

## Diabetes Mellitus and Impairment of Male Reproductive Function: Role of Hypothalamus Pituitary Testicular Axis and Reactive Oxygen Species

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**Received:** 07 March 2016

**Accepted:** 14 August 2016

**Published in January 2017**

### Abstract

Diabetes mellitus (DM) is one of the greatest public health threats in modern societies. Although during a few years it was suggested that DM had no significant effect in male reproductive function, this view has been challenged in recent years. From a clinical perspective, the evaluation of semen parameters, as well as spermatozoa deoxyribonucleic acid (DNA) integrity, are often studied due to their direct implications in natural and assisted conception. Testicular cells have their own glucose sensing machinery that react to hormonal fluctuations and have several mechanisms to counteract hyper- and hypoglycaemic events. Moreover, the metabolic cooperation between testicular cells is crucial for normal spermatogenesis. Sertoli cells (SCs), which are the main components of blood–testis barrier, are not only responsible for the physical support of germ cells but also for lactate production that is then metabolized by the developing germ cells. Any alteration in this tied metabolic cooperation may have a dramatic consequence in male fertility potential. Diabetes-related oxidative stress may also be the trigger for many alterations on sexual function (poor semen quality, erectile and testicular dysfunction, impotence, decreased fertility potential and retrograde ejaculations), which can also include decreased testicular mitochondrial function. In addition, diabetic men have decreased serum testosterone due to impaired Leydig cell function which is accompanied by low LH and FSH; the inability of the pituitary gland to respond appropriately to a decline in testosterone implying central effect of high serum glucose on the interaction between the nervous and endocrine system. Therefore, this review article highlights the impact of diabetes and associated, oxidative stress with reference to hypothalamus pituitary testicular axis.

**Keywords:** Diabetes, Hyperglycemia, Reproductive/ sexual dysfunction, Infertility, Insulin.

### Introduction

Infertility is already a major health problem in both the developed and developing world, with up to one in six couples requiring specialist investigation or treatments in order to conceive (1). It is estimated that 15% of couples attempting to conceive are not able to do so within one year. Male factor infertility is present in 20%–50% of these

couples, either independently or in conjunction with female factor infertility issues (2,3). Infertility is typically defined as the inability to achieve pregnancy after one year of unprotected intercourse. The global decline of fertility rates, specifically in developing and developed countries, has been an issue of concern (4), especially on the relative contributions of social, environmental and genetic factors. Diabetes has been associated with reproductive impairment in both men and women (5,6), and its impact on reproduction can be profound, as seen by diminution in fertility and increase in reproductive losses (7-10). Overall this is not surprising, considering diabetes commonly results in vascularization and endothelial dysfunction (11,12), potentially affecting, directly or indirectly, various functions of the reproductive system (13-16). In this review we highlight diabetes-induced alterations in male sexual/reproductive functions.

### **Brief Overview of Diabetes Mellitus**

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (17). As the oxidation or metabolism of sugars from carbohydrates is the major source of energy for the human body, diabetes can lead to major systemic problems. Insulin, a hormone released from the pancreas, allows glucose to be transported into cells so that they can produce energy or store the glucose until it is needed.

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes or juvenile-onset diabetes. The majority of type 1 diabetes is of the immune-mediated nature, in

which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin (18).

Type 2 diabetes is a heterogeneous disorder caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, over eating, lack of exercise, and stress as well as aging (19). It is typically a multifactorial disease involving multiple genes and environmental factors to varying extents (20). Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus (21). In Type 2 diabetes mellitus (non-insulin-dependent diabetes), the pancreas continues to manufacture insulin, sometimes at even higher than normal levels. However, the body develops resistance to its effects, resulting in a relative insulin deficiency (22).

### **Molecular Basis of Glucose Metabolism in Testis and Sperm**

Diabetes mellitus induced effects on testicular function have been attributed to the lack of insulin (26), which is the leading hormone responsible for glucose homeostasis regulation (27). Maintenance of spermatogenesis in vivo and the fertility capacity of male sperm depend on glucose metabolism (28,29). Sperm cells can effectively use several simple sugars such as glucose, fructose and mannose (30) and the spermatozoa energy production requires catabolism of glucose to pyruvate and lactate by the glycolytic pathway enzymes. Lactate production is made by Sertoli cells (SCs) to maintain germ cells survival and this process has been shown to be predominantly under the control of the endocrine system, primarily by sex steroid hormones (31-33), follicle-stimulating hormone (FSH) and insulin (34). An alteration in spermatozoa ability to utilize the substrates involved in ATP production would be expected to compromise sperm motility and subsequently fertility (35). Spermatozoa need specific carriers, known as glucose transporters (GLUTs) to mediate the glucose uptake from the surrounding medium

into the cell (36). Diabetes has been shown to be associated with a depletion of GLUTs (37). Therefore, diabetic individuals are known to possess an inability to transport glucose, which supports an association of this disease with disruptions in sperm metabolism and consequently sub fertility or even infertility.

