

**EFFECT ON THE PITUITARY-TESTICULAR AXIS IN
STREPTOZOTOCIN INDUCED DIABETIC RATS****Mr. Kishwor Bhandari^{*1}, Dr. Aruna Mukherjee², Dr. Ipseeta Ray³, Mrs. Sanju Acharya⁴**

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ABSTRACT

Diabetes affects sexual dysfunction in male. Testis represents the central organ of male reproductive system. Its function is primarily controlled by gonadotropins which are protein hormones secreted by gonadotrope cells of the pars anterior of the pituitary gland. The two principal gonadotropins are luteinizing hormone (LH) and follicle stimulating hormone (FSH). These hormones are central to the complex endocrine system that regulates normal growth, sexual development and reproductive function. LH acts on Leydig cells to produce testosterone. Follicle stimulating hormone and testosterone hormone both acts on Sertoli cells to regulate spermatogenesis. Diabetes mellitus is an endocrine disorder. To analyze the hormone of the pituitary testicular axis in diabetic conditions, adult male wistar rats

were induced into diabetes mellitus by intraperitoneal injection of streptozotocin. The rats were maintain in the diabetic state till one week, one month and six months according to the duration of study. Determination of the serum level of gonadotropins and testosterone hormone in the serum of both diabetic and non diabetic rats was done. Significant decrease ($p < 0.05$) in serum level of FSH, LH and testosterone hormone was found in the diabetic group after one and six months.

KEYWORDS: Diabetes mellitus, Follicle stimulating hormone, Luteinizing hormone, Testosterone.

INTRODUCTION

Diabetes mellitus is a syndrome that has been associated with long term complications which result in lesions and multiple processes in several tissues of the organism. It is one of the greatest public health concerns of the 21st century. Moreover, western lifestyle and lack of exercise leads to obesity in young individuals which strongly contributes to the incidence of diabetes mellitus particularly in the young generation. Research in diabetes mellitus is focused on insulin which is the first hormone to be affected. Besides insulin it is found that other hormones also fluctuate ultimately leading to the failure of their target organs. Increased rate of infertility in diabetes mellitus has led the attention of many researchers towards the effects of diabetes on reproductive hormones. Further, studies on the mechanisms involved in the reproductive hormones may help in the reduction of infertility in diabetic patients.

The fertility of men depends on the balanced endocrine interplay of hypothalamus, pituitary and the testis. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus is the key hormone to regulate the release of gonadotrophin.^[1] The gonadotropin binds with their corresponding receptors present in the target organ and elicits spermatogenesis.^[2] The Leydig cells which produce testosterone are under the control of LH whereas the Sertoli cells are under FSH. So FSH, LH and testosterone are prime regulators of germ cell development. FSH plays a key role in stimulating mitotic and meiotic DNA synthesis in spermatogonia.^[3] Therefore, the quantitative production of spermatozoa generally requires the balanced level of FSH, LH and testosterone in the blood. As diabetes mellitus is an endocrine disorder, alteration in its hormones will affect the hormones of the pituitary testicular axis which may lead to infertility. Therefore, this study was undertaken to evaluate the hormones of the pituitary testicular axis in experimentally induced diabetic rats.

MATERIALS AND METHODS

Adult male wistar rats weighing above 200 grams were used for the induction of diabetes mellitus. Only the euglycemic rats were included in this study. They were maintained in captivity in cages under natural light conditions, laboratory chow and water ad libitum were available. On the basis of duration of study; one week, one month and six months, the rats were divided into three groups. Each group contains control and diabetic rats. Diabetes was

induced by giving single dose of intraperitoneal injection of streptozotocin (STZ) at a dose of 50mg/kg dissolved in freshly prepared citrate buffer (pH 4.5). The control group were given the same volume of citrate buffer. The foods were given to the rats only after twelve hours of injection. After 72 hrs of STZ injection, the blood sample of STZ group rats were taken from the tail vein to measure the fasting blood glucose level by automated glucose analyzer. The fasting blood glucose level above 200mg/dl was considered diabetic rat. The fasting glucose level of control group was also measured to confirm the non diabetic. The fasting glucose level was monitored periodically to confirm the diabetic and non diabetic rats. The rats were sacrificed by cervical dislocation on the last day of their respective duration of study. The blood samples were taken by cardiac puncture to analyze the hormones.

RESULTS

Table 1: FSH, LH and testosterone level in the serum of diabetic and non diabetic rats

S.N	Rats	Duration of the study	FSH (ng/ml)	LH (ng/ml)	Testosterone (ng/ml)
1.	Control	One week	235.12± 4.69	3.85± 0.10	2.45 ±0.29
2.	Diabetic	One week	226.70± 3.58	3.65± 0.46	2.26 ± 0.26
3.	Control	One month	221.15 ± 4.39	3.77 ± 0.03	2.44 ± 0.13
4.	Diabetic	One month	156.82± 9.87*	2.58 ± 0.13*	1.38 ± 0.16*
5.	Control	Six months	240.20 ± 4.60	3.95 ± 0.06	2.36 ±0.09
6.	Diabetic	Six months	112.50 ± 12.73**	1.68± 0.11**	0.97± 0.09**

All results presented in Mean ± SEM;

* Significant: $p < 0.05$

**Highly significant: $p < 0.001$

The value of the FSH, LH and testosterone decreased significantly in diabetic group (after one month and six months) when compared to control group. The decrease in the hormones level in diabetic group, after a week is not considered significant as the p value is more than 0.05.

