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Low testosterone level increases fasting blood glucose level in adult males

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ABSTRACT

BACKGROUND
Total testosterone (TT) levels in males decrease with age. There has been a vigorous debate on the extent to which low testosterone causally contributes to diabetes and its complications. The aim of the present study was to determine the relationship between sex hormones and blood glucose levels in adult males.

METHODS
A cross-sectional study involving 259 males aged 41 - 70 years was conducted at Cilandak Subdistrict, South Jakarta. Sex hormone binding globulin (SHBG) and testosterone levels were measured by means of electro–chemiluminescent immunoassay (ECLIA), while blood glucose levels were measured enzymatically using a spectrophotometer. Free testosterone index (FTI) and body massa index (BMI) were calculated. Inter-variable relationships were tested by Pearson correlation analysis, followed by multiple linear regression analysis to determine the most influential factor on fasting blood glucose levels.

RESULTS
BMI was positively correlated with fasting blood glucose, but the correlation was statistically not significant (r=0.105; p=0.106). In contrast, total testosterone (TT) (r=-0.258; p=0.000) and SHBG (r=-0.193; p=0.02) had a significant negative correlation with fasting blood glucose level. Multiple linear regression showed that TT was the most influential factor on fasting blood glucose level (â=-0.044; p=0.008).

CONCLUSIONS
Low total testosterone level may increase fasting blood glucose level in adult males. SHBG levels did not predict fasting blood glucose levels.Assessment of testosterone in middle-aged men may allow early intervention for diabetes mellitus.

Keywords: Total testosterone, sex hormone binding globulin, blood glucose, male

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Kadar testosteron yang rendah meningkatkan kadar gula darah puasa pada laki-laki dewasa

ABSTRAK

LATAR BELAKANG

Pada laki-laki, seiring dengan meningkatnya usia terjadi penurunan kadar kadar testosteron total (TT). Masih banyak perbedaan pendapat mengenai kontribusi kausal dari kadar testosteron yang rendah terhadap diabetes dan komplikasinya. Penelitian ini bertujuan untuk menentukan adanya hubungan antara hormon seks dan kadar glukosa darah puasa pada pria dewasa.

METODE

Studi potong lintang dengan mengikut sertakan 259 pria yang berumur 41 – 70 tahun dilakukan di Kecamatan Cilandak, Jakarta Selatan. Pengukuran kadar sex hormone binding globulin *(SHBG) dan testosteron dilakukan dengan teknik* electro–chemiluminescent immunoassay *(ECLIA), dan glukosa diukur secara enzimatis menggunakan spektrofotometer. Dilakukan perhitungan indeks testosteron bebas (ITB) dan indeks massa tubuh (IMT). Hubungan antar variabel diuji menggunakan analisis korelasi Pearson, yang dilanjutkan dengan analisis regresi ganda linear untuk menetukan faktor yang paling berperan terhadap kadar glukosa darah puasa.*

HASIL

IMT berkorelasi positif dengan kadar gula darah puasa, tetapi secara statistik tidak bermakna (r=0.105; p=0.106). Sebaliknya TT (r=-0.258; p=0.000) dan SHBG (r=-0.193; p=0.02) berkorelasi negatif secara bermakna dengan kadar gula darah puasa. Analisis regresi ganda linear menunjukkan kadar TT yang paling berperan secara bermakna terhadap kadar gula darah puasa (â=-0.044; p=0.008).

KESIMPULAN

Kadar testosteron total yang rendah dapat meningkatkan kadar gula darah puasa pada pria dewasa. SHBG tidak dapat memprediksi kadar gula darah puasa. Pengukuran testosteron pada pria setengah baya memungkinkan intervensi dini terhadap diabetes melitus.

