Diabetes Care Publish Ahead of Print, published online March 3, 2010

TESTOSTERONE CONCENTRATIONS IN DIABETIC AND NON-DIABETIC OBESE MEN

Sandeep Dhindsa, MD
Michael G. Miller, Pharm.D.
Cecilia L McWhirter*, M.S.
Donald E. Mager, MD**
Husam Ghanim, Ph. D.
Ajay Chaudhuri, MD
Paresh Dandona, MD

Division of Endocrinology, Diabetes and Metabolism
State University of New York at Buffalo and Kaleida Health
3 Gates Circle
Buffalo, NY 14209

Solvay Pharamaceuticals Inc., Marietta, GA,*

Department of Pharmaceutical Sciences**
University at Buffalo, SUNY

Correspondence To:
Paresh Dandona, B.Sc., M.B. B.S., D.Phil., F.R.C.P.
E-mail: pdandona@KaleidaHealth.org

Additional information for this article can be found in an online appendix at
http://care.diabetesjournals.org

Submitted 3 September 2009 and accepted 23 February 2010.

Objective: To determine the prevalence of subnormal testosterone concentrations in patients with obesity and with type 2 diabetes in a primary care clinic population.

Research Design and Methods: FT concentrations of 1849 men(1451 non-diabetic and 398 diabetic) in the Hypogonadism In Males(HIM) study were analyzed. HIM study was a US based cross-sectional study designed to define the prevalence of hypogonadism in men older than 45 years. FT was measured by equilibrium dialysis.

Results: Prevalence of subnormal FT concentrations in lean, overweight and obese non-diabetic men was 26%(n=275), 29%(n=687) and 40%(n=489) respectively($p<0.001$ for trend); and 44% (n=36), 44%(n=135) and 50%(n=227) respectively in diabetic men ($p=0.46$ for trend within group, $p<0.05$ compared to non-diabetic men). Mean FT concentration of diabetic men was significantly lower than that of non-diabetic men. FT concentrations were negatively and significantly ($p<0.001$) related to age(r=-0.37), BMI(r=-0.18) and SHBG(r=-0.11) in multiple regression analysis. The average decline of FT concentrations was 7.8 pg/ml/decade in non diabetic and 8.4 pg/ml/decade in diabetic men.

Conclusion: 40% of obese non-diabetic and 50% of obese diabetic men above the age of 45 years have subnormal FT concentrations. In view of its high prevalence, obesity is probably the condition most frequently associated with subnormal FT concentration in males. The concomitant presence of diabetes is associated with an additional increase in prevalence of subnormal FT concentration.

We have previously shown that men with type 2 diabetes have a high prevalence of hypogonadotropic hypogonadism(1). In these patients, there was a significant inverse relationship of BMI with total and free testosterone (T) concentrations(1; 2). Our observations on the inverse relationship of BMI and T concentrations in patients with type 2 diabetes have been confirmed in other studies(3; 4). In another study we found that BMI and total T and free testosterone (FT) concentrations were also inversely related in type 1 diabetic men who rarely had hypogonadism(5). These observations raise the question whether obesity itself is associated with low FT.

Studies in the past have suggested that total T concentrations are lower in obese men than in men with normal BMI(6). The Hypogonadism In Males (HIM) study which confirmed the presence of frequent low T concentrations in men with diabetes(DM)(7) also showed that there was a tendency for total T concentrations to be low in patients with higher BMI. However, sex hormone-binding globulin(SHBG) concentrations are also low in obese and diabetic men. According to The Endocrine Society Guidelines, the diagnosis of hypogonadism should be dependent on the measurement of FT or bioavailable T(BT) in conditions that can alter SHBG concentrations.(8) FT concentrations should be measured by equilibrium dialysis or calculated using algorithms based on law of mass action and the methods of Vermeulen et al and Sodergard et al(9). Some previous relatively small studies showed that BMI is inversely related to total T, SHBG and FT but that only morbid obesity (BMI $\geq$40 kg/m^2) was associated with subnormal FT concentrations(10-12). Recent studies have examined the prevalence of hypogonadism in obesity in a much larger number of men. One of these studies is the above mentioned HIM study. It was designed to determine the prevalence of hypogonadism (defined as low total T) in men in the United States above the age of 45 years. This study, which included 2165 men, showed that 38.7% of men had a subnormal total T concentration. 52% of the obese men and 50% of DM men had subnormal total T concentrations. However, the publication did not address FT concentrations in men with obesity, with or without DM. A recently published Dutch study in 149 obese men (mean age 43 years) found a 36% prevalence of hypogonadotropic hypogonadism(13). Men with DM were included in the obese population in both the HIM and the Dutch studies. 23% of the study subjects in the HIM study and 37% in the Dutch study had DM. Data on non-DM obese men were not reported separately. Large studies on the prevalence of low FT in the non-DM obese men are thus not available. Nor are any data available on the differences in the prevalence of subnormal FT concentrations in the non-DM obese when compared to those with DM. Although the concentration of FT falls with increasing obesity in DM men, 25% of type 2 DM men have subnormal FT concentrations even when they have a normal weight(1).

