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Pattern of Gonadal Hormones in Oral Testosterone-Supplimented Male Wistar Rats with Diabetes-Induced Hypogonadism

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Research Article

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Abstract

Hypogonadotrophic hypogonadism, which is characterized by low serum testosteronee co-existing with elevated Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) has been reported to be a common finding in poorly managed diabetes mellitus (DM). This study is an attempt to investigate the impact of oral exogenous testosterone on the pattern of gonadal hormone profile in male Wistar rats, without any form of antidiabetic therapy.

Twenty-one male Wistar rats were divided randomly into three groups of seven rats each as follows; Healthy control (HC) rats which were neither induced with diabetes nor treated with testosterone, Diabetic control (DC) rats which were induced with DM but were not treated with testosterone, and Diabetic/Oral Testosterone (D/ORT) rats that were induced with DM and treated with oral testosterone daily for four weeks. DM was induced via single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg.

Result revealed a significantly elevated fasting blood sugar (FBS) in DC and D/ORT rats following induction of DM and throughout the study when compared to HC group ($p<0.05$). There was a significant decrease in the serum testosterone levels of DC when compared to either of D/ORT or HC groups. However, serum LH level of DC rats was significantly elevated when compared to both D/ORT and HC groups ($p<0.005$), while serum FSH was not significantly different ($p>0.005$) among all three groups.

It was therefore concluded, that oral testosterone supplementation results in elevated blood testosterone that corrects diabetes-induced hypogonadism despite persistently elevated blood sugar.

Keywords: Gonadal Hormones; Hypogonadism; Testosterone

Abbreviations

LH: Luteinizing Hormone; FSH: Follicle Stimulating Hormone; DM: Diabetes Mellitus; HC: Healthy Control; DC: Diabetic Control; STZ: Streptozotocin; FBS: Fasting Blood Sugar.

Introduction

Testosterone is the primary sex hormone and anabolic steroid in males. In humans, testosterone play key roles in the development of male reproductive organs, as well as

promoting secondary sexual characteristics like increased muscle mass, bone mass and body hair growth [1]. In addition, testosterone in both genders is involved in the maintenance of good health as it is pivotal in the prevention of certain morbidities including osteoporosis and also contribute to the regulation of mood and behaviour [2]. Testosterone is a steroid of the androstane class and contains a ketone and a hydroxyl group at positions three and seventeen respectively. It is mainly synthesized biologically through several steps from cholesterol and is converted in the liver to inactive metabolites. It exerts its action through binding to and activation of the androgen receptor in a wide range of body tissues [3].

Testosterone is widely referred to as a male reproductive hormone due to the predominantly high blood levels in them, although it is also produced in the females but in minute concentrations [4]. A multidirectional neuroendocrine signalling cascade involving the hypothalamus-pituitary-testicular axis controls the production and release of testosterone. A "feedback loop" closely regulates the level of the hormone in blood by regulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland, whose releases are in turn stimulated by gonadotropin releasing hormone (GnRH) from the hypothalamus [5].

Diabetes mellitus is a group of metabolic disorders characterized by elevated blood glucose level (hyperglycaemia) over a prolonged period of time [6]. Symptoms often include frequent urination, increased thirst and increased appetite and if left untreated, diabetes can cause many health complications [7]. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycaemic state or death [7]. Serious long-term complications include cardiovascular diseases like stroke, chronic kidney diseases, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment [8]. Additionally, complications affecting the reproductive system occur commonly, and prominently include male hypogonadism which is usually characterised by low levels of blood testosterone [9].

Hypogonadism can be explained simply as a situation of diminished functionality of the gonads (the testes or the ovaries) that may result in diminished production of sex hormones [10]. Hypogonadotrophic hypogonadism, is the specific type characterized by low serum testosterone in concurrence with elevated LH and FSH and this pattern has been reported to be a common finding in complicated or poorly managed type 2 diabetes mellitus [11]. This represents a classical picture expected in all forms of primary hypogonadism where the actual organ of pathology are the gonads, i.e. the testes in this case of male reproductive system.