### **Diabetes and the Hypothalamus - Pituitary - Testicular Axis**

In a normally functioning hypothalamus pituitary gonadal axis (HPG) axis, the hypothalamus releases gonadotropin-releasing hormone (GnRH) pulses that stimulate the pituitary to secrete both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH act on the Sertoli cells and the Leydig cells, respectively, to stimulate the process of spermatogenesis. The onset of diabetes is known to disrupt the HPG axis, resulting in impaired spermatogenesis and subsequent subfertility (38). Diabetic men had significantly low serum testosterone with low LH and FSH. The hypothalamic cells, which produce LHRH, do not function correctly to the feedback when testosterone level decreased. This inability of the pituitary gland to respond appropriately to a decline in testosterone implies that high serum glucose has a central effect on the interaction between the nervous system and endocrine system. The decrease in serum LH and FSH may result from impairment in its production and secretion (39). Increase in body lipids due to diabetes decreases serum testosterone level due to increased activity of the aromatase enzyme present in fat tissues there by converting testosterone and androstenedione to estrogens (40). Consequently in diabetic men, in addition to decreased testosterone production and metabolism, higher than normal percentages of testosterone and androstenedione are converted into estradiol and estrone, respectively. This increased conversion may account for the diminished testosterone. The increased estrogens appear to exert a negative feedback effect on LH and FSH production and may thereby contribute to

the suppression of these key reproductive hormones.

Apart from this, serum insulin has long been known to affect the central nervous system, and these effects might mediate whole body energy homeostasis, including the reproductive axis, through further signaling to the pituitary and, ultimately, the gonads. A research in 1977 (41) showed that peripheral insulin injection caused an increase in insulin levels in cerebral spinal fluids, suggesting that insulin could potentially be a signal to the brain regarding energy stores and promoting whole-body energy homeostasis. Thus, a lack of insulin would signal to the brain a lack of energy supply, and the central nervous system could potentially shut down extraneous energy-consuming processes such as reproductive function. Indeed, insulin levels have dramatic effects on the regulation of the HPG axis. The effects of diabetes on the reproductive axis are mediated, at least in part, by signalling in the brain. Insulin mediates its effects through binding with the insulin receptor, resulting in a signaling cascade. Through interactions with the insulin receptor substrate proteins, notably IRS-2, insulin potentiates signalling through phosphatidylinositol 3-kinase (PI3-kinase), which then activates PKB (Protein kinase B), an important mediator of energy signalling (42). Insulin signalling in the brain can happen at multiple sites. Insulin receptor expression has been detected in the hypothalamus, the olfactory bulb, and the pituitary (43). Additionally, insulin concentrations in the brain are markedly higher than plasma insulin levels, suggesting that insulin in the brain is not simply a reflection of serum levels, but that this signaling hormone has a crucial function in the central nervous system (44).

The precise impact that insulin in the brain has on changes in the reproductive axis is unknown. It has been shown in animals that hyperinsulinaemia (induced by hyperinsulinaemic clamp) stimulates LH secretion, an expression of the hypothalamic activity of GnRH (45). The same investigators

demonstrated in hypothalamic neurons in culture that GnRH secretion and expression was stimulated directly and dose-dependently by insulin (46,47). Neuron-specific insulin receptor knockout mice exhibit hypogonadism secondary to impaired GnRH secretion (47). In their research they also revealed in histological examination that, although many of the semeniferous tubules appear normal, about 20% do not possess a lumen and have little or no mature sperm cells. Additionally, the Leydig cells appear shrunken, suggesting that the lack of proper insulin signalling in the brain reduces the hormonal output necessary to retain the Leydig cell population for the successful promotion of spermatogenesis in all tubules.

Other studies examining the mechanism of insulin signalling in the brain have found that insulin signalling in the brain is required for the inhibition of glucose production. Injection of insulin directly into the brain results in a decreased production of glucose independently of serum insulin levels. Injection of insulin signalling inhibitors cause increased glucose production, despite circulating serum insulin levels. This study further demonstrates that neuronal insulin action is regulated separately from plasma insulin and might be involved in overall energy homeostasis mediated by the central nervous system (48).

Insulin's effects on the reproductive axis are not solely mediated by insulin interactions with receptors in the brain. Insulin levels are also known to be correlated directly to circulating levels of leptin, an important molecule involved in maintaining energy homeostasis. Leptin is a crucial hormone secreted by the fat cells that signals to the hypothalamus, and regulates the reproductive system. It serves as a metabolic signal that informs the brain of nutritional status and provides information regarding an animal's ability to meet the energy demands of reproduction (49). Insulin has been shown in vitro to have direct effects on leptin synthesis (50-52). In fat cells, the administration of insulin promotes an increase in leptin

production in vitro. In vivo, long-term exposure to hyperinsulinemia was shown to promote an increase in circulating leptin levels (53).

