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# THE EFFECTS OF TYPE 2 DIABETIC MELLITUS ON THE LEVELS OF TESTOSTERONE, ESTRADIOL, GONADOTROPINS, AND RETINOL BINDING PROTEIN 4

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**ABSTRACT**
The present study was aimed to investigate the effects of Type 2 diabetic mellitus on the levels of sex hormones (Testosterone and Estradiol), gonadotropins (Luteinizing hormones (LH) and Follicle stimulating hormones (FSH), beside Retinol binding protein (RBP4).The study included 60 Iraqi males diabetic patients aged (40-50) years with mean of 46.86 ±0.85 years and apparently healthy (20) as control subjects aged (40-50) years with mean of 45.35 ±0.78 years who visited the specialist center of Endocrine and diabetes at Baghdad province. The study begun from 1 November 2016 to 30 January 2017.The level of fasting blood glucose (FBG mg/dL) was increased significantly (P<0.01) in testo, normal diabetic group (264 ±28.27) and in Testo. The low diabetic group (247 ±14.51) in comparison with control group (63.50 ±2.80). The level of glycated hemoglobin HBA1C was increased significantly (P<0.01) in both diabetic groups (9.772 ± 0.47; 8.767 ±0.20) respectively in comparison with control group (4.935 ±0.16).The level of testosterone (ng/ml) was decreased significantly (p<0.01) in Test. Low diabetic group (1.49 ±0.07) in comparison with control group (3.11 ±0.15) and Testo. Normal diabetic group (3.36 ±0.25).There was no significant difference (p>0.05) level in testosterone between Test. Normal diabetic (3.36 ±0.25) and control group (3.11 ±0.15).The level of Luteinizing hormone (LH) (miu/ml) shows non-significant difference (p>0.05) in both two diabetic groups (4.20 ±1.05, 4.87 ±0.44 ) in comparison with control group (4.81 ±0.56 ). The level of follicle stimulating hormone (FSH) (miu/ml) in Testo. Low diabetic group (6.69 ±0.64) was increased significantly (p<0.05) however, the value was within normal range (1-11miu/ml) in comparison with control group (4.61 ±0.62) and Testo. Normal diabetic group (3.34 ±0.80). Estradiol levels (pg/ml) were increased significantly (p<0.01) in Testo. Normal diabetic (72.90 ±4.42) and Testo. Low diabetic (71.54 ±3.06) groups in comparison with control group (22.74 ±1.75). The levels of Retinol binding protein (ng/ml) increased significantly (p<0.01) in the two diabetic groups (1.254 ±0.11); (1.253 ±0.07) respectively in comparison with control group (0.387 ±0.03). Our study showed that Type2 Diabetic mellitus reduce the level of testosterone at (81.66%) among diabetic subjects groups. The decreasing in testosterone was accompanied by an increase in the level of estradiol hormone. Both diabetic patients show increasing in the estradiol level that could lead to reduce the level of testosterone through paracrine/autocrine effects. The level of Gonadotropin (LH, FSH) was within the normal range. The increasing in the level of Retinol binding protein 4 (RBP4) seems to be act as insulin resistance that associated with increasing the fasting blood glucose (FBG) and HBA1C.

**KEYWORDS**: Diabetes Mellitus, Testosterone, Estradiol, Gonadotropins, Retinol binding protein.

## INTRODUCTION

Diabetes mellitus is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin, that resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism (Njolstad *et al*., 2003). The prevalence of diabetes is increasing rapidly worldwide and the World Health Organization (2003) has predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 439 millionin 2030 (Shaw *et al*., 2010). The high plasma glucose produces the classical symptoms of polyuria, polydipsia and polyphagia (Rother, 2007). In obesity and in hyperinsulinaemia secondary to insulin resistance (IR), often present in patients with T2DM, there is a decrease in total Testosterone (TT) related to lower sex hormone–binding globulin (SHBG) levels resulting from decreased hepatic synthesis of this protein (Laaksonen *et al*., 2003). However, in obesity, as well as in hyperinsulinaemia and DM2, there is also a decrease in free T (FT) and bioavailable T (BT) which implies a real decline in T production (Knoblovits *et al*., 2010; Dandona and Dhindsa, 2011). It is hypothesized that in the presence of obesity and IR, Leydig cell steroidogenesis might be impaired either because of IR at this level or by the action of hormones such as insulin, leptin or cytokines from visceral fat (Pitteloud *et al*., 2005). That T has effects on insulin sensitivity in men low T levels predispose to central obesity and predict the development of metabolic syndrome and T2DM (Laaksonen *et al*., 2004). Over 90% of people with diabetes mellitus are type 2 diabetics and it is reported to be associated with