Due to the rising prevalence of obesity in the US and the rest of the world, it is imperative that the prevalence of low FT in obese men be defined. Furthermore, the magnitude of the contribution of obesity to subnormal FT associated with type 2 DM needs to be quantitated. To answer these issues, we analyzed FT concentrations in the obese non-DM and DM men in the HIM study. The hypotheses tested were that (1) the obese have a higher prevalence of subnormal FT concentrations than the non-obese and (2) that men with DM have a higher prevalence of subnormal FT concentrations than the non-DM in both the obese and the non-obese.

PATIENTS AND METHODS

The HIM study recruited 2165 men in 95 centers across United States. Study criteria have been published previously(7). Briefly, all men age 45 years or older had a blood sample taken in the morning (between 8 am and noon). Blood was analyzed for concentrations of total T, FT, BT and SHBG. Total T and SHBG were determined by radioimmunoassay, FT by equilibrium dialysis and BT by ammonium sulphate precipitation method (Esoterix, Calabasas Hills, CA, USA). The lower limit for FT of the reference laboratory was 50 pg/ml (0.174 nmol/L). The lower limit for BT was 90 ng/dl (0.313 nmol/L).

The HIM study had 474 men with self-reported DM. 1691 men did not have DM. Judging by their medication lists, we excluded men who were on anti-retroviral drugs(33 men), narcotics(78 men) or T replacement therapy(33 men). We also excluded men currently using steroids, narcotics, antibiotics(to eliminate men with acute illnesses) or drugs that affect the dopaminergic pathway(34 men). The prevalence of low FT in all these excluded men (n=178) was 50%. Men with missing data on BMI or hormone concentrations were also excluded from analysis(n=138). We report on the findings from 1849 men. 398 of these men had DM.

Group comparisons were carried out by one-way ANOVA, t-tests, Mann-Whitney rank sum tests and chi-square (χ^2) tests as appropriate. Data that were not normally distributed were log-transformed to carry out the parametric statistical tests. Total T, FT, BT, SHBG, age and BMI were not normally distributed and were log transformed. Pearson correlation and multiple linear regression analyses between variables were done using Sigma stat software (SPSS Inc, Chicago, Illinois). Using the statistical program SYSTAT-12 we ran a stepwise regression with a forward and backward stepping GLM analysis to find out the equation that relates the effects of BMI and age in DM and non-DM men on FT levels. Adjustment for age and BMI in group comparisons was done with ANCOVA and generalized linear model analysis. For purposes of uniformity in comparisons, data were adjusted to the mean age (60.9 years) and BMI (29.7 kg/m^2) of the whole population. Data are presented as means ± S.D.

RESULTS

Thirty-five percent of all men had subnormal FT concentrations, irrespective of DM status. The mean age, BMI and hormone concentrations of DM and non-DM men are depicted in table 1. The crude prevalence of subnormal FT concentrations in DM and non-DM men was 51% and 31% respectively. DM men were older and heavier than non-DM men. Even after adjusting for age and BMI, the prevalence of subnormal FT concentrations in the DM men was higher than that in non-DM (45% versus 33%). As expected, there was an increase in the prevalence of subnormal FT concentrations with age. Figure 1 shows the prevalence of subnormal FT concentrations in study subjects divided into quartiles of age.

