The Effect of Metformin Therapy on Testosterone Level in Men with Type 2 Diabetes Mellitus among Iraqi Population

Article *in* Biochemical and Cellular Archives · January 2021

CITATIONS
0

READS
175

1 author:

Hatem Hadeed
pharmacy college -anbar
8 PUBLICATIONS **2** CITATIONS

SEE PROFILE

Biochem. Cell. Arch. Vol. 21, No. 1, pp. 1229-1235, 2021	www.connectjournals.com/bca	*ISSN 0972-5075*
DocID: https://connectjournals.com/03896.2021.21.1229		*eISSN 0976-1772*

EFFECT OF METFORMIN THERAPY ON TESTOSTERONE LEVEL IN MEN WITH TYPE 2 DIABETES MELLITUS AMONG IRAQI POPULATION

Hatem M. Hadeed[1], Muhannad Shweash[1*] and Thulfiqar Fawwaz Mutar[2]

[1]Department of Clinical Laboratories Sciences, College of Pharmacy, University of Anbar, Anbar, Ramadi, Iraq.
[2]Department of Medical Laboratory Techniques, Al-Maarif University College, Ramadi, Al-Anbar, Iraq.
*e-mail : dr.muhannadshweash@gmail.com

(Received 2 November 2020, Revised 14 January 2020, Accepted 25 January 2021)

ABSTRACT : Diabetes is a global health problem, type 2 diabetes mellitus (T2DM) is the main kind of disease, which is related with several healthy problems. Measurements of serum testosterone are useful in the diagnosis and monitoring of sexual activity and secondary hypogonadism. Males with small amount of testosterone are at developed possibility for the progress of T2DM with reason the stimulation of insulin resistance because of the free testosterone levels is contrary associated with insulin resistance. This study aimed to evaluate the level of serum testosterone among T2DM with further analysis the possible effect of diabetic pharmacotherapy (metformin beside uncontrolled without metformin user) upon these changes. The study included 100 patients divided into four groups: one subject with uncontrolled, second subjects with metformin alone, third subject with insulin alone, fourth subject with insulin plus metformin and the fifth subject healthy group. The results indicated that the concentration of testosterone was significantly low in the uncontrolled group as compared with metformin, insulin and both group. The same finding was observed with insulin alone. Whereas, the findings of both treatments were significantly different compared to other groups. Besides, metformin has more effect on testosterone level, while insulin alone has lower than metformin. Metformin may have a specific interaction with mechanisms involved in reversible secondary hypogonadism particularly through enhancement of testosterone secretion and other relative hormones production. These data indicated for the first time the role of metformin function in mediating the enhancement of testosterone level on T2DM patients.

Key words : Testosterone, metformin, T2DM.

How to cite : Hatem M. Hadeed, Muhannad Shweash and Thulfiqar Fawwaz Mutar (2021) Effect of metformin therapy on testosterone level in men with type 2 diabetes mellitus among Iraqi population. *Biochem. Cell. Arch.* **21**, 1229-1235. DocID: https://connectjournals.com/03896.2021.21.1229

INTRODUCTION

The incidence of diabetic disease is increasing fast in the world and its morbidity and mortality extended due to several complications related to this healthy problem. Clinically, this disease includes several signs such as a high glucose level in blood, poor blood supply within limbs vessels, blood vessel, heart, retina, beta cell of the pancreas damage as well as damage of neurons around the limbs and other organs (Rask-Madsen and King, 2013). Basically, diabetic divided into two types, the first one is related to autoimmune condition inducing damage of the beta cells in pancreas known with Type 1 diabetes (T1D). The second one is type 2 diabetes (T2D), this is highly prevalence, considered a primary trouble of increasing defect of glucose regulation via mechanism of insulin resistance and pancreatic beta cells dysfunctional (Alberti and Zimmet, 1998). T2DM is a disorders of endocrine system documented for 90% of diabetic patients, described by many symptoms like hyperglycemia, polyuria, polydipsia, loss of weight because damaged insulin secretion or action and polyphagia (Kerner and Brückel, 2014). Metformin is most widely used oral antidiabetic drug in human. It is remain managed in all types of diabetes as a first-choice treatment, but more essential with type 2 diabetes mellitus (Del Barco *et al*, 2011). Metformin regulates glucose level through many mechanisms such as decreases production of hepatic glucose, enhance glucose used by peripheral tissues and promote the sensitivity of insulin. Importantly, metformin is only observed in patients with T2DM to decrease blood glucose levels (Inzucchi *et al*, 2012). Also, metformin has other mechanisms to regulate blood sugar through targeting the liver to decrease the process of gluconeogenesis and metabolism of skeletal muscles to

