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Short Communication

Early-life exposure to omega-9 monounsaturated fatty acid results in gonadal-regression and elevated stress levels in pubertal male rat

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Abstract

Corticosterone decreases hormones in the hypothalamic-pituitary-gonadal axis in stressful situations as well as when nutritional factors are modulated. The effect of *in-utero* exposure to omega-9 fatty acid on the gonadal hormones were compared with corticosterone in pubertal male rats. Pregnant rats were distributed on a random basis into five groups of five rats each as follows: $F0_{C}$: the control group was administered 1ml/kg of olive oil; $F0_{EXBM}$ was administered 1000mg/kg of oleic acid for 7days before mating; $F0_{GD1-7}$ was administered 1000mg/kg of oleic acid from gestation day (GD)1-7; $F0_{GD8-15}$ was administered 1000mg/kg of oleic acid from GD8-15. At puberty, hormonal analysis was carried out. Testicular prolactin, testosterone, follicle-stimulating and luteinizing hormone levels were decreased while corticosterone level was increased. Since corticosterone levels may adversely affect the male reproductive system, omega-9 fatty acid should be considered as a possible contributing factor in any decline of male fertility rates.

Keywords: omega-9, corticosterone, testosterone, prolactin

1. Introduction

Unsaturated fatty acids, (viz: omega-3, omega-6 and omega-9) are important components of all cell membranes, also, the proportion of these different fatty acids in tissues of the reproductive tract reflect dietary consumption (Wathes, Abayasekara, & Aitken, 2007). The beneficial roles of omega-3 polyunsaturated fatty acid on male reproductive capacities abound in man (Safarinejad & Safarinejad, 2012) and boars (Estienne, Harper, & Crawford, 2008; Strzezek, Fraser, Kuklińska, Dziekońska, & Lecewicz, 2004). In some other studies in man (Conquer, Martin, Tummon, Watson, & Tekpetey, 2000) and boars (Castellano *et al.*, 2010) however, there was no effect of n-3 fatty acid supplementation on semen quality. The ratios of n-3/n-6 polyunsaturated fatty acids (PUFAs) on reproductive functions have also been exploited. A study by Am-in, Kirkwood, Techakumphu, and Tantasuparuk (2011) reported that the ratios of n-3/n-6 PUFAs in boar sperm were negatively correlated with sperm motility, viability, normal morphology, and normal plasma membranes, which suggested that the appropriate ratio of n-3/n-6 PUFAs in males was important for sperm quality. Omega-3 also plays an important role in acrosome biogenesis (Argov-Argaman, Mahgrefthe, Zeron, & Roth, 2013; Connor, Lin, Wolf, & Alexander, 1998). Although there is little information concerning the effect of omega-9 on the male reproductive indices, researches have shown its beneficial roles. Omega-9 fatty acid (oleic acid) is a dietary mono-unsaturated fatty acid whose highest concentration has been measured in olive and canola oils (Esmaeili, Shahverdi, Moghadasian, & Alizadeh, 2015). It is known to build a healthier balance between omega-3 fats and omega-6 fats (Ferrara *et al.*,2000) while in the mammalian biological systems, as observed in sperma-

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tozoa, omega-9 is a major fatty acid among the monounsaturated fatty acids (Alizadeh, Esmaeli, Shahverdi, & Rashidi,2014). Dietary intake has a role to play in several hormonal control systems which will lead to permanent effects on the set-points of internal homeostatic systems that will extend into postnatal life. It has been observed that the major and minor fatty acids in membranes can affect fluidity of biological compounds such as milk fat triglycerides and sperm phospholipids. The adequate membrane fatty acids' mean melting point is also necessary for functional performance of biological compounds like milk and probably sperm fatty acids (Alizadeh *et al.,* 2014).

The most significant long-term alterations occur in the hypothalamic-pituitary-gonadal axis. Glucocorticoid levels have been documented, to correlate to an increased set-point in the hypothalamic-pituitary-adrenal (HPA) axis (Duthie & Reynolds, 2013). The fetus has mechanisms to protect itself against these excesses, but if hormone levels rise sufficiently, they may become saturated and fail. Corticosterone (the analogy of glucocorticoid in rat secretion) can be modulated by nutritional factors provided either in prenatal or postnatal conditions. In a study, 10-day-old offspring of dams fed with fat-rich diets secreted less corticosterone after ether stress (Trottier *et al.,*1998), whereas adult rats fed with the same type of diet secreted more corticosterone than regular chow fed rats (Tannenbaum *et al.,* 1997).