Kata kunci: *Testosteron total,* sex hormone binding globulin*, glukosa darah, pria*

INTRODUCTION

In males, the age-related reduction in total testosterone and adrenal androgens such as dehydroepiandrosterone sulfate (DHEAS) has been documented and linked to changes in physiological functions, including abdominal obesity, insulin resistence, lung function, and cardiovascular disease.[1-3] Testosterone levels in males are low before puberty and increase with puberty, reaching a peak in the age group of 40 – 49 years and decreasing gradually with advancing age.[4] Testosterone is synthesized by Leydig cells in the testis and is the main sex hormone in males. Part of the testosterone in the testes is used for spermatogenesis and the rest is secreted into the circulation. The total amount of testosterone in the circulation is called total testosterone. In general, total testosterone (TT) in the circulation is divided into 4 main parts, viz. testosterons bound to sex hormone binding globulin (SHBG) (44%), testosterone bound to albumin (50%), testosterone bound to cortisol binding globulin (4%), and free or unbound testosterone (2%). One third of US men older than 65 years have type 2 diabetes, and a similar percentage have low or subnormal testosterone levels, compared

with reference ranges based on healthy young men.[5]

Dhindsa et al.[6] reported that 43% of males with type 2 diabetes had low total testosterone levels, with a decrease in 57% of the free testosterone. On the other hand, among patients with type 1 diabetes, 1.7% had decreased TT and 20% had low free testosterone levels. A high prevalence of hypogonadism was reportedly found in patients with type 2 diabetes. The variable prevalences of hypogonadism found in several studies was presumably caused by differences in diagnostic criteria and the instruments used to determine testosterone levels.[7] One study reported a high prevalence of hypogonadism in type 2 diabetes, on the basis of free testosterone, and showed that one-third of males with type 2 diabetes (aged between 31 – 75 years) had a low free testosteron level.[7]

Supplementation of testosterone have been found to improve body composition and insulin sensitivity.[8] In hypogonadal male diabetics older than 30 years, testosterone improved insulin resistance, glycemic control, and visceral obesity. On the other hand, abrupt cessation of testosterone supplementation in men with idiopathic hypogonadotropic hypogonadism caused worsening of insulin sensitivity, without detectable changes in body fat content [9] Another study reported that lower testosterone levels may promote insulin resistance through impairment of mitochondrial function.[10] Because is is still uncertain whether insulin resistance and diabetes may be causing male hypogonadism or vice versa, the mechanisms regulating any preventive effect of conserved testosterone levels need to be investigated further.

It has been observed that low testosterone is inversely associated to both diabetes and the metabolic syndrome, and may be a predictor of these disorders. These associations are of moderate strength, corresponding to a decrease of 2–3 nmol/L in total testosterone.[11] Total testosterone has a stronger association with diabetes than does free testosterone. Since changes in total testosterone (but not free testosterone) are proportional to those of SHBG, it may be suggested that SHBG also plays a causal role in the above associations. However, the possibility of a reverse causation or a common third factor cannot be excluded, because of the strong inverse association of SHBG with insulin resistance and visceral adiposity. Further study is required to SHBG levels add additional information to conventional risk factors for diabetes and related complications and whether SHBG mediates risk via androgenic or testosterone-independent mechanisms.

In the meta-analytical study conducted by Ding et al.[12] on 20 cross sectional studies, involving 850 diabetic men and 2000 nondiabetic controls, it was found that in each study, total testosterone levels were lower in diabetic men compared with nondiabetic controls. However, the meta-analysis did not address the association of free testosterone levels with future diabetes, this has been reported by six prospective studies.[13] Based on the variable results of the abovementioned studies, it was felt necessary to conduct a study aiming to determine any relationships between sex hormone levels and other determinant factors, such as BMI and SHBG, and blood glucose levels in adult males.

METHODS

Study design

This study used a cross-sectional design and was conducted between February 2011 and October 2011 at Cilandak Subdistrict, South Jakarta.

Study subjects

The study subjects were healthy males who were at least 40 years old and were residents of Cilandak Subdistrict, South Jakarta. Inclusion criteria were a good health status as evidenced by physical examination by the investigators, and normal serum albumin level (3.5 - 5.0 g/dL). Exclusion criteria were: i) liver cirrhosis, hypogonadal or hypergonadal status; ii) consumption of drugs related to liver functions,

e.g. phenytoin; iii) consumption of drugs affecting reproductive organs, e.g. androgens, growth hormones, high-dose glucocorticoids; iv) consumption of drugs affecting steroid production and metabolism, e.g. diazoxide; and v) consumption of drugs affecting insulin production, e.g. epinephrine, cortisol, progestins, sulfonylureas. The subjects gave written informed consent before participating in the study.