improve the utilization of peripheral glucose, with a probable target the intestine to increase production of levels glucagon-like peptide 1 (GLP-1) (Rena *et al*, 2017). Since the metformin is deliberated as an agent for oral anti diabetes. It is commonly used as a first medication for the treating of T2DM and its have a potential role in the alleviation of some of the inflammatory and immunological markers in diabetic patients. Variable reduction in concentrations of these biomarkers in response to metformin, insulin or both was established (Shweash *et al*, 2018). Numerous studies have indicated that the mechanisms of metformin to reduce diabetes. However, Stumvoll *et al* (1995) demonstrated that metformin suppressing the production of hepatic glucose to reducing glucose level. Also, Abbasi *et al* (1998) revealed another mechanism via reduced production of free fatty acids from adipose tissue leading to the effect of metformin on reducing glucose level. In the same context, Mueller *et al* (2000) indicated from a study on cultured adipocyte cells metformin inhibits leptin secretion and increases glucose uptake.

Many cases of the disorder appear in patients when they go to a urologist, including erectile dysfunction and hypogonadism, where the diagnostic evidence indicates the significant link between these disorders and T2DM disease (Jones, 2007). Several studies have reported that T2DM causes impotence and hormonal imbalance in men. However, Johannes *et al* (2000) reported that men with T2DM are three more times possible to progress the incidence of erectile dysfunction than healthy males. Also, Várkonyi and Kempler (2014) found that in men with diabetes the levels of reproductive hormones such as testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are decreased. Moreover, Farias *et al* (2014) reported that the deficiency in testosterone levels in serum patients with T2DM may lead to atherosclerotic disease and mortality because of its inversely related to cardiovascular risk factors.

Muller *et al* (2005) reported that metabolic syndrome in men is related with small circulating levels of total and free testosterone hormone. The lipoprotein lipase enzyme activity would be enhanced through the decrease levels of testosterone amount leading to the formation of new fat cells elevating fat storage via utilization of triglyceride into the adipocytes. This mechanism will lead to drives the cycle to further reduce testosterone levels through the resistance of insulin (Jones, 2007). Furthermore, not enough information for the effects of metformin therapy related to testosterone levels in both diabetic and not diabetic males. Consequently, our work aimed to estimate the level of serum testosterone in T2DM beside to analyze the possible effect of diabetic pharmacotherapy (drug against uncontrolled without metformin user) upon these changes.

MATERIALS AND METHODS

Patient's sample collection

This is a revising study of patients diagnosed, treated and untreated during their lifetime for diabetes mellitus. Clinical samples (blood) were obtained from four patient groups, two of categories are under medication with antidiabetic therapy. 100 diabetic patients from men, all those enrolled were from of the middle age group, two groups of these samples referred for treatment at Al-Ramadi Teaching Hospital between the periods of January 2019 to April 2019. This study included 100 male patients, and patients were divided into five groups according to the specific criteria. Again, The were 100 male patients in five categories (25 users of diabetic metformin only, 25 diabetic non-metformin users, 20 users of diabetic insulin only, 20 users of diabetic insulin with metformin and 10 users of nondiabetic balanaced groups). The certain patient giving blood samples for the analysis was obtained wrritin information concent. The research was authorized by Ethical standard of Al-Ramadi Teaching Hospital, Anbar.

Serum preparation

Blood samples have been collected from men by venipuncture after the patients had been in a recumbent position for 15 min in the temperature-controlled room (22-25^{0}C). The samples were transferred to a dry clean and sterile tube without anticoagulant substances and allow it to clot. The name, gender, age, medication have been written on the tube from the provided patient's list history. Blood samples allowed to stand for 20-30 min for clot formation and centrifuged. The supernatant serums were stored in the Eppendorf tube at (-20^{0}C to -80^{0}C) for subsequent analysis or use.

Laboratory assay for testosterone hormone

When starting laboratory analysis, patient information including age, personal medical, and family history was documented by interviewer-administered questionnaire form. Testosterone level was measured using a commercially available kit (DRG Diagnostics, Marburg, Germany) using an enzyme-linked immunosorbent assay (ELISA).