certain endocrine disorders, in particular hypogonadism in men (Burtis *et al.,* 2008). The hypogonadal-obesity-adipocytokine cycle have been offered to explain the effect of adiposity on circulating testosterone based on known scientific findings (Cohen, 1999 and Kapoor *et al*., 2005). The amount of testosterone synthesized is regulated by the hypothalamic-pituitary-testicular axis. When testosterone levels are low, gonadotropin- releasing hormone (GnRH) which in turn stimulates the pituitary gland to release FSH and LH. These latter two hormones stimulate the testis to synthesize testosterone (Swerdkiff *et al*., 1992). This means that when the feedback mechanism is functioning properly, low testosterone level will induce secretion of high FSH and LH levels. The most causes of decreasing the level of testosterone in diabetic patients and obese individuals may be resulting from conversion of testosterone to estradiol by the actions of aromatase enzyme located in adipose tissue. Therefore, a reduction of testosterone is inevitable with increased expression of aromatase, which is a result of an increased number of adipocytes in diabetic men (Kelly and Jones, 2013). RBP4 has been proposed as an adipokine involved in the pathogenesis of insulin resistance (Yang *et al.,* 2005; Graham *et al*., 2006). That decrease in insulin sensitivity was associated with reduced phosphoinositide 3-kinase activity and increased the phosphoenol pyruvate carboxykinase expression in the liver which is the same mechanism by which RBP4 induces insulin resistance in which Serum RBP4 levels are increased in subjects with impaired glucose tolerance,T2DM (Yang *et al*., 2005).

**Aims of the study**

To investigate the effects of type 2 diabetes mellitus in Iraqi male patients on the levels of Testosterone, Gonadotropins (LH and FSH), Estradiolhormones, Retinol binding protein, RBP4, lipid profile through the following items by estimation of:

1- Body mass index (BMI) $kg/m^2$.
2- Fasting blood glucose (FBG) mg/dl and glycated hemoglobin (HBA1C %).
3- Testosterone level ng/ml.
4- Luteinizing hormonemiu/ml.
5-Follicle stimulating hormone miu/ml
6-Estradiol pg/ml.
7- RBP4 ng/ml
8- Lipid profile mg/dl.

## MATERIALS & METHODS

**Collection of Information and Patients Selection**

The study was carried out on (60) Iraqi males diabetic patients aged (40-50) years with mean (46.86 ±0.85) and apparently healthy who visited the Specialist Center for to Endocrine and Diabetes at Baghdad province, control subjects with total number of (20) aged (40-50) years with mean (45.35 ± 0.78) were included in this study and they were diagnosed according to the level of fasting blood glucose (FBG) and glycated hemoglobin (HBA1C).

**Collection of blood sample**

Eight milliliters of blood was drawn from each individual after (12- 14) hours fasting via venipuncture, by using 10 ml disposable syringes between (8.00–10.30A.M). The blood sample was divided into two aliquots; 2 and 6 ml. The first aliquot blood was dispensed in a tube containing ethylene diamine tetra acetic acid (EDTA K3) as anticoagulant and stored at (2-8°C) for analyses of HBA1C, while the second aliquot was transferred into Gel tubes without anti-coagulant; blood was left to clot for 20-30 minutes at (37°C) in an incubator. Sera were separated by centrifugation at 3000 rpm for 10 minutes divided into three small eppendorf tubes capacity 1.5 ml, the serum in first eppendorf used for the determination of FBG, Estradiol, the second eppendorf used for the determination of LH, FSH, testosterone and the third eppendorf kept at (-20°c) for Retinol binding protein analysis.