Prevalence of subnormal FT in lean, overweight and obese men: We analyzed the hormonal concentrations of study subjects after stratifying them in lean (BMI<25 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI ≥30 kg/m^2) categories (table 1). The data were adjusted for age and SHBG differences. There was a significant decline in FT concentrations with BMI in both non-DM and DM men. FT concentrations in lean, overweight and obese non-DM men were 62.0±19.4 pg/ml (0.215±0.067 nmol/L), 60.9±19.3 pg/ml (0.211±0.067 nmol/L) and 55.5±19.7 pg/mL (0.193±0.068 nmol/L) respectively ($p<0.001$ by ANOVA). FT concentrations in lean, overweight and obese DM men were 58.8±19.5 pg/ml (0.204±0.068

nmol/L), 56.9±19.5 pg/ml (0.198±0.068 nmol/L) and 50.7±19.4 pg/mL (0.176±0.068 nmol/L) respectively (p=0.002 by ANOVA).

The prevalence of subnormal FT concentrations was 26% in lean, 29% in overweight and 40% in obese non-DM men, while it was 44%, 44% and 50% in lean, overweight and obese DM men respectively. DM men had a significantly higher prevalence of subnormal FT concentrations in all BMI categories (see table 1). Similar results were obtained when hormonal concentrations were log-transformed and compared (online supplement table 2 which is available at http://care.diabetesjournals.org).

51 non-DM and 57 DM men were morbidly obese (BMI ≥40 kg/m^2). The mean FT concentrations in morbidly obese non-DM and DM men were 51.1±19.4 (0.177±0.067 nmol/L) and 45.1±19.4 pg/mL (0.157±0.067 nmol/L) respectively (p=0.15 by *t*-test). The prevalence of subnormal FT in morbidly obese non-DM and DM men was 49% and 55% (p=0.09 by chi-square) respectively.

Relation of FT with age and BMI: FT concentrations were significantly (p<0.001) and negatively related to age (r= -0.38) and BMI (r= -0.10). The final equation that described the decline in FT concentrations with an increase in age and BMI in non-DM men was

FT=123.57 – 0.7815 x Age – 0.5761 x BMI

(FT is in pg/ml, age in years and BMI in kg/m^2)

In DM men, the equation was:-

FT=123.57 – 0.8382 x Age – 0.5761 x BMI

Thus, the average reduction in FT concentration with age in non- DM and DM men was 7.8 and 8.4 pg/ml/decade respectively. The average reduction in FT concentration if the BMI of the subject increases by 1 kg/m^2 was 5.76 pg/ml in both DM and non-DM men.

FT concentrations were negatively related to SHBG (r= -0.18, p<0.001). Age was negatively related to BMI (r= -0.16, p<0.001) and positively to SHBG (r= 0.34, p<0.001). BMI was negatively related to SHBG (r= 0.28, p<0.001). We carried out multiple linear regression analysis in a model with FT as the dependent variable and age, BMI and SHBG as the independent variables. Age, BMI and SHBG were all independent predictors of serum FT (R^2 of the model was 0.18; standardized (β) coefficients for age, BMI and SHBG were -0.37, -0.18 and -0.11).

We also analyzed the effect of age and BMI separately in lean, overweight and obese DM and non-DM men. The results are presented in figure 2. These data show that the relationship of FT with age was stronger in the non-obese men. On the contrary, the relationship of FT with BMI was strongest in obese men.

Prevalence of subnormal FT in DM men on oral hypoglycemic and insulin: Out of 398 men with DM, 67 were diet controlled, 201 were on metformin, 204 were on sulfonylureas, 120 were on thiazolidinediones and 60 men were on insulin (17 on insulin monotherapy and 43 in combination with oral hypoglycemic drugs). The prevalence of subnormal FT was similar (p=0.40 by chi-square) in men treated with diet (45%), metformin (47%), sulfonylureas (54%), thiazolidinediones (48%) and insulin (57%), whether alone or in combination with oral agents.

Bioavailable testosterone (BT): Log transformed BT concentrations were lower in DM men as compared to non-DM men (online supplement table 2). Serum BT concentrations were independently predicted by age, BMI and SHBG (R^2 of the model was 0.31; β coefficients for age, BMI and SHBG were -0.39, -0.15 and -0.32). The age, BMI and SHBG adjusted prevalence of subnormal BT concentrations in DM and non-DM men was 51% and 43% respectively (p=0.004). The prevalence of subnormal BT concentrations in obese non-DM men (52%)

was higher than lean (37%, p<0.001) and overweight (38%, p<0.001) non-DM men. The prevalence of subnormal BT concentrations in obese DM men (58%) was higher than overweight (49%, p=0.05) but not lean (51%, p=0.42) DM men.