RESULTS

Comparison of serum testosterone level between nondiabetic (healthy) and diabetic (uncontrolled) group

To assess the normal values of testosterone hormone

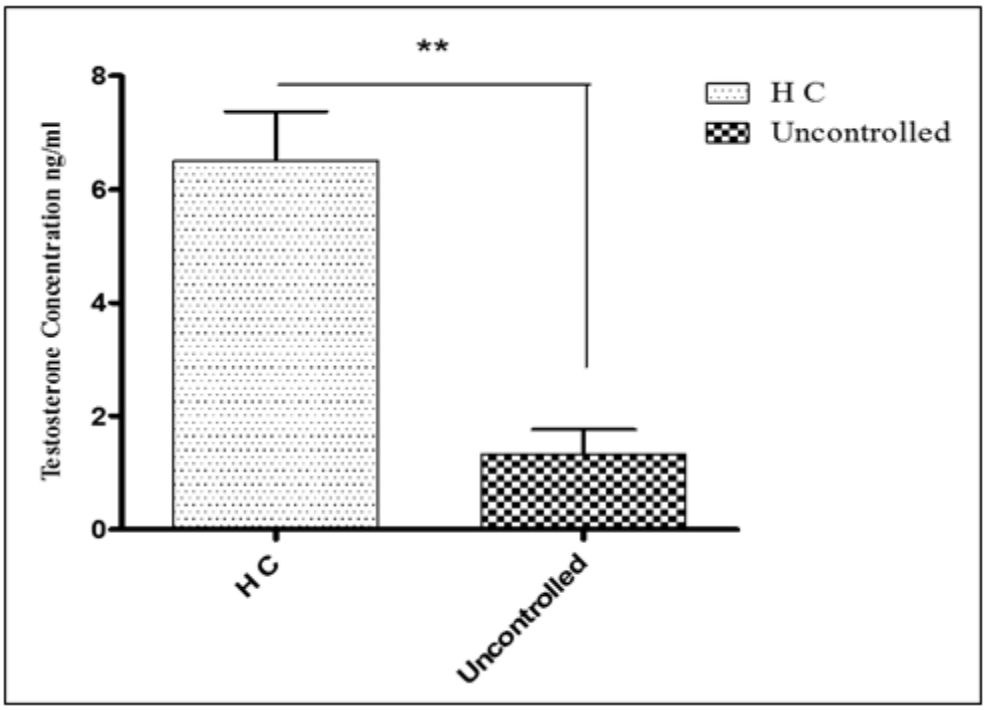

Fig. 1 : The effect of uncontrolled type 2 DM on serum testosterone concentration in male patients. Serum samples were analysed for testosterone concentration for the indicated group.

levels in healthy people, samples have been collected as a control group then uncontrolled T2DM patient's serum samples were utilized. However, serum healthy control was suggestively evident and high concentration. While serum uncontrolled group of testosterone levels in patients with T2DM were slightly lower if compared to obtain controls (mean ± SEM 6.512 ± 0.863 versus 1.326 ± 0.438, p = <0.005) as shown in (Fig. 1). The effect of metformin was then compared in serum patients. As expected, patients with uncontrolled diabetic (without treatment) group had significantly lower serum concentrations for testosterone (p = <0.005). Also, experiments were conducted to determine whether uncontrolled diabetes could enhance the decrease in testosterone concentration. For this purpose, serum samples were assessed for both group and the results were significantly inhibited the testosterone concentration.

Characterization of serum testosterone level following treatment with metformin in patients with class 2 DM

Characterization of treatment for serum patient's upon testosterone concentration in response to metformin therapy is shown in Fig. 2. The Figure shows that healthy control (HC) indicate a significant normal value of testosterone concentration as predicted (mean ± SEM 6.512 ± 0.863) and these values were more highly but not significantly increased at metformin group of treatment patients respectively compared with the healthy group. Metformin therapy has a higher effect on testosterone concentration (Metformin= mean ± SEM 8.341 ± 1.115).

Characterization of serum testosterone level following treatment with insulin in class 2 DM patients

The insulin treatment effect alone on serum testosterone was also examined in Fig. 3, insulin group samples were assessed for the above hormone, then compared to a healthy group in the same manner as an experiment. Insulin caused a normal secretion of testosterone with lower values to the healthy group. Which was not high but within the normal references of production. However, the examination of testosterone at the insulin agent is significantly different by the kinetic increase which was highly inhibited in the uncontrolled group. This was noticeable compared to the healthy group (healthy= mean ± SEM 6.512 ± 0.863 and insulin = mean ± SEM 4.104 ± 0.443).