DISCUSSION

Gonadotrophin and testosterone are required to obtain full reproductive potential. In the testis, somatic Leydig cells and Sertoli cells transduce signals for the production of factors that are required by germ cells as they mature into spermatozoa. Both Leydig cells and Sertoli cells functions are regulated by luteinizing hormone and follicle stimulating hormones. Therefore, spermatogenesis and its outcome are directly under the control of these hormones.

So, alternations in any of these hormones in pathological conditions like diabetes mellitus affect the fertility of an individual.

The function of Leydig cell is not only regulated by luteinizing hormone but also by many other hormones. There are evidences that Leydig cell steroidogenesis is further modulated locally by circulating hormones, growth factors, and cytokines.^[4] In the diabetic state Leydig cell function, particularly testosterone synthesis may be impaired by changes in the production of these hormones and cytokines in the target tissue and in adipose tissue. Adipose tissue, which is considered an endocrine organ and produces a host of hormones and cytokines, may modulate insulin action and regulate Leydig cell function.^[5] The production of leptin hormone by adipose tissue may play a key role in steroid biogenesis and reduced testosterone levels. Leptin levels have been shown to be inversely correlated with serum testosterone levels (Haffner et al., 1997; Wabitsch et al., 1997; Luukkaa et al., 1998).^[6-8] The increased circulating leptin in the blood may be involved in the pathogenesis of Leydig cell dysfunction (Isidori et al, 1999).^[9] The presence of leptin receptors in Leydig cells and the inhibition of hCG stimulated testosterone secretion from rat Leydig cells (Caprio et al., 1999).^[10] This suggest the role of leptin hormone in the biogenesis of testosterone. Hong et al., (2004).^[11] proposed that tumour necrosis factor alpha (TNF- α) inhibits steroid biosynthesis in Leydig cells and proposed a molecular mechanism by which proinflammatory factors could contribute to inhibition of androgen biosynthesis.

Adult concentrations of intratesticular testosterone in the rat are approximately 50 to 100-fold higher than that found in serum. Although the physiological advantages of elevated testosterone levels in the testis are not understood, the higher testicular levels of testosterone are important because full spermatogenic capacity requires 70 ng/ml and spermatogenesis is dramatically compromised at testosterone concentrations below 20 ng/ml.^[12] Decrease in serum testosterone are linked with insulin resistance and implicated in hyperglycemia, hypertension, dyslipidemia and an increased risk of vascular disease.^[13-26] Serum testosterone levels reflect the integrity of the hypothalamic-pituitary-gonadal axis (HPG), and low testosterone levels noted in cases of diabetes may indicate a defect at functional levels of the hypothalamic-pituitary-gonadal axis. In our study, there is a decrease in the testosterone and gonadotropins in the diabetic group after one week but this is not significant as compared to nondiabetic rats. The decrease in luteinizing hormone and follicle stimulating hormone in diabetes as shown by our results and other investigators,^[27-29] indicates the pituitary-gonadal

axis (HPG) is altered in streptozotocin induced diabetes. In our study, the marked decrease in testosterone after six months is strongly correlated with the decrease in luteinizing hormone, as steroidogenesis by Leydig cells is under the control of luteinizing hormone. Since testosterone has been shown to exhibit feedback effects at the pituitary gland,^[30] and the hypothalamus,^[31] this decreased testosterone in diabetes is unable to increase gonadotropin levels through negative feedback mechanism. This clearly states that decrease in serum gonadotropins in the diabetic animals is due to the effects of diabetes on hypothalamo-pituitary axis. Paz et al.^[29] and Howland and Zebrowski,^[32] have reported that the pituitary gland of Streptozotocin-induced diabetic rats is capable of responding normally to gonadotropin-releasing hormone (GnRH), indicating that the primary effect of diabetes on the neuro-endocrine axis may be on the hypothalamus. Other investigators,^[29,33] have presented evidence that the pituitary gland is directly affected by diabetes, leading to decreased release of gonadotropins.