Due to the almost direct connection between the hormonal control of testosterone secretion and the hormonal basis of hypogonadism, many studies have created awareness on the existence of an influence of testosterone supplementation as a therapeutic procedure for the conventional forms of Hypogonadism [12,13]. However, studies have yet to show the role of testosterone supplementation in possible amelioration of hypogonadism that is diabetes-induced, without directly managing the diabetes mellitus therapeutically. This study is an attempt to investigate the impact of oral exogenous administration of testosterone on the pattern of gonadal hormone profile in male Wistar rats with diabetes-induced hypogonadism, without any form of antidiabetic therapy.

Materials and Methods

Study Animals

A total of Twenty-one (21) male Wistar rats with weight range of 150g to 200g were used for this study. The animals were kept in clean cages under standard conditions (25 ± 2ºC, 12 h light and 12 h dark cycle) in a separate room in the animal house of the Physiology Department, School of Basic Medical Sciences, Federal University of Technology, Akure, Ondo State, Nigeria. After two weeks of acclimatization all animals were randomly grouped into three groups of seven rats each.

Experimental Design

Each group was housed in different plastic cages with iron gauze bottom grid and a wire screen top. The experimental groups were as follows:

Group 1 (Healthy control – HC) rats were neither induced with diabetes nor treated with testosterone all through the experiment, but had food and water ad libitum.

Group 2 (Diabetic control – DC) rats were induced with diabetes mellitus but were not treated with testosterone all through the experiment.

Group 3 (Diabetic/Oral Testosterone – D/ORT) rats were induced with diabetes mellitus and treated with oral testosterone daily.

Induction of Diabetes Mellitus

Diabetes was induced via single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg. Rats were fasted for 12hrs overnight before diabetes induction and allowed access to food and water immediately after STZ injection. STZ was dissolved in citrate buffer (0.1M) on ice at a pH of 4.5 and prepared freshly before use. For the intraperitoneal injection, the rats were held in one hand in dorsal position, the injection site was swabbed using alcohol and the designated amount of STZ was injected. Blood sugar

level was recorded 48hours after and rats with readings of 200 mg/dl and above were considered diabetic. Fasting blood sugar (FBS) was then monitored weekly throughout the duration of the experiment.

Preparation of Testosterone Stock Solution

Oral testosterone undecanoate (Testoheal®) were used for this study. The procedure for preparation includes the following;

Oral testosterone dose of 1mg/kg body weight was used in this study [14]. One tablet (40 mg) was dissolved in 100 ml of normal saline to get an initial stock solution of 0.40 mg/ml. The testosterone dosage for each rat was delivered based on body weight. The volume of the stock preparation given to each rat daily was calculated as;

(Body weight in grams × 0.0025) ml.

Admnistration of Testosterone

Oral testosterone was administered by gavage with the aid of an oropharyngeal cannula. The rats were handled appropriately to restrict movement and prevent trauma to the rats during drug administration. Administration was done daily.

Collection of Samples for Analysis

All animals were anaesthetized using chloroform inhalation and euthanized via exsanguination. Blood samples obtained via cardiac puncture from each rat and were put in plain serum bottles which were clearly labelled and allowed to clot at room temperature for 20 minutes before been centrifuged for 15 minutes at 350 rpm to obtain serum. Freezing of the samples was done immediately and remained frozen till assayed.

Hormonal Assays

Serum luteinizing hormone (LH) and Follicle stimulating hormone (FSH) levels were determined using the MILLIPLEX® MAP Rat Pituitary Magnetic Bead Panel (RPTMAG-86K), employing Luminex® xMAP® technology. While serum testosterone level was determined by radioimmunoassay technique using testosterone rat/mouse enzyme-linked immunosorbent assay (ELISA) kit (DEV9911) in accordance with the manufacturer's specifications (Demeditec®)

Statistical Analysis

Data were expressed as mean ± SEM and analysed with the aid of GraphPad Prism version 8.2. ANOVA was used, followed by Krushkal-Wallis post-hoc test. A value for P < 0.05 was considered to be statistically significant.