Subjects were selected by cluster random sampling of the *kelurahan* (villages) in Cilandak Subdistrict, South Jakarta. In each selected *RW* (hamlet), subject selection was by simple random sampling.

Measurement of body mass index (BMI)

Height and weight were measured by means of weighing scales with a built-in device for measuring height. BMI was computed as the ratio of weight to the square of height (kg/m^2). The anthropometry was performed in standing subjects wearing light clothes and no shoes.

Laboratory measurements

Venous blood samples were collected in the morning from the subjects after a 10-hour fast. Fasting blood samples were drawn from the cubital vein in the supine position. Serum aliquots were prepared for immediate analysis and for storage at - 80°C for further analysis. Blood glucose levels were measured enzymatically using a spectrophotometer and were expressed in mg/dL. Total testosterone levels were determined by electrochemiluminescence (ECLIA) immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, IN, USA), and levels of SHBG were measured by chemiluminescent immunometric assay (Immulite, DPC, Los Angeles, CA, USA).

Free testosterone index (FTI) values were calculated using the formula TT/SHBG x 100 and were expressed in percentages, in accordance with Vermeulen's method. [14] FTI was used to determine the testosterone status, to indicate binding abnormalities between SHBG and testosterone. Determination of SHBG and total testosterone levels was performed at the Prodia Laboratories, Jakarta. Testosterone levels were expressed in nmol/L.

Ethical clearance

The study protocol was approved by the Research Ethical Committee, Faculty of Medicine, Trisakti University, Jakarta.

Data analysis

Descriptive statistics, means and SD for continuous variables were used to describe the study population. The correlations between age, BMI, serum hormone levels and blood glucose levels were tested by Pearson's test. Multivariate regression linear analysis was conducted in order to identify the most influential variable on fasting blood glucose levels. All p-values were two-tailed, and $p < 0.05$ was considered statistically significant. All analyses were performed with SPSS 17.0.

RESULTS

A total of 237 males participated in this study, with mean age of 53.19 ± 8.39 years and mean BMI of 24.35 ± 3.74 kg/m^2. Mean fasting blood glucose level was 106.59 ± 33.78 mg/dL, TT 529.76 ± 190.75 nmol/dL, and SHBG 40.59 ± 17.58 nmol/L (Table 1).

Table 1. Demographic and clinical characteristics of the study subjects (n=237)

Characteristics	All subjects (n = 428)
Age (years)	53.19 ± 8.39
BMI (kg/m^2)	24.35 ± 3.74
Glucose (mg/dL)	106.59 ± 33.78
TT (nmol/dL)	529.76 ± 190.75
SHBG (nmol/L)	40.59 ± 17.58
FTI (%)	48.17 ± 14.15

Data are expressed as mean ± SD; BMI : body mass index; TT: total testosterone; SHBG: sex hormone binding globulin; FTI: free testosterone index

Table 2 . Correlation between several covariates and fasting blood glucose level

Covariate	Fasting blood glucose	p
Age	0.077	0.224
BMI	0.105	0.106
TT	-0.258	0.000*
SHBG	-0.193	0.002*
FTI	-0.068	0.283

BMI: body mass index; TT: total testosterone; SHBG: sex hormone binding globulin; FTI: free testosterone index

Table 2 above shows that BMI was positively correlated with fasting blood glucose, but statistically the correlation was not significant (r=0.105; p=0.106). In contrast, TT (r=-0.258) and SHBG (r=-0.193; p=0.002) had a significant negative correlation with fasting blood glucose. It may be concluded that high TT and SHBG levels will result in decreased fasting blood glucose levels, while low TT and SHBG levels will result in increased fasting blood glucose levels.

Although BMI did not show a significant relationship with fasting blood glucose level, theoretically BMI affects fasting blood glucose level, which was the reason for its inclusion in the multiple linear regreassion model. Multiple linear regression analysis showed that TT was the most infuential factor on fasting blood glucose, in comparison with BMI and SHBG levels (â=-0.044; p=0.008). This means that low TT levels result in high fasting blood glucose levels (Table 3).