Omega 9 is a fatty acid of interest in this study because the relationship between unsaturated fatty acids with hormonal parameters is inconsistent. Although, most studies have shed light on the crucial role of fatty acids especially polyunsaturated fatty acids (PUFA) in sperm biology, the beneficial and detrimental roles of dietary fatty acids are the current focus of research in the field of nutrition and reproduction in males (Esmaeli *et al.,* 2015). This study thus sought to investigate the effect of *in-utero* exposure to omega-9 on reproductive hormonal indices and if corticosterone presumably affects the mechanisms that modulate the pituitary-gonadal axis in male pubertal rats.

2. Materials and Methods

2.1 Animals and experimental design

This study was conducted in accordance with the Use of Laboratory Animals committee of the Central Animal House, University of Ibadan in accordance with the National Institute of Health (NIH publication no.85-23, revised 1996) guidelines. Pro-oestrus female rats weighing between 190-220g were purchased from the Central Animal House, University of Ibadan and animals were maintained in an airy room under a controlled 12h: 12 h light/dark cycles, relative humidity was 50±5% as well as a room temperature of 21±1º C. Food and water were available *ad libitum* throughout the experiment. The detection of sperm in the vaginal smear was determined as day 1 of gestation. Pregnant rats were distributed on a random basis into five groups of five rats each as follows: $F0_{C}$: the control group was administered 1ml/kg of olive oil; $F0_{EXBM}$ was administered 1000mg/kg of oleic acid for 7days before mating; $F0_{GD1-7}$ was administered 1000mg/kg of oleic acid from gestation day (GD)1-7; $F0_{GD8-15}$ was administered 1000mg/kg of oleic acid from GD8-15. Dams delivered naturally. The litters were culled. An advantage of culling is that food will not be restricted despite a large litter size, so that male puberty attainment will not be delayed (Engelbregt, Houdijk, Popp-Snijders, & Delemarre-van de Waal, 2000). At puberty, hormonal analysis was carried out.

2.2 Hormonal analysis

Dissections were conducted between 8.00 a.m and 10.00 a.m. to minimize circadian influences on testosterone as well as other hormones (Meerts *et al*., 2004). The serum levels of FSH, LH, testosterone and testis homogenate for prolactin were determined using the enzyme linked immunoassay (ELISA) which was according to the manufacturer's instruction. The ELISA kits were manufactured by Diagnostics Automation Inc., Calabasas, California, USA. Total plasma corticosterone levels were determined by RIA using a kit specific for rats and mice, from ICN Biomedicals (Costa Mesa, CA). The limit of detection was 0.2 μg/dl. The inter-assay variability was 12%, and intra-assay variability was 3%.

2.3 Statistics

Data obtained were expressed as mean ± standard error of mean (mean ± SEM). The significance of the results was evaluated using analysis of variance (ANOVA) and the mean was compared using Tukey-Kramer Multiple comparison Test. Student's T-test was used to determine $P<0.05$, which was regarded as being statistically significant.

3. Results

There was a significant decrease in the testosterone, follicle-stimulating hormone, luteinizing hormone as well as the testicular prolactin levels in the $F1_{EXBM}$, $F1_{GD1-7}$, and $F1_{GD8-15}$ groups compared with the $F1_{C}$ group (Table 1). The corticosterone level in the $F1_{EXBM}$, $F1_{GD1-7}$, and $F1_{GD8-15}$ groups was significant increased compared with the $F1_{C}$ group (Figure 1).

4. Discussion

This study investigated the effect of *in-utero* exposure to omega-9 on hormonal reproductive indices and whether corticosterone affected the mechanisms that modulate the pituitary gonadal axis in pubertal rats. Studies by Yan *et al.* (2013) and Feng *et al.* (2015) revealed that the ratio of omega-3 to omega-6 polyunsaturated fatty acids caused a

Table 1. Effect of omega-9 on serum testosterone, gonadotropins and testis homogenate prolactin levels.