Results

Levels of Fasting Blood Sugar in All Experimental Groups

As shown in Table 1, there was no significant difference among the baseline fasting blood sugar (FBS) levels across all three experimental groups. FBS became significantly elevated in DC and D/ORT rats following induction of DM and after two weeks of testosterone administration, when compared to HC group ($p<0.05$).

However, at the third and fourth weeks, FBS levels remained significantly elevated in DC but not in D/ORT group when compared to HC group ($p<0.05$).

	HC	DC	D/ORT
	[Milligram per deciliter (mg/dL)]		
Baseline	67.4±3.61	65.8±2.15	69.6±1.63
Week 1	49.6±3.93	248.2±4.77*	252.4±10.75*
Week 2	69.6±6.72	287.2±43.2*	375.0±19.17*
Week 3	62.8±3.51	318.4±22.03*	249.6±20.25
Week 4	63.4±2.50	302.0±19.68*	243.8±18.39

Table 1: Levels of fasting blood sugar in all experimental groups at baseline and for four weeks of treatment
Values expressed as mean ± SEM, n=7
*= p <0.05 when compared to healthy control group.

Effects of Testosterone Administration on Serum Fsh Levels

This result Figure 1 shows that there was no significant difference in the serum FSH levels across all groups ($p>0.005$) after the administration of testosterone.

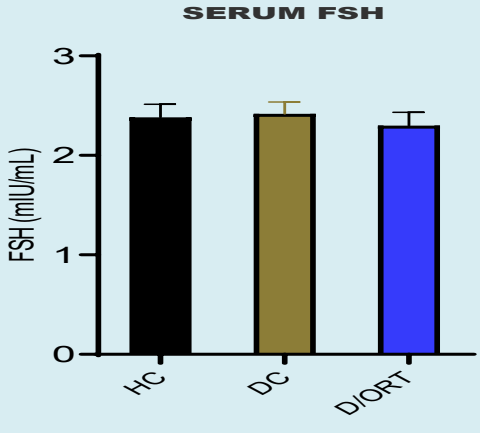

Figure 1: Serum FSH levels across all three experimental groups.

Effects of Testosterone Admnistration on Serum Lh Levels

As shown in Figure 2, serum LH level of DC rats was significantly elevated when compared to the HC group ($p<0.005$). However, the serum LH level in D/ORT rats was not significantly different ($p > 0.05$) from levels in HC group and was statistically significantly lower ($p<0.005$) when compared to the level from DC rats.

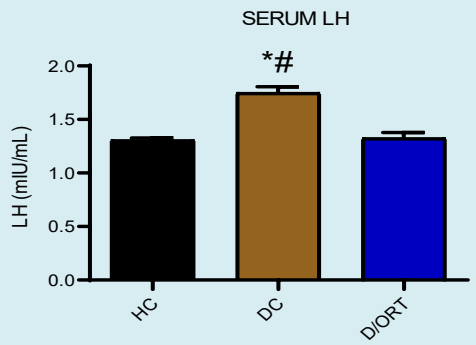

Figure 2: Serum LH levels across all three experimental groups
(*p<0.05 Vs HC, #p<0.05 Vs D/ORT)

Effect of Testosterone Admnistration on Serum Testosterone Levels

This result Figure 3 shows that there was no significant difference in the serum testosterone levels when readings from D/ORT rats was compared to that from HC group ($p > 0.05$). However, there was a significant decrease in the serum testosterone levels of DC when compared to either of D/ORT or HC groups.