**Fasting blood glucose (FBG) and HBA1C measurement**

Fasting blood glucose was estimated by the Reflotron Plus method which is an in vitro diagnostic device designed for the quantitative determination of clinical chemistry parameters using Reflotron test strips. It works on the principle of reflectance photometry and ensures rapid and reliable results while being simple to use.(Price and koller, 1988).The HBAIC determination is based on the fluorescence immunoassay technology for hemolyzed whole blood in i-CHROMA $^{TM}$system (Brooks *et al*., 1999).

**Normal value for FBG**

70-110 mg/dl; Normal values for HBA1C: 4.5-6.5 %

**Measurement of serum total testosterone**

Testosterone determination is based on the competitive immunofluorescence assay. The fluorescence intensity of the anti-testosterone antibody reflects the amount ofantigen captured and is processed in ichroma™ Reader to determine the testosterone concentration in the specimen (Tulsidas and Shrivastav, 2002).

**Normal values**: Men 2.5-10.0 ng/ml.

**Estimation of Serum Luteinizing hormone (LH)**

LH is used as an aid in the screening or monitoring of determination of evaluating fertility issues, function of reproductive organs(ovaries or testicles), or detection of the ovulation.(South *et al.,*1993).**Normal values:** Men 1.0 – 8.0 mIU/ml.

**Estimation of Serum Follicle-stimulating hormone (FSH)**

FSH is synthesized and secreted by gonadotrophs of the anterior pituitary gland (Kim *et al*., 2011). The FSH determination is based on immunoassay system using antigen antibody interaction and fluorescence technology (Beastall *et al.,* 1987). Normal values: Men 1-11 mIU/ml.

Estimation of Serum estradiol ($E_2$) The quantitative determination of Estradiol concentration in human serum or plasma by a microplate enzyme immunoassay (Bergquist *et al.,* 1983). Normal values: Men 10-36 pg/ml.

Estimation of Serum RBP4-The quantitative measurement of RBP4 in serum was performed using a leptin enzyme immunoassay or ELISA kit.(Sell and Eckel, 2007).

**Normal values:** 0.053-0.628 ng/ml.

## RESULTS & DISCUSSION

### Levels of FBG and HBA1C

The level of fasting blood glucose (FBG mg/dl) was increased highly significant (p<0.01) inTesto. Normal diabetic group (264 ±28.27) and Testo low diabetic group (247.86 ±14.51) in comparison with control group (63.50 ± 2.80)Table (1).

**TABLE1:** Comparison among difference groups in FBG and HBA1C

| The group | Mean ± SE | |
|---|---|---|
| | FBG (mg/dl) | HBA1C (%) |
| Control | 63.50 ± 2.80 b | 4.935 ± 0.16 c |
| Testo. Normal | 264.09 ± 28.27 a | 9.772 ± 0.47 a |
| Testo. Low | 247.86 ± 14.51 a | 8.767 ± 0.20 b |
| Normal value | 70-110 | 4.50-6.50 |
| LSD value | 56.989 ** | 0.859 ** |
| P-value | 0.0001 | 0.0001 |

Means having with the different letters in the same column differed significantly ** (P<0.01).

