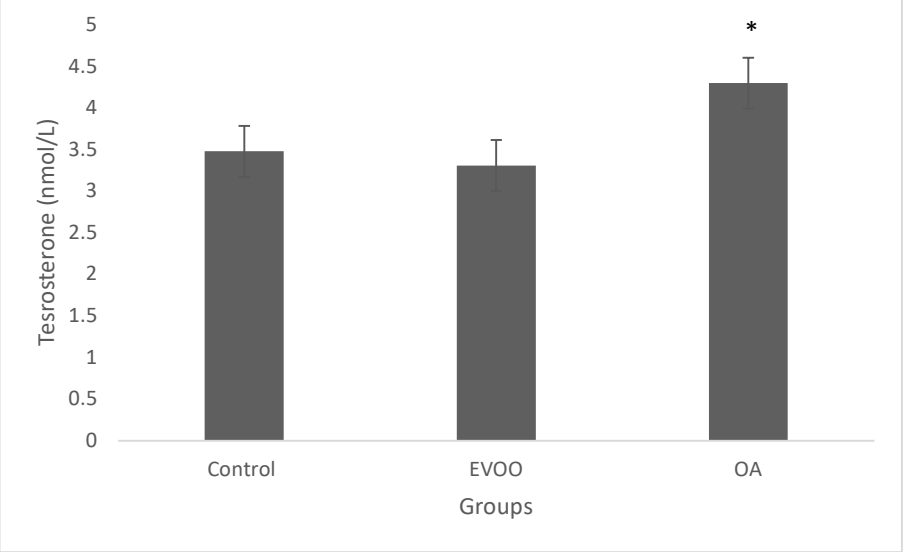


**Figure 1- Testicular testosterone level of rats administered EVOO and OA for four weeks.** Values are expressed as mean ± SEM, n=6 per group. *p< 0.05 was significant when compared with control.

**Biochemical assessment**

There was a significant decrease in the serum total cholesterol level of the oleic acid administered group when compared with the control. There was a significant increase in the serum LDL-C and glucose levels in both EVOO and OA groups when compared with the control. The serum HDL-C levels were significantly decreased in both EVOO and OA groups when compared with the control. The serum triglyceride level was significantly increased in the EVOO group but was significantly decreased in the OA group when compared with the control. The total protein

serum level was significantly decreased in the EVOO group however, the level, was significantly increased in the OA group when compared with the control (Table 3).

**Table 3- Serum biochemical profile of rats administered EVOO and OA for four weeks.**

| Biochemical variables | Control | EVOO | OA |
|---|---|---|---|
| T. Cholesterol (mg/dl) | 40.34 ± 1.36 | 40.63 ± 2.06 | 36.07 ± 2.65* |
| HDL-C (mg/dl) | 31.50 ± 2.25 | 26.36 ± 2.05* | 24.78 ± 3.69* |
| LDL-C (mg/dl) | 9.68 ± 0.98 | 13.23 ± 2.25* | 23.84 ± 2.13* |
| Triglyceride (TG) (mg/dl) | 23.02 ± 1.04 | 30.91 ± 2.75* | 22.29 ± 1.08* |
| Glucose (mg/dl) | 31.16 ± 1.78 | 43.63 ± 1.21* | 47.23 ± 1.73* |
| T. protein (mg/dl) | 5.21 ±0.21 | 4.91 ± 0.14* | 6.16 ± 0.10* |

Values are expressed as mean ± SEM, n=6 per group. *p< 0.05 was significant when compared with control.

There was a significant increase in the testicular total cholesterol level in both EVOO and OA groups when compared with the control. There was a significant increase in the testicular LDL-C level in the OA group when compared with the control. The HDL-C level was however not significant in both groups when compared with the control. There was also a significant increase in the testicular glucose level in the EVOO and OA groups when compared with the control. The testicular triglyceride was significantly increased in both groups when compared with the control. The testicular total protein was significantly decreased in the EVOO group when compared with the control (Table 4).