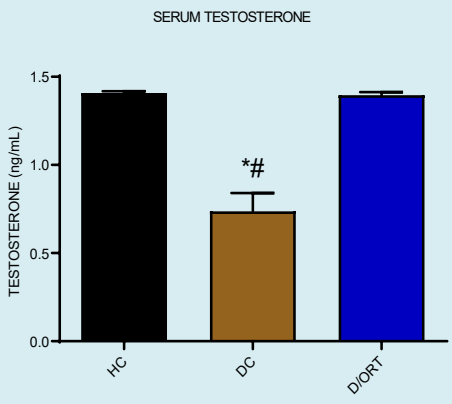

Figure 3: Serum Testosterone levels across all three experimental groups.
(*p<0.05 Vs HC, #p<0.05 Vs D/ORT)

Discussion

The findings in this study as reported in the result section basically indicated the correction of the primary hypogonadotropic pattern observed in the diabetic control group by oral testosterone administration, as seen in the treated group. This appeared to have occurred in spite of a persistently elevated blood sugar in the diabetic rats after four weeks of testosterone administration. The observation further adds credence to the previous report of improved metabolic and reproductive functions in diabetic men undergoing testosterone replacement therapies for hypogonadism [15]. Additionally, an earlier study by Arokoyo et al. (2017A) reported the observation of an almost complete recovery in testicular endocrine function in diabetic rats following a short-term administration of an antioxidant-rich plant extract [6]. The consistency of these reports may be sufficient to postulate that diabetes induced hypogonadism, even though usually primary in nature, does not result from an organic damage to the primary endocrine cells of the testes (Leydig cells), but rather from a reversible dysfunction of the cells. The hypothalamic-pituitary-gonadal axis is a neuro-endocrine pathway that plays a major role in regulating testosterone levels. The hypothalamus secretes GnRH, which travels down the hypothalamo-hypophyseal portal system to act on the anterior pituitary, which in turn secretes luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH are two gonadotropic hormones that travel through the blood and act on receptors in the gonads to stimulate the production of androgens. LH, in particular, acts on the Leydig cells to increase testosterone production. Testosterone limits its own secretion by exerting a negative feedback effect on the hypothalamus as well as the anterior pituitary gland. High level of testosterone in the blood causes negative feedback signals to the hypothalamus to suppress the secretion of GnRH and also to the anterior pituitary, making it less responsive to GnRH stimuli [16]. In this present study, the FSH levels was not significantly different between diabetes groups treated and those untreated with testosterone supplement, while there was a prominent reduction in LH levels in with testosterone supplementation. This is an indication of a preponderance of the feedback regulatory effect of blood testosterone for LH over and above that for FSH. The observation is in consonance with an earlier report that the administration of exogenous testosterone in normal male subjects resulted in near absolute lowering of serum LH level and only a partial reduction in serum FSH levels [17].

The findings in this study is limited in scope to the endocrine pattern obtainable within the regulatory framework of the hypothalamus-pituitary-testicular axis, and did not explore the gametogenic aspect of testicular function. Therefore, even though the endocrine picture

of hypogonadism seen in the untreated diabetic rats appeared to be ameliorated significantly in those treated with oral testosterone, it may not be safe to conclude that gametogenesis is also automatically restored to normal in the treated rats. As has already been reported in numerous previous studies, diabetes-induced hypogonadism is often accompanied by subnormal sperm parameters that are indicative of dysfunctional testes [6,11,18-20]; It is therefore expedient to further expand the scope of this present study to accommodate the exploration of the effects of oral testosterone supplement of the sperm parameters and other fertility indices of diabetic males in addition to the gonadal hormone pattern. This is the focus of a subsequent study.

Conclusion

Primary hypogonadism as a complication of untreated diabetes mellitus in confirmed in this study. In the male, this appears to be as a result of a direct effect on the Leydig cells of the testes thereby preventing adequate testosterone production and consequently resulting in elevated blood levels of LH and FSH.due to the absence of negative feedback regulation from testosterone. However, oral testosterone supplementation results in elevated blood levels of testosterone that corrects the diabetes-induced hypogonadism in spite of a persistently elevated blood sugar.

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Conflict of Interest

There is no conflict of interest with regards to any component of this manuscript.

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