DISCUSSION

Our study results showed reduced TT levels in the study subjects, accompanied by increased fasting blood glucose levels. This is consistent with the results of other studies reporting that decreases in the levels of androgenic hormones, i.c. testosterone, is one of the causal factors for increased blood glucose levels in the subjects,[15] with zinc deficiency and adipokines (such as leptin and tumor necrosis factor alpha) being two of the possible causes.[16] Increased insulin levels decrease SHBG levels leading to a decrease in TT. The correlation between low testosterone levels and diabetes may also be the result of decreased functioning of Leydig cells. Reductions in TT and SHBG levels are closely associated with male abdominal obesity.[17,18] Although its mechanism is uncertain, abdominal osetity in males may be caused by increased lipid uptake and increased accumulation of visceral fat. Other studies found that testosterone replacement therapy significantly decreases insulin resistence and improves blood glucose levels. In addition, it has been documented that men with impaired glucose tolerance (IGT) have low TT levels and that TT levels are inversely associated to fasting blood glucose.[19]

TT levels in male diabetics were lower than those in normal males, possibly because free testosterone (FT) levels in the male subjects with diabetes were also lower than in normal males. Furthermore, FT has autoregulatory functions for a feedback mechanism to both the hypothalamus and the pituitary gland.

Table 3. Multiple linear regression analysis of several covariates and fasting blood sugar level

Covariate	B	Beta	p
BMI	0.006	0.000	0.933
TT	-0.044	-0.262	0.008*
SHBG	0.039	0.021	0.834

BMI: body mass index; TT: total testosterone; SHBG: sex hormone binding globulin

Our findings that SHBG levels were inversely related to fasting blood glucose are consistent with the results of the studies of Ding et al.[20] as well as Lakshman et al.[21] Similar results were obtained in male diabetics, showing a negative correlation of SHBG with TT.[22,23] The current evidence about the impact of SHBG on the prediction of later diabetes suggests that previous data on the impact of TT on later diabetes might have been confounded by SHBG. Therefore, we repeated the analyses intending to explore the independent contribution from total testosterone and SHBG when included in the same multivariate models. TT but not SHBG was still independently predictive of subsequent diabetes.

Our study showed that BMI was not correlated with blood glucose level. Kaplan et al.[23] clearly demonstrated that obesity and fasting hyperglycemia are the key components of the metabolic syndrome that exacerbates the age-related decrease in TT levels. It is well recognized that obesity and type 2 DM are both conditions associated with low TT levels. [5,25] Decreased testosterone levels are known to be associated with increased glucose concentration, and our study confirmed this association. The clinical implication of our findings is that administration of testosterone may be beneficial to males. Testosterone replacement therapy may have beneficial effects on insulin sensitivity in older diabetic men with late hypogonadism. Since testosterone replacement therapy results in reduced fat mass and consequently decreased circulating free faty acids, the end result may be improved insulin sensitivity.[16] However, routine administrtaion of testosterone therapy to diabetic men with low-normal testosterone levels should await the outcomes of well-conducted clinical trials. Males with subnormal testosterone levels should implement lifestyle measures such as weight reduction and exercise, which may raise testosterone levels and provide multiple health benefits.

Some limitations should be recognized in the current study. First, FT were calculated from TT and SHBG, using the Vermeulen's equation. Second, we used a single blood sample to determine TT and SHBG levels. Increasing the number of samples would have yielded greater precision in measurement. Daily testosterone levels may fluctuate greatly, and therefore repeated tests are indicated for a diagnosis of hypogonadism. Our use of a single blood sample did not allow us to account for the intra-individual daily variation in our study. Observational studies of cross-sectional design as used in our study cannot explain the temporal nature of the association between testosterone and blood glucose levels.

CONCLUSIONS

Total testosterone level is the most influential factor on fasting blood glucose level. A low total testosterone level may increase fasting blood glucose level in adult males. SHBG levels did not predict fasting blood glucose levels. However, a follow-up study is needed to confirm these associations in men. Assessment of testosterone in middle-aged men may allow early intervention for diabetes mellitus.

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REFERENCES

1. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005; 26:833–76.
2. Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen TP, Salonen R, et al. Sex hormones, inflammation and the metabolic syndrome: a population-based study. Eur J Endocrinol 2003;149:601–8.