Groups	T (ng/mL)	FSH (u/l)	LH (u/l)	T. Prolac (u/l)
$F1_{C}$	7.99±0.24	0.26±0.02	0.11±0.01	0.08±0.04
$F1_{EXBM}$	4.10±0.1*	0.24±0.01*	0.06±0.01*	0.05±0.01*
$F1_{GD1-7}$	3.40±0.1*	0.25±0.01*	0.09±0.01*	0.06±0.01*
$F1_{GD8-15}$	4.19±0.1*	0.25±0.01*	0.07±0.02*	0.06±0.01*

Values are expressed as Mean ± SEM, N= 45/group, *$P< 0.01$ compared with control. T= testosterone, FSH =Follicle-stimulating hormone, LH= luteinizing hormone, T. Prolac= Testicular prolactin.

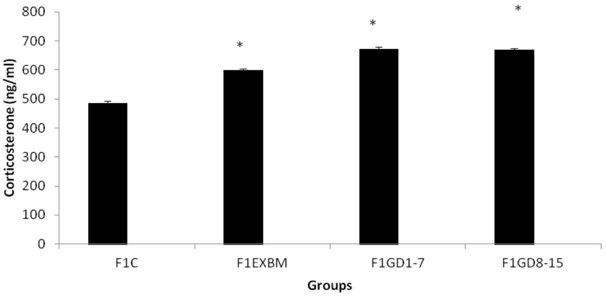

Figure 1. Corticosterone levels in male rats. Values expressed as mean ± S.E.M (n=45) *P<0.01.

significant increase in the follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) levels in rats and roosters respectively. This was not the same pattern as the results in this study because omega-9 caused a significant reduction in the hormones. This can be an indication of omega-9 modulating the hormones differently. FSH plays a role in spermatogenesis while LH stimulates the interstitial cells located in the testes to produce testosterone. The activities of these hormones were altered in the study. Consequently, it is presumed that omega-9, possesses some degree of stress, as such testosterone levels decreased via the inhibition of the hypothalamic-pituitary-gonadal (HPG) axis caused by the elevated corticosterone level since the rise in cortisol (corticosterone) is a universal stress response. Corticosterone levels increase via activation of the hypothalamic-pituitary- adrenal (HPA) axis. Under the regulation by the hypothalamus, the pituitary gland produces pituitary gonadotropins such as LH and FSH. LH stimulates the Leydig cells, which have surface receptors for LH, and produce as well as secrete testosterone. When testosterone is released it controls spermatogenesis, probably by influencing Sertoli cells in the seminiferous tubules. It is well known that testosterone has important androgenic roles, which includes promotion of spermatogenesis and development of spermatogenic tissues (Russell, Ettlin, SinhaHikim, & Clegg, 1990). However, if corticosterone levels become elevated by stress, such as in the exposure to omega-9 *in-utero*, production of testosterone in Leydig cells declines and apoptosis of Leydig cells is induced (Gao *et al.*,2002). High fat diet has been related to high cortisol and low testosterone, which is similar to this study on omega-9 fatty acid (Trottier *et al.*, 1998).

Studies in rats have suggested that gonadotropin-inhibitory hormone (GnIH) is a likely channel for the effect of stress on the reproductive axis (Kirby *et al.*, 2009). GnIH is a peptide that is produced in neurons of the dorsomedial nucleus (DMN) of the hypothalamus. In the male rats, either acute or chronic restraint stress has been shown to increase GnIH gene expression in the DMN, which was associated with a reduction in LH levels (Kirby, Geraghty, Ubuka, Bentley, & Kaufer, 2009). It was reported that over half of the GnIH cells express the glucocorticoid receptor (GR) and proposed that GnIH cells were the intermediary for the stress effect on reproduction, by the action of cortisol (Kirby *et al.*, 2009). Low birth weight especially on postnatal day 1 as was revealed in this study (data not shown) has been associated with increased susceptibility to stress (Gale & Martyn, 2004). Studies have indicated that animals exposed to early-life hardship, such as maternal deprivation, poor maternal care, prenatal stress are less affected by stressful situations in adulthood (Champagne, de Kloet, & Jöėls, 2009). This may not be the same situation in pubertal rats according to this study.