The increasing in the level of FBG was in agreement with many researchers (Njostad *et al.,* 2003; Hussein and Al-Qaisi, 2012).The chronic diabetes is a group of metabolic diseases characterized by hyperglycemia, the elevation in FBG level may be resulting from defects in insulin secretion, insulin action or both (ADA, 2014). The FBG test is directly proportional to the severity of the diabetes mellitus (Rother, 2007; Ngugi *et al*., 2012). So the increase in the level of FBG in this study was also in agreement with that reported by (ADA, 2015) that stated FBG level ≥ 126 mg/dl, in both diabetic patients groups (Testo. Normal and Testo. Low ) showed high level of FBG in comparison with the control group. Hyperglycemia is the main feature of diabetic and its increase may associated with the increase of glucagon level that characterized by hepatic glucose production, the major factor that participate in fasting and postprandial hyperglycemia (Lefebvre, 2006).The level of glycated hemoglobin HBA1C% was increased significantly (p<0.01) in both diabetic groups (9.772 ±0.47; 8.767 ±0.20) respectively in comparsion with control group (4.935 ±0.16) Table1.The high level of FBG was associated with the increased level of HBA1C % in both two diabetic groups, that testing HBA1C is attracting as measures chronic glycaemia in diabetic patients. It has been used as objecting marker of average glycemic control in the monitoring of patients with diabetes (d' Emden, 2014), that the major consequences of hyperglycemia are excessive non-enzymatic glycosylation of various body proteins like hemoglobin, albumin. So the increasing of HBA1C levels in our study indicates poor control of FBG levels or poor glycemic index (Tayde *et al*., 2013). The increasing in the HBA1C levels in two diabetic groups was in accordance with that reported by Mohsen, 1999 in Saudi population (9.7%) in T2DM subjects and with that recorded by (Ahmed *et al*., 2013) who found HBA1C level (9.5% vs 6.0% in control).The International Diabetes Federation (IDF) recommend HBA1C ratio below 6.5% while HBA1C below 7.0% was recommend by American Diabetes Association (ADA, 2014) for most patients to indicate good glycemic control.

### Levels of testosterone, LH, FSH, Estradiol and RBP4

The levels of testosterone (ng/ml) was decreased highly significant (p<0.01) in Test. Low diabetic group (1.49 ±0.07) in comparison with control group (3.11 ±0.15) and Testo. Normal diabetic group (3.36 ±0.25). There was no significant difference (p>0.05) between Test. Normal diabetic and control group Table (2).

**TABLE 2:** Comparison among difference groups in level of hormones

| The group | Mean ± SE | | | | |
|---|---|---|---|---|---|
| | Testosterone ng/ml) | LH (miu/ml) | FSH (miu/ml) | Estradiol (pg/ml) | RBP4 (ng/ml) |
| Control | 3.115 ± 0.15 a | 4.811 ± 0.56 a | 4.610 ± 0.62 ab | 22.74 ± 1.75 b | 0.387 ± 0.03 b |
| Testo. Normal | 3.362 ± 0.25 a | 4.203 ± 1.05 a | 3.346 ± 0.80 b | 72.90 ± 4.42 a | 1.254 ± 0.11 a |
| Testo. Low | 1.491 ± 0.07 b | 4.877 ± 0.44 a | 6.694 ± 0.64 a | 71.54 ± 3.06 a | 1.253 ± 0.07 a |
| Normal value | 2.50-10 | 1-8 | 1-11 | 10-36 | 0.053-0.628 |
| LSD value | 0.407 ** | 1.963 NS | 2.559 * | 11.867 ** | 0.276 ** |
| P-value | 0.0001 | 0.796 | 0.022 | 0.0001 | 0.0001 |

* (P<0.05), ** (P<0.01), NS: Non-significant.
Means having with the different letters in same column differed significantly.

The level of Luteinizing hormone (LH) (miu/ml) shows non-significant difference (p>0.05) in both two diabetic groups (4.20 ±1.05; 4.87 ±0.44) in comparison with control group (4.81 ±0.56). The levels of follicle stimulating hormone (FSH) (miu/ml) in Testo. Low diabetic group (6.69 ±0.64) was significant increase (p<0.05) although was with normal range (1-11) (miu/ml) in comparison with control group (4.61 ±0.62) and Testo. Normal diabetic group (3.34 ±0.80) respectively. Estradiol levels (pg/ml) were increased significantly (p<0.01) in Testo. Normal diabetic (72.90 ±4.42) and Testo. Low diabetic (71.54 ±3.06) groups in comparison with control group (22.74 ±1.75 ).The levels of RBP4 (ng/ml) shows significant difference (p<0.01) in two diabetic groups (1.254 ±0.11);(1.253 ±0.07) respectively in comparison with control group (0.387 ±0.03). The decrease in the