Characterization of serum testosterone level following treatment with metformin plus insulin (both) in type 2 DM patients

Anti-diabetic drugs gave the expectation is that the treatment will be functional and able to increase and enhance the production of testosterone levels. However, a reduced level of testosterone is observed in previous (Fig. 1), or as demonstrated previously in the laboratory, following treatment group, having established two agents which were able to generate testosterone with diabetes

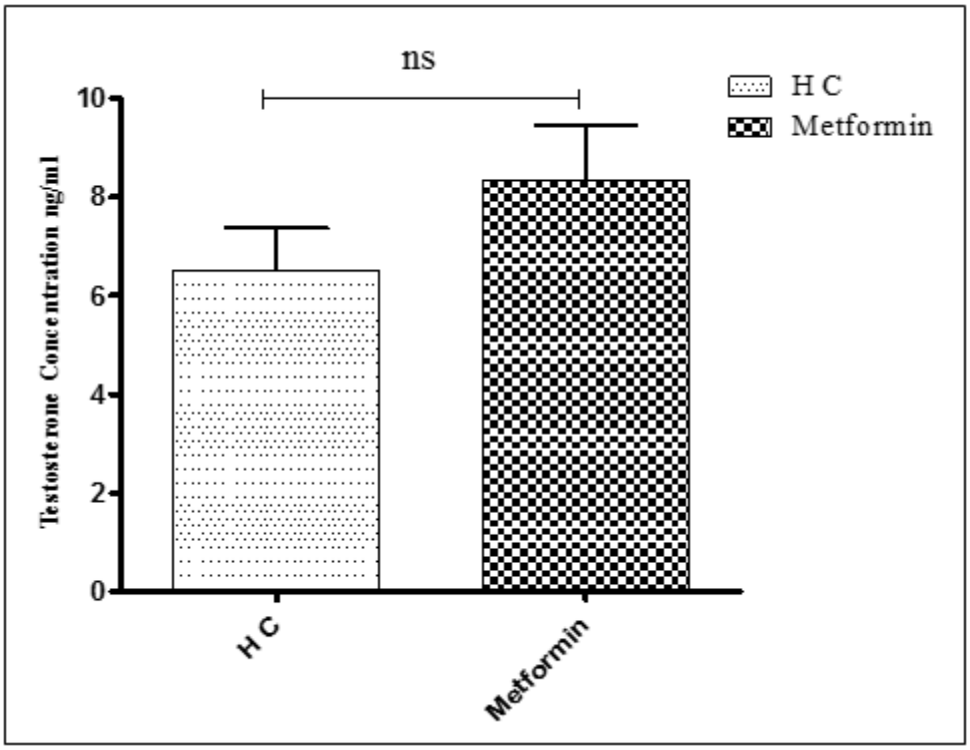

Fig. 2 : The effect of metformin on serum testosterone concentration in type 2 DM patients. Serum samples were analysed for testosterone concentration for the indicated group.