**Table 4- Testicular biochemical profile of rats administered EVOO and OA for four weeks.**

| Biochemical variables | Control | EVOO | OA |
|---|---|---|---|
| T. Cholesterol (mg/dl) | 24. 21 ± 0.46 | 24.75 ± 0.82* | 27.81 ± 0.64* |
| HDL-C (mg/dl) | 9.96 ± 0.37 | 9.94 ± 0.29 | 10.90 ± 0.34 |
| LDL-C (mg/dl) | 13.64 ± 0.65 | 14.33 ± 0.61 | 16.32 ± 0.49* |
| Triglyceride (TG) (mg/dl) | 105.90 ± 3.25 | 134.20 ± 3.51* | 131.10 ± 3.72* |
| Glucose (mg/dl) | 3.00 ± 0.12 | 6.07 ± 0.12* | 3.93 ± 0.17* |
| T. protein (mg/dl) | 1.80 ±0.01 | 1.58 ± 0.01* | 2.20 ± 0.01 |

Values are expressed as mean ± SEM, n=6 per group. *p< 0.05 was significant when compared with control.

## DISCUSSION

Currently, there is renewed interest in the relationship between diets and the male reproductive axis and by extension male infertility. It has been observed that the function of the pituitary-gonadal axis is very sensitive to adverse lifestyle metabolic disorders such as diabetes and obesity[18]. There are however other factors that may be responsible for male infertility, e.g., hormonal deficits, physical causes, sexually transmitted problems, environment and lifestyle, and genetic factors amongst others[23]. Diets of healthy individuals thus require re-evaluation from time to time. This is necessary because dietary fatty acid composition in the diet, for example, affects infertility in mammals and birds[17] although the mechanisms of action remain unclear. There are several mechanisms proposed regarding male reproductive dysfunction, and studies have focused on alterations in the hormonal profile as the major cause[24].

The Mediterranean fatty acids have been reported to ameliorate sperm dysfunctions that occur because of reproductive adverse effects observed in animals fed diets to induce metabolic diseases. This study however reported a boost in the LH and FSH levels of healthy male rats fed normal rat chow. The significant increase in the Luteinizing Hormone (LH) level observed in both the EVOO and OA groups

indicates that EVOO and OA have positive effects on the level of LH in normal diet-fed animals which positively affects spermatogenesis and thus improve sperm quality. A study reported that rats fed with high-protein diet supplemented with oleuropein, (a component of olive oil) had higher levels of LH when compared with those that received the vehicle alone[25]. These results show that olive oil and its components whether being used in ameliorating a disorder or not, have positive effects on the LH level. The feeding of a normal diet with EVOO and OA did not appear to depress Leydig cell function, signifying the importance of dietary lipids in Leydig cell function even in the normal diet-fed rats. Studies show that the availability of free cholesterol from cholesterol esters is influenced by both the quality and quantity of fatty acids as well as by hormonal levels[26]. In the rat Leydig cells, the cholesterol sidechain cleaving enzyme is subjected to LH regulation in a complex way which depends on both the time and the intensity of the gonadotrophic stimulus[26]. This could be the reason for the significant increase in the LH levels in both EVOO and OA groups as well as follicle-stimulating hormone (FSH) levels in the OA group. In a previous study, there was also a significant increase in LH level in male rats exposed to OA *in utero* on gestation days 18 and 19 when compared to the control rats[14].