testosterone hormone level in Testo. Low diabetic patients group in our study was in agreement with that reported by (Onah *et al*., 2013; Asare-Anane *et al*., 2013 and Shahin *et al*., 2015 and Abdul- Hadi, 2016).There are several mechanisms for the association between low serum testosterone level and T2 diabetes with IR and obesity as central features of the association between low serum testosterone (LST) and DM has recently received substantial attention (Ghazi *et al.*, 2012; George, *et al*., 2013) Studies have reported that T2DM men have a high prevalence LST. (Tamler *et al*., 2010) Further, low total testosterone (TT) levels have been accompanied with insulin resistance and subsequent risk for developing T2DM (Grossmann *et al.*, 2008; Soriguer *et al.*, 2012). The main signs of LST are decrease libido/ erectile dysfunction, decreasing muscle mass and strength, increased adiposity, osteoporosis/low bone mass, depressed mood, fatigue, low energy, and impaired quality of life (Zhang *et al.*, 2012; Al Hayek, *et al.*, 2013). Testosterone decrease has a high prevalence in men with T2DM (Dhindsa *et al*. 2004, Ding *et al*., 2006; Kapoor *et al.*, 2007). Furthermore, low testosterone is accompanied with impaired insulin sensitivity, increased percentage of body fat, truncalobesity, dyslipidaemia, hypertension and CVD (Wang *et al*., 2011 and Daniel *et al.*, 2013). Testosterone biosynthesis regulation primarily by pulsatile secretion of luteinizing hormone (LH) and serum testosterone levels reflect the integrity of the hypothalamic-pituitary-gonadal (HPG) axis. Therefore low testosterone levels estimated in cases of insulin resistance may indicate a defect at one or more functional levels of the HPG axis. In the IR state, Leydig cell function, particularly steroidogenesis, may be deteriorate by alterations in the production of hormones and cytokines locally in the target tissue and in adipose tissue that hyperinsulinemia, as encountered in insulin resistance, might impair testosterone secretion by the Leydig cell, maybe directly since Leydig cells has insulin receptors on them. Although several studies suggest that rising in insulin resistance may be associated with a decrease in testosterone secretion in men, it is not completely clear how the HPG axis mediates the interplay between testosterone and insulin levels (Verma *et al.*, 2013).The decrease of testosterone in Testo. Low level diabetic group in our study may be related to a decrease in the level of SHBG that (Hu *et al*., 2016) found that an increase of estradiol levels in males may related to decrease in the levels of sex hormone-binding globulin (SHBG) and the men who have high estradiol level and low levels of SHBG had develop type 2 diabetes risk. Vermeulen *et al*., 1993 who reported that increased BMI in males is associated with decline plasma concentrations of (SHBG) and testosterone with a concomitant increase in plasma concentration of estrogen. The normal levels of testosterone in Testo. Normal diabetic group in our results was agreement with at reported by (Esmaeel, 2013) who recorded non-significant changes (P>0.05)in testosterone levels between ten healthy men aged (25-53) years and ten diabetic men with in same age in Babylon province. It is estimated that 30 to 40% T2DM men have low T levels, assessed as total T (TT), free T (FT) or bioavailable T (BT). (Dhindsa *et al*., 2004) measured FT levels by equilibrium dialysis method in 103 males with T2DM. They found that 33% of patients had levels in the hypogonadal range. The levels of LH and FSH hormones were within normal ranges in our study in both diabetic groups, inspite such significant elevation of FSH was showed in Testo. Low diabetic group. The normal levels were in agreement with that reported by (Ando *et al*., 1984) reported low TT and normal LH and FSH levels in diabetics; whereas, (Ali *et al*.,1993) found that subjects with diabetic neuropathy had low testosterone, high LH and FSH levels. In hypogonadal patients with T2DM, gonadotropin levels are usually normal or low, that enhance the diagnosis of hypo- or normogonadotrophic hypogonadism in most of these men. This reinforces the possibility of a failure at central level, which may be related to a hypothalamic defect and/or to an absence of pituitary response to GnRH. As (Dhindsa *et al.*, 2004) reported the LH and FSH levels were significantly lower in the hypogonadal group in comparison with patients with normal FT levels (3.15 - 0.26 *vs.* 3.91 - 0.24 mIU/ml for LH and 4.25 -0.45 *vs.* 5.53 - 0.40 miu/ml for FSH; $P < 0.05$). In a study by (Onah *et al.*, 2013) reported mean level of FSH is significantly elevated in T2DM than in control. However, the level of LH is increased in T2DM than in control but it was non-significant. The reports on the levels of gonadotrophic hormones (FSH and LH) were conflicting (Natah *et al.*, 2013 and Ali *et al.*, 1993) reported high significant increasing in FSH and LH levels in diabetics than in control. Our present study shows an elevation in level of estradiol in both diabetic groups in comparison with control group. That refers Estrogens are synthesized from androgens by the aromatase complex, which contains the cytochrome P450 enzyme encoded by the *CYP19* gene. Aromatase expression is revealed in Sertoli-Leydig cells, spermatogonia, spermatocytes, elongate spermatids and spermatozoa in adult mice and rats (Carreau *et al.*, 2002), and in Sertoli- Leydig cells, spermatocytes, spermatids and spermatozoa in man (Carreau *et al.*, 2008). Elevated estrogens in obese men may, in part, result from the increased mass of white adipose tissue. White adipose tissue is responsible for aromatase activity and adipose-derived hormones and adipokines, which are increased in obese men (Wake *et al.*, 2007). The aromatase cytochrome P450 enzyme is produced by many tissues, involving adipose tissue and Leydig cells. In men, aromatization activity converts testosterone to estrogens. It is suggested that increased estrogen levels in obese men may result from an increased conversion of androgens to estrogens by white adipose tissue (Phillips *et al.*, 2010). This contributes to the increased plasma estrogen levels (Katib, 2015).