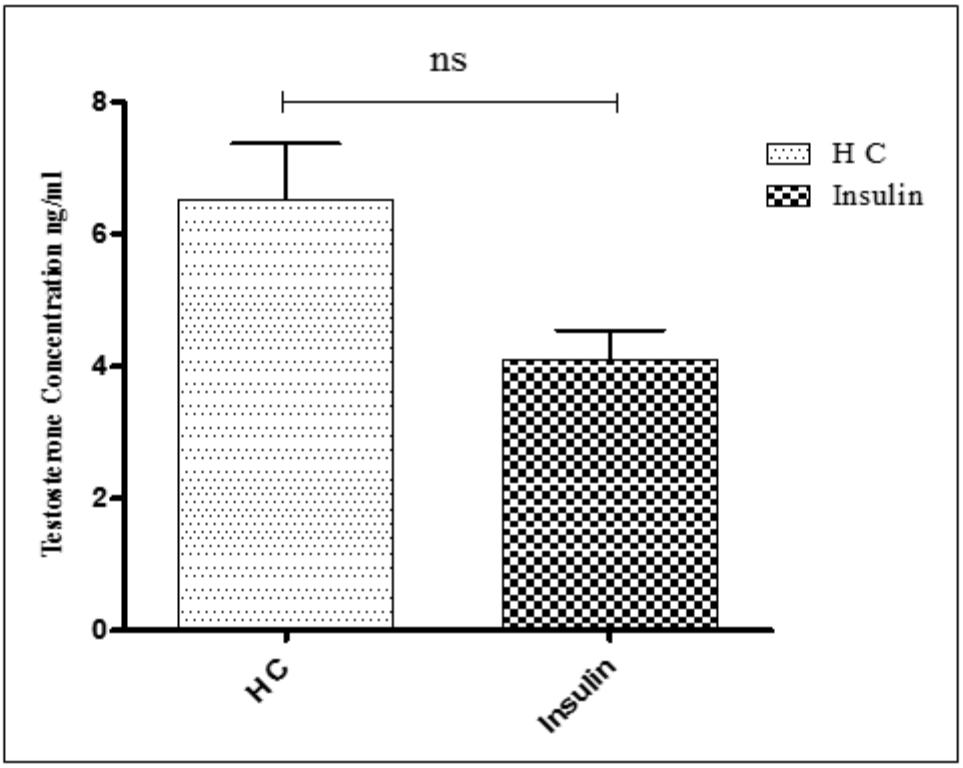

Fig. 3 : The effect of insulin on serum testosterone concentration in type 2 DM patients. Serum samples were analysed for testosterone concentration for the indicated group.

patients, the effect of the same drugs for testosterone was examined. Fig. 4 shows the effect of metformin plus insulin upon testosterone with more response to metformin or insulin alone (Metformin= mean ± SEM 8.341 ± 1.115, insulin= mean ± SEM 4.104 ± 0.443 and both= mean ± SEM 12.208 ± 0.444). Treatment with both significantly induced an increase in testosterone at all samples tested comparing to all groups. Although the

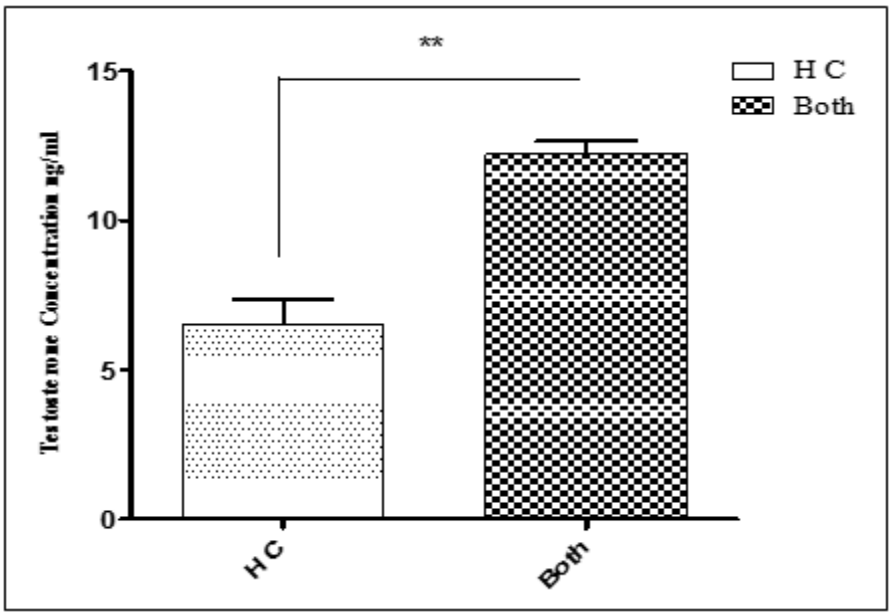

Fig. 4 : The effect of metformin plus insulin on serum testosterone concentration in type 2 DM patients. Serum samples were analysed for testosterone concentration for the indicated group.

response was greater with metformin and insulin together compared with other previous groups.

DISCUSSION

This project focused on the effects of treatment with metformin and uncontrolled patients on serum levels of testosterone in patients with type 2 DM (T2DM) among Iraqi males. The main findings were a significant increase in serum testosterone concentrations during metformin therapy and, conversely, a significant decrease with uncontrolled patients or without metformin therapy.

The results of this study showed that, compared to normal healthy men, male T2DM patients are associated with low testosterone serum levels, this result is compatible with Chuang *et al* (2017) the incidence of a low testosterone levels has been reported to be associated with age and obesity, and to be a risk factor for the progression of both the T2DM and metabolic disease in men. In the same context, Fernández-Miró *et al* (2016) finding that patients with T2DM were found to have a prevalence of frank hypogonadism and a decrease in the total testosterone levels in serum. Also, Korani and Sonbol (2018) informed that testosterone regulates nearly every component of erectile function, so the low percentage of testosterone contributes to erectile dysfunction (ED) in patients with type 2 diabetes mellitus. Ali and El Askary (2018) reported many health issues in patients with the development of T2DM can be observed in hypogonadism and low levels of testosterone together with hyperinsulinism and decrease insulin sensitivity. On the other hand, Fink *et al* (2018) showed that tumor necrosis factor-alpha (TNFα) and Interleukin-1 beta (IL-1β) stimulate the intracellular pathways promoting both T2DM and insulin resistance. Gianatti and Grossmann (2019) reported that testosterone deficiency is common in patients with T2DM and testosterone levels are partly affected by insulin resistance.