3. Mawi M, Nirmalasari. High free testosterone index increases lung function in adult males. Univ Med 2012;31:113-9.
4. Agledahl I, Skjærpe PA, Hansen JB, Svartberg J. Low serum testosterone in men is inversely associated with non-fasting serum triglycerides: the Tromsø study. Nutr Metab Cardiovasc Dis 2008;18:256–62.
5. Cowie CC, Rust KF, Byrd-Holt DD, Gregg EW, Ford ES, Geiss LS, et al. Prevalence of diabetes and high risk for diabetes using A1C criteria in the U.S. population in 1988–2006. Diabetes Care 2010;33:562–8.
6. Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A, Dandona P. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J Clin Endocrinol Metab 2004;89:5462–8.
7. Svartberg J, von Mu¨ hlen D, Schirmer H, Barrett-Connor E, Sundfjord J, Jorde R. Association of endogenous testosterone with blood glucose level in men : the Tromsø study. Eur J Endocrinol 2004;150:65–71.
8. Laaksonen DE, Niskanen L, Punnonen K, Nyysso¨nen K, Tuomainen TP, Valkonen VP, et al. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. Diabetes Care 2004;27:1036–41.
9. Simon D, Charles MA, Lahlou N, Nahoul K, Oppert JM, Gouault-Heilmann M, et al. Androgen therapy improves insulin sensitivity and decreases leptin level in healthy adult men with low plasma total testosterone: a 3-month randomized placebo-controlled trial. Diabetes Care 2001;24:2149–51.
10. Kapoor D, Goodwin E, Channer K, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol 2006;154:899–906.
11. Pitteloud N, Mootha VK, Dwyer AA, Hardin M, Lee H, Eriksson KF, et al. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care 2005;28:1636–42.
12. Kupelian V, Page ST, Araujo AB, Travison TG, Bremner WJ, McKinlay JB. Low sex hormone-binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in nonobese men. J Clin Endocrinol Metab 2006; 91:843–50.
13. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2006;295:1288–99.
14. Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, Lenzi A, et al. Type 2 diabetes mellitus and testosterone: a meta-analysis study. Int J Androl 2010 doi:10.1111/j.1365-2605.2010. 01117.x.
15. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
16. Grossman M, Thomas MC, Panagiotopoulos S, Sharpe K, MacIsaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. J Clin Endocrinol Metab 2008;93:1834–40.
17. Kim MJ, Rolland Y, Cepeda O, Gammack JK, Morley JE. Diabetes mellitus in older men. Aging Male 2006;9:139-47. Doi: 10.1080/136855306 00907977.
18. Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I. Free testosterone in elderly men. Aging Male 2002;5:98–102.
19. Muller M, den Tonkelaar I, Thijssen JH, Grobbee DE, van der Schouw YT. Endogenous sex hormones in men aged 40–80 years. Eur J Endocrinol 2003;149: 583–9.
20. Halmenschlager G, Rhoden EL, Riedner CE. The influence of age on bioavailable and free testosterone is independent of body mass index and glucose levels. World J Urol 201;29:541–6. DOI 10.1007/s00345-011-0724-x.
21. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med 2009;361:1152–63.
22. Lakshman KM, Bhasin S, Araujo AB. Sex hormone-binding globulin as an independent predictor of incident type 2 diabetes mellitus in men. J Gerontol A Biol Sci Med Sci 2010;65: 503–9.
23. Bremner WJ, Vitiello MV, Prinz PN. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metabol 2006;56:1278–81.
24. Bolelli G, Muti P, Micheli A, Sciajno R, Franceschetti F, Krogh V. Correlation studies of testosterone hormones level in serum with the development of diabetes. Epidemiol Biomark Prev 2005;4:509–13.
25. Kaplan SA, Meehan AG, Shah A. The age related decrease in testosterone is significantly

exacerbated in obese men with the metabolic syndrome. What are the implications for the relatively high incidence of erectile dysfunction observed in these men? J Urol 2006;176:1524–8.

26. Kapoor D, Aldred H, Clark S. Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes. Diabetes Care 2007;30:911–7.