The hypothalamic-pituitary-testicular (HPT) axis of the male reproductive system forms a finely tuned system that is controlled through a classic negative feedback mechanism. As testosterone level in the blood rises, the anterior pituitary becomes less responsive to stimulation by gonadotropin-releasing hormone (GnRH), resulting in reduced luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion. In this study, the serum LH levels were significantly increased in the EVOO and OA groups when compared to the control group while the serum testosterone levels were not affected. It can be deduced that the action of OA may not be through the gonadotropins but may be directly on the testes. This is because the testicular testosterone level was significantly increased in the OA group when compared to the control group. Testosterone is majorly a testicular steroid produced by the Leydig cells and it is released to respond to LH secreted from the anterior pituitary gland[27]. The upregulation of LH from the Leydig cell was not affected by serum testosterone but the testicular testosterone probably because LH is the primary driver of testicular testosterone in rats of pubertal age and even adults[28]. Testosterone can be converted to oestrogen through the process of aromatization[29]. Serum oestradiol levels can hinder testosterone production, thus in the presence of high aromatase enzyme activity, there would be reduced testosterone production but an increase in oestradiol levels as observed in the OA group in this study. The EVOO group on the other hand had a significantly reduced oestradiol level when compared to the control group. A significant increase in oestradiol level in the presence of a decrease or no significant difference in the testosterone level and a rise in aromatase activity is responsible for infertility states[30]. EVOO comprises oleic acid as well as phenols and other compounds while OA is purely a fatty acid. The OA-administered group had a significant increase in serum LH levels and an increase in serum oestradiol levels with no effect on the serum testosterone level. The increase in oestrogen level with a reduction or no significance in testosterone level has also been reported in animals fed a high-fat diet[31] indicating that OA, a fatty acid might possess modulatory effects on the pituitary-testicular axis. EVOO on the other hand might be protected by its other properties like phenols and not necessarily by oleic acid. The *in-utero* exposure to oleic acid in late pregnancy has also been reported to alter LH, FSH, and testosterone levels in male offspring[14]. EVOO improved levels of testosterone, and LH in human patients with poor semen quality but not in normal individuals[8,32].

The key function of prolactin is to control milk production. Apart from this breastfeeding role, prolactin is involved in reproduction[33]. Basic physiology explains that the synthesis and release of prolactin are controlled by the dopaminergic inhibition mechanism[29]. In this study, the level of prolactin was significantly increased in the EVOO group when compared with the control, while the level in the OA

administered group was significantly reduced when compared to the control. When serum concentration of prolactin is normal, it exerts tolerable functions on the male reproductive tract[34], nevertheless, when hyperprolactinaemia occurs, as observed in the EVOO administered group, reproductive capabilities such as hypogonadism, impotence, and infertility may occur[35].

The serum and testicular glucose levels were significantly increased in the EVOO and OA groups when compared with the control. A study[36] reported an increase in serum glucose level when a high-fat diet rich in saturated and polyunsaturated fatty acids was administered to Wistar rats. The consumption of saturated fat can cause metabolic changes, there is a need however for further research into the Mediterranean fat which comprises monounsaturated fatty acids, and their metabolic roles especially in normal feeding.

Studies have reported the ameliorative effects of EVOO and virgin olive oil (VOO) in hypercholesterolemic diets[2,5,11]. There are however some differences observed when rats were fed EVOO or VOO with normal diets. A study[18] reported that in a diet supplemented with VOO (20%), HDL-C levels were significantly reduced when compared to the control as reported also in our study, although there were no significant differences in the TG, TC, LDL-C levels when compared with the control. In another study[9], animals fed with isocaloric diets supplemented with different oils, and olive oil-fed animals had higher plasma TC and LDL-C levels. There have been scientific reports that have confirmed that dietary fat sources and changes in lipid levels are related to the synthesis of testosterone and impaired testicular dysfunction suggesting that hypercholesterolemia is an independent risk factor for testicular dysfunction[18]. The above could be the reason for the increase in oestradiol level in the OA group when compared to the control, increase in prolactin level in EVOO when compared to the control, and no significant difference in testosterone level in EVOO and OA groups when compared to the control, increase in testicular total cholesterol in EVOO and OA groups when compared to the control, and an increase in serum TG level in the EVOO group when compared to the control as well as an increase in serum LDL-C level in EVOO and OA groups when compared to the control.

The protein content of the testicular tissue is a marker of tissue injury, as well as damage[37] as observed in this study in the reduction of testicular tissue protein and serum levels in the EVOO administered group when compared to the control animals. The reduction in testicular protein content can be connected to testicular dysfunction[38]. There are also suggestions that testicular protein and steroid hormones are linked as steroid hormones are sensitive to protein synthesis inhibition[39].

## CONCLUSION

This study has been able to further buttress the fact that fatty acids and oils play different roles in diseases and normal states. In conclusion, we report that a normal diet plus EVOO or OA feeding resulted in diminished serum testosterone levels, a significant increase in the serum LDL-C levels, and a significant decrease in serum HDL-C levels. A more urgent and critical look into the roles of fatty acids and oils in healthy males is important to avoid an unconscious increase in the number of dyslipidemic infertile men.

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None

The authors declare no conflicts of interest.

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