Obese men have been exhibited high circulating estrogen levels predominantly due to increase aromatase activity, that irreversibly converting testosterone (T) to estradiol (E2) leading in decreased T and elevated E2serum levels (Bulun *et al*., 2003). The retinol binding protein was elevated in both diabetic groups this result was in acordnance with that reported by **(**Mohasseb, and Khalil, 2014) that RBP4works as an adipokine that supports a possible link between expression of adipose GLUT4 in adipocytes and insulin resistance. Many studies reported that a decreased GLUT4 expression by adipose tissue

causes an increased RBP4 synthesis and secretion, suggesting that RBP4 might be the link between adipose tissue and insulin resistance induction in the muscle and liver (Abel *et al* ., 2001; Yang *et al.*, 2005). In the present study, serum RBP4 levels were increased in overweight that Lin *et al*., 2008, showed reducing insulin sensitivity in hepatic androgen receptors knockout male mice, without impaired development of genital organs and subsequent hypogonadism. This decrease in insulin sensitivity was associated with reduced phosphorinositide 3-kinase activity and increased the phosphoenolpyruvate carboxykinase expression in the liver, which is the similar mechanism by which RBP4 causes insulin resistance (Yang *et al*., 2005). Serum RBP4 levels are increased in subjects with impaired glucose tolerance, T2D, and correlate inversely with insulin sensitivity in non-diabetic subjects with a family history of T2D (Yang *et al.,* 2005; Cho, 2006). Serum RBP4 levels correlate with the degree of insulin resistance in these patients and relationship is independent of obesity. RBP4 is elevated in the early stages of the occurrence of T2D. It could be indicate as an additional marker for early detection of patients predisposed to develop T2D contributing an early and vigorous intervention. Since levels of RBP4decrease with weight loss and exercise, RBP4 could also be used as an additional parameter in the evaluation of the success of the intervention (Kotnik *et al.,* 2011).Therefore, decreasing glut-4 level in adipose or muscle is considered as a hallmark of IR. So RBP4 expression in adipocytes has been recorded to be related to its plasma levels. High plasma RBP4 levels appear to be positively correlated with insulin resistance, T2DM, and dyslipidemia (Graham *et al.*, 2006). We concluded from our study that Type2 Diabetic Mellitus reduce the level of testosterone at (81.66%) among diabetic subjects groups. The decreasing in testosterone was accompanied by an increase in the level of estradiol hormone. The levels of Gonadotropin (LH, FSH) were within normal range. The increasing in the level of Retinol binding protein 4 (RBP4) seems to be act as insulin resistance that associated with increasing the fasting blood glucose (FBG) and HBA1C.

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