All of the enrolled patients in this study were from the middle age group. This was done to exclude the effect of aging on serum testosterone levels, since aging leads to a decrease in serum androgen levels caused by the adefect in the testicular-pituitary-hypothalamic axis (Souteiro *et al*, 2019). The majority of our patients, indeed were overweight and obese. A study conducted by Zhao *et al* (2016) low testosterone levels were directly affected by obesity and high BMI due to oxidative stress induced by adipose tissue and high adipokine levels such as leptin inhibit-testosterone secretions through direct inhibition of testicular Leydig cell function. Besides, the peripheral conversion of testosterone to estrogen is increased, causing negative inhibition of feedback on the production of luteinizing hormones and then inhibition of the production of testicular androgen. As shown in the current research, this leads to sexual dysfunction and little sex drive. Moreover, Al-Kuraishy and Al-Gareeb (2016) a substantial proportion of men with T2DM reported low serum levels of testosterone. Low serum levels of testosterone are associated with the occurrence of T2DM because testosterone leads to increases in muscle mass and decreases in fat mass, leading to a significant decrease in insulin resistance and type 2 DM prevention. Also, Oh *et al* (2002) indicated that a low endogenous testosterone level can independently predict T2DM and

obesity.

Considering the effect of diabetic pharmacotherapy on serum levels of testosterone, in the present study, metformin showed a significant elevation of testosterone concentratin compared with the untreated levels, this research is unlike published researchers who showed that metformin effectively decrease total testosterone levels; cytochrome P450-C17a, which is an enzyme involved in total testosterone synthesis and LH hormone secretion reduction is inhibited (Cheraghi *et al,* 2016). Metformin therapy in T2DM also leads to a significant decrease in total testosterone through leptin secretion modulation (Diwan *et al*, 2018). Furthermore, testosterone levels were significantly higher in sulfonylurea-treated patients compared to metformin-treated patients, according to Wong *et al* (2019) they reported that T2DM sulfonylurea therapy resulted in a significant increase in total testosterone and free testosterone due to pro-11b-hydroxysteroid dehydrogenase type 1 inhibition, as a result of a reduction in glucocorticoid biosynthesis and stimulation of testosterone synthesis, glucocorticoid reduces the level of testosterone. In addition, it is well known that sulfonylureas stimulate the secretion of insulin, which plays an important role in regulating testicular function and the hypothalamic-pituitary-testicular axis, thereby improving the levels of testosterone and the indicators of testosterone secretion in T2DM (Barella *et al*, 2019).

On the other hand, the positive effect of metformin therapy has been demonstrated by research findings on the pharmaceutical action of metformin. For example, in some published articles, the mechanisms by which metformin stimulates positive effects on sperm quality have been clarified. One apparent mechanism is that metformin reduces oxidative stress levels (Banihani, 2016), the imbalance between oxidants and antioxidants for the benefit of the former and lipid peroxidation (Attia *et al*, 2009). It has been shown that metformin supplementation in diabetic male rats, it restores antioxidant functions such as decreased glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase (Adaramoye and Lawal, 2014). When using diet-induced obesity rats (*i.e.* higher superoxide dismutase and glutathione peroxidase and lower malondialdehyde), significant effects of improving antioxidant function were observed (Fang *et al*, 2012). Metformin has been recognized by reactive oxygen species as having antioxidant activity against oxidative damage (Cahova *et al*, 2015). In addition, metformin has been found to improve the phosphorylation of 5'-AMP-activated protein kinase (AMPK), a cellular energy status sensor protein, which is a strategic enzyme for regulating energy balances (i.e., glucose and lipid metabolism) (Ashabi *et al*, 2014; Ma *et al*, 2015). Thus by increasing the activity of AMPKK, metformin may improve sperm function.

In addition, the effect of metformin on pituitary-gonadal hormones has also been mentioned in some *in vivo* system studies. The study research done by Adaramoye and Lawal (2014) in diabetic male rats, metformin was shown to restore follicle-stimulating, luteinizing, and testosterone hormones. Moreover, oral administration of metformin in diabetic rats realized to restore testosterone levels to normal (Ayuob *et al*, 2015).