The testicular prolactin level was significantly decreased in the omega-9 exposed groups compared with the control group. Although the role of prolactin in males is yet to be understood, it is a versatile compound that has dual function, as a circulating hormone and as a cytokine. The prolactin receptor is a member of the cytokine receptor superfamily, linked to the activation of signaling pathways that promote cell growth and survival. Through these mechanisms, prolactin regulates diverse physiological functions via its effects on cellular processes such as proliferation, differentiation, and cell survival (Vera-Lastra, Jara, & Espinoza, 2002). Prolactin may also represent a peripheral regulatory factor for reproductive function in males, and/or a feedback mechanism that signals CNS centers controlling sexual arousal and behavior. Hyperprolactinemia in males induces hypogonadism by inhibiting gonadotropin-releasing hormone pulsatile secretion and, consequently, FSH, LH and testosterone release. This leads to spermatogenic arrest, impaired motility, as well as sperm quality and results in morphologic alterations of the testes like those observed in prepubertal testes (De Rosa *et al.,* 2003). The present results on decreased testicular prolactin levels in omega-9 fatty acid exposure disagree with the aforementioned. Some factors such as the pubertal age of the rats, the testis organ where the prolactin level was assessed could explain this discrepancy.

The consequence of the reproductive hormones and corticosterone levels after omega-9 administration in this study can also be addressed as follows. Lipids are made up of a wide-range class of molecules that play important roles in the structure and function of cells in mammals. They serve as cellular metabolism, signaling molecules, storage compounds, and various membrane-related functions like trafficking, regulation of proteins, and creating membrane sub-compartments. The carbon chain may either be saturated, or it may contain one or more double bonds as in mono- and polyunsaturated fatty acids, respectively. In PUFA the number, position, and cis/trans state of the double bond have dramatic effects on cell function as well as membrane fluidity. The first carbon of the methyl group is called omega in the omega system and based on the distance of other carbons from omega, they are called omega-3 (n-3), omega-6 (n-6), and omega-9 (n-9) (Esmaeli *et al.,* 2015). It appears that C18:0 is a more important saturated fatty acid than other saturated fatty acids and omega-9 (C18:1) is transformed from this fatty acid. Some sperm-saturated fatty acids might be affected by dietary saturated or unsaturated fatty acids (Esmaeli *et al.,* 2015). Researches show that the concentration of C18:0 was higher in infertile men Aksoy, Aksoy, Altinkaynak, Aydin, and Ozkan (2006), and Zalata, Christophe, Depuydt, Schoonjans, and Comhaire (1998) while a substantial decrease in C18:0 concentration was demonstrated by dietary manipulation in oligozoospermia men. Research has revealed that hormones such as luteinizing hormone (LH) and adrenocorticotropin hormone (ACTH) possibly change unsaturated fatty acid composition in the testis by changing activities of the enzymes (de Catalfo, Mandon, & de Gomez Dumm, 1992). Such enzyme activities include the fact that the testes designed sperm fatty acids to maintain membrane fluidity to achieve their biological goal since it has already been established that testes have high capacities for desaturation and elongation of unsaturated fatty acids (Alizadeh *et al.,* 2014). The mean melting point (MMP) is proposed as an index of membrane fluidity. The enzymes' ability and quantity are areas of concern which need to be thoroughly studied in the future. The desaturase index is also a factor to be considered. Oleic acid (Cis9-18:1) is transformed from stearic acid (C18:0) by stearoyl-CoA desaturase to maintain membrane fluidity (Alizadeh *et al.,* 2014). Apart from the enzyme activities, there could also be other factors to consider. Research into literature has shown that fatty acids are able to modulate lipid metabolism by complex mechanisms involving a series of interdependent and cross-regulated molecular events. Unsaturated fatty acids have been demonstrated to increase lipid peroxidation. Unsaturated fatty acid supplementation has been shown to disturb ram sperm parameters when used without vitamin E supplementation (De Graaf, Peake, Maxwell, O'Brien, & Evans, 2007). Although dietary fatty acid administration and antioxidant supplementation were not the scope of this study, the level of oxidative stress in the testis of male rats exposed to omega-9 *in utero,* was assessed by malondialdehyde (MDA), a marker of lipid peroxidation. The MDA showed a strong correlation with corticosterone, FSH, LH, and testosterone (data not shown).

5. Conclusions

The results of this study provide evidence that omega-9 fatty acid may influence hormone levels associated with the male reproductive system in rats and increase the corticosterone levels. Since corticosterone levels may adversely affect the male reproductive system, omega-9 fatty acid should be considered as a possible contributing factor in any decline of male fertility rates.

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