There are several studies which state disturbance in the sex hormone in diabetes but the mechanism involved in the interplay of the pituitary gonadal axis in diabetic patients is still not clear. Pitteloud et al.,^[17,18,34] studied the effect of suppression of endogenous reproductive hormones. He stimulated pituitary gland by GnRH and testes by hCG. HCG-stimulated testosterone levels at 48 hours were positively correlated with insulin sensitivity as well as with baseline serum testosterone levels. The authors suggested that alteration in Leydig cell function may account in part for the mechanisms by which a decrease in testosterone leads to insulin resistance. Pitteloud et al.,^[17,34] did not observe a correlation between insulin sensitivity and parameters of luteinizing hormone secretion or its response to exogenous gonadotropin releasing hormone, suggesting that low testosterone levels associated with insulin resistance are not attributable to a major decrease in hypothalamic or pituitary hormone secretion. The authors have demonstrated a strong positive correlation between hCG-stimulated testosterone secretion and insulin sensitivity in men using physiological doses of hCG in the presence of experimentally induced hypogonadism. Ballester et al.,^[35] proposed several potential mechanisms that affect testosterone biogenesis in diabetes. The authors suggested that diminished Leydig cell function and testosterone production in insulin-dependent diabetes is attributed to reduced or absent stimulatory effect of insulin on Leydig cells.

The effect of insulin on Leydig cells has been previously reported and is related to the control of cell proliferation and metabolism. Addition of insulin to the medium increased the incorporation of thymidine into DNA in cultured Leydig cells (Khan et al; 1992).^[36] In this regard, luteinizing hormone mediates the proliferation of Leydig cells through a mechanism that involves insulin and IGF-I signaling (Feng et al; 1999).^[37] Moreover, insulin partially restored alteration in lipid metabolism in cultured Leydig cells from diabetic rats (Cataifo et al; 1998).^[38] There is a wide discrepancy concerning the effects of insulin treatment on luteinizing hormone and follicle stimulating hormone levels in diabetic rats, from a total recovery of luteinizing hormone, follicle stimulating hormone (Ben-tez and Peı rez Dı'az, 1985; Seethalakshmi et al, 1987)^[39,40] and to a lack of recovery of LH and FSH (Hutson et al, 1983, Sudha et al, 1999).^[41,42]

The testosterone synthesis disorder may be caused by molecular changes at the level of Leydig cells and may lead to other disorders in all target organs and tissues. The close correlation between Leydig and Sertoli cells function, through decreased testosterone results in certain anomalies in diabetic patient's spermiograms. Parallel lesions associated with diabetes mellitus, through central nervous system (hypothalamus-hypophysis), and endocrine profile are indirectly intensified or induced by these disorders, which reflect dysfunction of homeostatic balance in carbohydrate metabolism.

The primary hormonal controls on spermatogenesis involve the action of FSH and testosterone on Sertoli cells. Within the seminiferous tubules only Sertoli cells possess receptors for testosterone and FSH and thus these cells are the major targets of the ultimate hormonal signals that regulate spermatogenesis. The studies done by Hutson et al., 1983;^[41] and Sudha et al, 1999:^[42] indicate that one of the most important regulatory roles of insulin on spermatogenesis is the modulation of serum follicle stimulating hormone levels. Sperm production is a follicle stimulating hormone-regulated process that requires normal Sertoli cell function (Ward et al., 1991).^[43] Sertoli cells metabolic function is highly dependent on glucose^[44,45] and insulin concentration.^[46] Therefore, any metabolic alteration in the sertoli cell derived from diabetes mellitus may be responsible for spermatogenesis disruption, playing a crucial role in fertility. The adjacent sertoli cell forms the blood-testes barrier and protects the developing germ cells from the blood. Therefore, blood-to-germ cells transport of glucose and other metabolic intermediates is highly controlled, particularly due to the presence of the blood–testis barrier (BTB). This barrier not only physically divides the

seminiferous epithelium in two compartments but also is responsible for the maintenance of different levels of substances and metabolites. Diabetes mellitus alters the overall physiological cellular condition and modifies the metabolic environment surrounding the blood-testis barrier. The blood-testis barrier cells metabolism is also altered, particularly under conditions of prolonged hyperglycemia. Under deprivation of glucose in the Sertoli cell it maintain spermatogenesis through monocarboxylic acids, fatty acids and ketone bodies,^[47,48] It has been reported that β -oxidation pathway is used by Sertoli cells to produce ATP, especially by using free fatty acids or the recycling lipids of apoptotic spermatogenic cells and residual bodies that are phagocytized and degraded.^[49] The exact role for blood-testis barrier in glucose dynamics during diabetes mellitus is also unknown, although the blood-testis barrier plays a crucial role in the maintenance of spermatogenesis that is expected to be compromised by diabetes mellitus.

CONCLUSION

On the basis of our result, we can hypothesize that the reduced secretion of testosterone by Leydig cells may be due to effect of hyperglycemia on the Leydig cells or on the pituitary gland. Hyperglycemia may affect the hypothalamus also which leads to reduce in GnRH which in turn affects the gonadotrophin release from the pituitary gland. These various factors may all come to play and further studies are required to reveal the potential, biochemical and physiological mechanism involved in hypothalamo-pituitary axis, which leads to the reduction of reproductive hormones in the hyperglycemic diabetic rats.

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