CONCLUSION

In conclusion, positive effects of metformin on serum testosterone levels are presented by the public of research, however there are still few reports considering some negative effects. Subsequently, when considering its direct effect on serum concentration, metformin, usually at the therapeutic dose, appears to be impressive. This may be due to the ability of metformin to decrease oxidative damage and lipid peroxidation, improve the activity of AMPK and restore normal pituitary-gonadal hormone levels. However, further studies typically clinical studies, are still very applicable for verifying these findings. In order to standardize the favorable and unfavorable concentrations of metformin for human sex hormones, particularly human testosterone DNA, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in patients with diabetes, future research requires the conduct of a clinical study using flow cytometry and molecular study.

ACKNOWLEDGMENTS

We would like to thank all the patients who were involved in the research.

Conflict of interest

The authors do not have any conflict of interest.

REFERENCES

Abbasi F, Carantoni M, Chen Y-D and Reaven G M (1998) Further evidence for a central role of adipose tissue in the antihyperglycemic effect of metformin. *Diabetes Care* **21**, 1301–1305.

Adaramoye O A and Lawal S O (2014) Effect of kolaviron, a biflavonoid complex from G arcinia kola seeds, on the antioxidant, hormonal and spermatogenic indices of diabetic male rats. *Andrologia* **46**, 878–886.

Alberti K G and Zimmet P Z (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet. Med.* **15**, 539–553.

Ali T M and El Askary A (2018) The association between fetuin-A and testosterone levels and markers of arterial stiffness in Saudi subjects with type 2 diabetes mellitus. *Diabetes Metab. Syndr. Clin. Res. Rev.* **12**, 1045–1050.

Al-Kuraishy H M and Al-Gareeb A I (2016) Erectile dysfunction and low sex drive in men with type 2 DM: The potential role of diabetic pharmacotherapy. *J. Clin. Diagnostic Res.* **10**, FC21.

Ashabi G, Khodagholi F, Khalaj L, Goudarzvand M and Nasiri M (2014) Activation of AMP-activated protein kinase by metformin protects against global cerebral ischemia in male rats: interference of AMPK/PGC-1α pathway. *Metab. Brain Dis.* **29**, 47–58.

Attia S M, Helal G K and Alhaider A A (2009) Assessment of genomic instability in normal and diabetic rats treated with metformin. *Chem. Biol. Interact.* **180**, 296–304.

Ayuob N N, Murad H A S and Ali S S (2015) Impaired expression of sex hormone receptors in male reproductive organs of diabetic rat in response to oral antidiabetic drugs. *Folia Histochem. Cytobiol.* **53**, 35–48.

Banihani S A (2016) Effect of metformin on semen quality. *Brazilian J. Pharm. Sci*. **52**, 591–594.

Barella L F, Rossi M, Zhu L, Cui Y, Mei F C, Cheng X, Chen W, Gurevich V V and Wess J (2019) β Cell–intrinsic β-arrestin 1 signaling enhances sulfonylurea-induced insulin secretion. *J. Clin. Invest.* **129**, 3732-3737. https://doi.org/10.1172/JCI126309.

Cahova M, Palenickova E, Dankova H, Sticova E, Burian M, Drahota Z, Cervinkova Z, Kucera O, Gladkova C and Stopka P (2015) Metformin prevents ischemia reperfusion-induced oxidative stress in the fatty liver by attenuation of reactive oxygen species formation. *Am. J. Physiol. Liver Physiol.* **309**, G100–G111.

Cheraghi E, Mehranjani M S, Shariatzadeh M A, Esfahani M H N and Ebrahimi Z (2016) N-Acetylcysteine improves oocyte and embryo quality in polycystic ovary syndrome patients undergoing intracytoplasmic sperm injection: an alternative to metformin. *Reprod. Fertil. Dev.* **28**, 723–731.

Chuang S M, Lee C C, Chien M N, Sun F J and Wang C H (2017) The Associations between Serum total Testosterone Levels, Anthropometric Measurements and Metabolic Parameters in Elderly and Young Male Patients with Type 2 Diabetes Mellitus in Taiwan. *Int. J. Gerontol.* **11**, 220–224.

Del Barco S, Vazquez-Martin A, Cufí S, Oliveras-Ferraros C, Bosch-Barrera J, Joven J, Martin-Castillo B and Menendez J A (2011) Metformin: multi-faceted protection against cancer. *Oncotarget.* **2**, 896.

Diwan A G, Kuvalekar A A, Dharamsi S, Vora A M, Nikam V A and Ghadge A A (2018) Correlation of serum adiponectin and leptin levels in obesity and type 2 diabetes mellitus. *Indian J. Endocrinol. Metab.* **22**, 93.