In humans, leptin levels are also affected by type 1 diabetes. Leptin levels are decreased in newly diagnosed type 1 diabetic patients before the administration of insulin treatment, but these levels normalize after the onset of insulin treatment (54). This leads to the conclusion that serum leptin is not simply readout of body fat stores; but that it is also regulated by insulin levels (55) and hence have effect on reproductive axis.

### **Diabetes and Erectile Dysfunction**

A very common pathology experienced by some men with diabetes is the consistent inability to achieve and maintain penile erection sufficient for adequate sexual relations, or erectile dysfunction (ED) (56,57). Indeed, ED is three times more prevalent in men with type 1 or 2 diabetes than in non diabetic men, it occurs at an earlier age in diabetic males, and its effects increase with the duration of the disease. The pathophysiology of ED in diabetes is not well understood, but associated neuropathy or vascular and endothelial dysfunctions are considered the most important factors (57), given that ED is predominantly a vascular disease. It has been estimated that 50–75% of diabetic men have ED of some degree. The mechanisms for ED in men with diabetes are endothelial dysfunction, dysfunction of the nitric oxide (NO) pathways (down regulation of NO synthase and degeneration of nitrergic nerves), dysfunction of other signal transduction pathways, corporal smooth muscle degeneration, and tissue remodeling (58,59). Sexual stimulation activates the non-adrenergic, noncholinergic nerve and activates the neural NO synthase/cGMP pathway. The release of NO facilitates the relaxation of penile cavernosal arteries and resistance arterioles, which causes vasodilation, and increases blood flow to the corpus cavernosum. The increased blood flow

stimulates the endothelium lining of the lacunar spaces of the corpus cavernosum to release endothelial NO from the endothelium NO synthase. These biochemical and physiological processes result in trabecular smooth muscle relaxation and expansion of the sinusoids within the corpora cavernosa, leading to penile engorgement. This expansion of the corpora cavernosa against the tunica albuginea results in venoocclusion and trapping of blood under pressure. This process is referred to as the 'venoocclusive' mechanism. Neural and endothelial NO synthases are regulated by androgens. In addition, the tissue histo-architecture is dependent on androgens. Thus, any perturbations or alterations in the neural, vascular or erectile tissue fibroelastic properties will contribute to ED, by altering the veno-occlusion mechanism (60). Also, in diabetes it has been shown that endothelium dependent smooth muscle relaxation is impaired, thus leading to ED *via* smooth muscle dysfunction in the microvasculature tree of the penis.

### **Relation between Diabetes, Oxidative Stress and DNA**

Systemic complications are the major causes of morbidity and mortality in patients with diabetes (11). Hyperglycaemia, a key clinical manifestation of diabetes mellitus, not only generates reactive oxygen species (ROS), but also attenuates anti-oxidative mechanisms by scavenging enzymes and antioxidant substances (61-65). Hyperglycemia causes glucose auto-oxidation, glycation of proteins, activation of polyol metabolism and subsequent formation of ROS. It has also been demonstrated that hyperglycemia is associated with an increased production of free radicals in the mitochondria and may contribute to a greater DNA damage (66-69). As ROS cause strand breaks in DNA and base modifications including the oxidation of guanine residues to 8-oxo, 2'-deoxyguanosine (8-oxodG), 8-oxodG can serve as a sensitive biomarker of oxidative DNA damage (70). 8-OxodG was

increased in the kidneys of diabetic rats, and insulin treatment reduced both urinary albumin excretion and 8-oxodG formation in the kidney (71). A recent study reported an increase in the 8-oxodG content in mononuclear cells and ROS level in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients when compared with control subjects (72). Several reports have shown that diabetes increases the oxidative damage to DNA (71,72). Another study confirmed a significant positive correlation between urinary 8-oxodG excretion and the content of 8-oxodG in mononuclear cells. These findings suggest that urinary 8-oxodG excretion possibly is a useful marker of oxidative DNA damage in diabetic patients (73-74). Goodarzi et al. report on a significant positive correlation between urinary 8-OHdG and fasting blood glucose and HbA1c (75). These findings provide evidence that the increased oxidative stress in diabetes contribute to the pathogenesis of DNA damage.