Fang X, Xu Q-Y, Jia C and Peng Y-F (2012) Metformin improves epididymal sperm quality and antioxidant function of the testis in diet-induced obesity rats. *Zhonghua nan ke xue= Natl. J. Androl.* **18**, 146–149.

Farias J M, Tinetti M, Khoury M and Umpierrez G E (2014) Low testosterone concentration and atherosclerotic disease markers in male patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **99**, 4698–4703.

Fernández-Miró M, Chillarón J J and Pedro-Botet J (2016) Testosterone deficiency, metabolic syndrome and diabetes mellitus. *Med .Clínica* **146**, 69–73.

Fink J, Matsumoto M and Tamura Y (2018) Potential application of testosterone replacement therapy as treatment for obesity and type 2 diabetes in men. *Steroids* **138**, 161–166.

Gianatti E J and Grossmann M (2019) Testosterone deficiency in men with Type 2 diabetes: pathophysiology and treatment. *Diabet. Med.* **37**(2), 174-186.

Inzucchi S E, Bergenstal R M, Buse J B, Diamant M, Ferrannini E, Nauck M, Peters A L, Tsapas A, Wender R and Matthews D R (2012) Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the European Association for the Study of Diabetes (EASD). *Diabetes Spectr.* **25**, 154–171.

Johannes C B, Araujo A B, Feldman H A, Derby C A, Kleinman K P and McKINLAY J B (2000) Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. *J. Urol.* **163**, 460–463.

Jones T H (2007) Testosterone associations with erectile dysfunction, diabetes and the metabolic syndrome. *Eur. Urol. Suppl.* **6**, 847–857.

Kerner W and Brückel J (2014) Definition, classification and diagnosis of diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes* **122**, 384–386.

Korani M A and Sonbol A (2018) Study of risk factors for erectile dysfunction in patients with type 2 diabetes mellitus: Correlation to serum testosterone level. *Alexandria J. Med.* **54**, 319–321.

Ma J, Yu H, Liu J, Chen Y, Wang Q and Xiang L (2015) Metformin attenuates hyperalgesia and allodynia in rats with painful diabetic neuropathy induced by streptozotocin. *Eur. J. Pharmacol.* **764**, 599–606.

Mueller W M, Stanhope K L, Gregoire F, Evans J L and Havel P J (2000) Effects of metformin and vanadium on leptin secretion from cultured rat adipocytes. *Obes. Res.* **8**, 530–539.

Muller M, Grobbee D E, Den Tonkelaar I, Lamberts S W J and Van Der Schouw Y T (2005) Endogenous sex hormones and metabolic syndrome in aging men. *J. Clin. Endocrinol. Metab.* **90**, 2618–2623.

Oh J-Y, Barrett-Connor E, Wedick N M and Wingard D L (2002) Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* **25**, 55–60.

Rask-Madsen C and King G L (2013) Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab.* **17**, 20–33.

Rena G, Hardie D G and Pearson E R (2017) The mechanisms of action of metformin. *Diabetologia* **60**, 1577–1585.

Shweash M, Hadeed H M, Farhan Y M and Mutar T F (2018) Serum Levels of C-Reactive Protein, Complement 3 and Complement 4 in Iraqi Diabetic Patients on Metformin Therapy. *Int. J. Pharma Bio Sci.* **8**, 1–9.

Souteiro P, Maia R, Santos-Silva R, Figueiredo R, Costa C, Belo S, Castro-Correia C, Carvalho D and Fontoura M (2019) Pituitary incidentalomas in paediatric age are different from those described in adulthood. *Pituitary* **22**, 124–128.

Stumvoll M, Nurjhan N, Perriello G, Dailey G and Gerich J E (1995) Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl. J. Med.* **333**, 550–554.

Várkonyi T and Kempler P (2014) Sexual dysfunction in diabetes. In: *Handbook of Clinical Neurology.* Elsevier, pp 223–232.

Wong C K H, Man K K C, Shi M, Chan E W, Ho C W, Tse E T Y, Wong I C K and Lam C L K (2019) Intensification with dipeptidyl peptidase-4 inhibitor, insulin, or thiazolidinediones and risks of all-cause mortality, cardiovascular diseases and severe hypoglycemia in patients on metformin-sulfonylurea dual therapy: A retrospective cohort study. *PLoS Med.* **16**,

Zhao S, Mugabo Y, Ballentine G, Attane C, Iglesias J, Poursharifi P, Zhang D, Nguyen T A, Erb H and Prentki R (2016) α/β-Hydrolase domain 6 deletion induces adipose browning and prevents obesity and type 2 diabetes. *Cell Rep.* **14**, 2872–2888.