### **Relation between Insulin and SHBG**

Sex hormone-binding globulin (SHBG) is a circulating plasma globulin binding sex hormones, both oestradiol and testosterone and is produced primarily by the liver. In addition to inducing DNA damage, insulin levels have also been shown to influence the levels of sex-hormone-binding globulin (SHBG), by inhibiting their biological activity as carriers. High circulating insulin levels inhibit SHBG synthesis in the liver, whereas weight loss has been shown to increase SHBG levels (76). In type 2 diabetic males, the decrease in SHBG means that less estrogen will be bound, resulting in more biologically active, free estrogen. In addition to the conversion of testosterone to estrogen in diabetic patients, the decreased ability of SHBG to sustain homeostatic levels of free testosterone also contributes to abnormal testosterone levels (77). This failure to maintain homeostatic levels might magnify the negative feedback effect of elevated total estrogen levels. Even

when the presence of SHBG is accounted for, an independent relationship between insulin resistance and testosterone production can still be demonstrated (78). Therefore, the levels of SHBG might be important only as a marker of altered hormone profiles in diabetic infertile men.

Interestingly, there is a significant difference in plasma testosterone levels between men with diabetes type 1 (who have normal levels) and type 2 (who have subnormal levels) (79). This difference was attributed to the differences in circulating levels of insulin (low in type 1 and high in type 2). There is an inverse relationship between insulin levels and sex hormone-binding globulin (SHBG) and, consequently, plasma levels of total testosterone are lower in men with type 2 diabetes. Low total testosterone and SHBG levels independently predict development of the metabolic syndrome and diabetes in middle-aged men (80). Thus, hypoandrogenism is an early marker for disturbances in insulin and glucose metabolism that may progress to the metabolic syndrome or diabetes and may contribute to their pathogenesis.

### **Diabetes and Spermatogenesis Studies**

Diabetes causes the reduction of spermatogenic cells and decreases the tubules diameter by cell apoptosis and seminiferous tubules atrophy. These changes are indicative of morphologic disorders in spermatogenesis (81,82). In another research, atrophy and reduction of seminiferous tubules diameter and spermatogenic cells were seen in the diabetics. Diabetes increases the thickness of basal lamina in seminiferous tubules which accompanies reduction of sperm production and total size of seminiferous tubules (83). Also, diabetes leads to reduction of sertoli and germinal cells. Moreover, reduction in sertoli cells causes reduction in sperm number. Sertoli cells have an important role in spermatogenesis in providing physical and nutrition protection and necessary hormone

signals for successful spermatogenesis (84), therefore, when sertoli cells reduce, the number of germinal cells decreases intensively (85). Diabetes effect on testicular function is due to inadequate insulin production and subsequent reduction of it deteriorates sertoli and leydig cells function. Moreover, reduction of insulin levels weakens the spermatogenesis process by reduction of FSH (86).

### **Reproductive Impairments in Diabetic Animal Models**

Animal studies using rodent models of Streptozotocin (STZ) induced diabetes have demonstrated diminished reproductive organ weight, lower testicular and epididymal sperm content, and lower sperm motility (87-89). Additionally, histological studies revealed a considerable reduction of the seminiferous tubules and epididymal lumen in STZ-treated rats (89). It has also been demonstrated that fewer diabetic rats complete ejaculation when compared to control rats, in accordance with the observed reduction in copulatory behaviour. Moreover, a marked decrease in serum testosterone levels, and of in vitro testosterone secretion, was observed in diabetic rats by same researchers. Testosterone synthesis disorder is caused by changes in leydig cells, and may lead to other disorders in all target organs and tissues. In addition, a marked reduction in fecundity has been observed after as little as 15 days following the injection of streptozotocin (88). Other groups have reported similar findings after longer periods of induced diabetes (2–6 months) (90). The associated reduction in fertility is more pronounced when diabetes is induced in pre-pubertal animals (91). Furthermore, spontaneously occurring diabetes in the BB Wistar rat, is also associated with a significant reduction in fertility (92), thus eliminating any possible confounding effects of diabetogenic agents as a primary cause. These studies support the hypothesis that diabetes mellitus impairs male reproductive function.

## Conclusion

Disruptions in the HPG axis have severe reproductive consequences. Diabetes can impact many aspects of the functional axis, resulting in subfertility. However, the diabetic outcomes on fertility are probably mediated not only through the HPG axis, but also by the detrimental effects of hyperglycemia and oxidative DNA damage to the testes and sperm cells. Free radical formation along with antioxidant deficiency in diabetes mellitus increases over time due to prolonged hyperglycemic status and play an important role in the oxidative DNA damage and development of diabetic complications including infertility. Strict glycemic control along with antioxidant therapy can help to

reduce the risk of developing diabetic complications. Additionally, the presence of insulin transcripts in testes and sperm raises the possibility that insulin signaling is important within the testes and plays a part in the diabetic pathogenesis of infertility. Further work in the field is necessary to establish the precise role of direct insulin signalling in the testes and sperm cells and to determine whether diabetes has an impact on this local signaling.

## Acknowledgement

The authors are thankful to Department of Biotechnology, New Delhi, for providing valuable assistance through DBT-IPLS-BU programme.

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