Prevalence of subnormal total T concentrations: The prevalence of subnormal total T concentrations (less than 300 ng/dl or 10.4 nmol/L) in non-DM and DM men. 33% of non-DM and 44% of DM men (p<0.001 by χ^2) had subnormal total T concentrations after adjusting for age, BMI and SHBG concentrations. 30% of lean, 29% of overweight and 39% of obese non-DM men had subnormal total T concentrations (p<0.001 by χ^2). 33% of lean, 44% of overweight and 46% of obese DM men had subnormal total T concentrations (p=0.56 by χ^2). Serum total T concentrations were independently predicted by age, BMI and SHBG (R^2 of the model was 0.45; β coefficients for age, BMI and SHBG were -0.30, -0.16 and 0.65).

The age, BMI and hormonal concentrations of men with subnormal total T, FT or both are compared in online supplement table 3.

DISCUSSION

In this, the largest analysis undertaken to answer this question, the data show clearly that 40% of all obese non-DM men and 49% of morbidly obese non-DM men had subnormal FT concentrations. Furthermore, there was an inverse relationship between FT concentrations and BMI. According to NHANES 2003-2004 data, 31% of all adult men in US are obese and 2.8% are morbidly obese(14). Thus, in view of the fact that almost one third of the US is obese, these observations have profound pathophysiological, clinical, epidemiological and public health implications. This study is also the first to comprehensively assess the comparative prevalence of subnormal FT concentrations with obesity and diabetes separately and together when they co-exist.

Our study shows that DM men with and without obesity have lower FT concentrations than non-DM men after adjustment for age or BMI. The effect of having DM on FT concentration in a 60 year old man was similar to that of an increase in BMI of 6 kg/m^2 (equal to weight gain of 25 kg in a 180 cm (6 feet) tall man) in a man without DM. The elevated insulin resistance of type 2 DM men as compared to obese men might explain these findings. Cross-sectional analysis of the NHANES III data (101 DM and 1312 non-DM men) has shown that low androgens are associated with the presence of DM in men (15). The prevalence of low FT concentrations was not mentioned in that analysis. The mean calculated FT concentrations were similar in DM and non-DM men. We found a small (6%) but statistically significant difference in the age and BMI adjusted FT concentrations of DM and non-DM men. The larger number of DM subjects in our study might explain the difference in our data and that from NHANES III. The mean age and BMI of NHANES III subjects (57 years and 29.5 kg/m^2) were lower than our DM study subjects. Consistent with the NHANES III study, we did not find a difference in SHBG concentrations of DM and non-DM men.

The prevalence of low FT in DM men was higher in all BMI categories when compared to non-DM men. While non-DM men showed an increase in prevalence of subnormal FT across BMI categories, there was no significant change in the prevalence of low FT with increasing BMI in the DM men. This is attributable to the high prevalence of low FT in lean DM males (45% after adjustment for age). Nevertheless, we found that FT concentrations decreased significantly with increasing BMI in both DM and non-DM men. In the morbidly obese subjects, we found that there was a non-significant trend

towards lower FT concentrations and higher prevalence of subnormal FT in DM men as compared to non-DM men. This could be due to smaller numbers of morbidly obese men in the study (51 DM and 57 non DM).

FT concentrations were negatively related to age in our study. We found that obese men had a smaller age-related decline of FT concentrations as compared to non-obese men. This suggests an independent effect of BMI related factors on FT concentrations. While our study cannot answer questions about the causes of low FT in the obese and type 2 DM men, several prior studies have addressed this question(10; 12; 16).

It has been suggested that the increase in adipose tissue mass in obesity may result in increased aromatase activity and thus to a greater conversion of T into estradiol(12). An increase in estradiol concentrations would lead to the suppression of hypothalamic gonadotropin releasing hormone and pituitary gonadotropin secretion. This would result in the reduction of both T secretion by Leydig cells and spermatogenesis in the seminiferous tubules. Young overweight and obese men are indeed known to have a decrease in sperm count(17). However, there is hitherto no study demonstrating that estradiol concentrations are actually elevated in obese or diabetic patients with subnormal testosterone concentrations. If indeed, this is confirmed, aromatase inhibition could be a therapeutic strategy in future. Unfortunately, estradiol concentrations are not available in our study.

The other possible mechanism involved in the pathogenesis of obesity related low FT is insulin resistance. The selective deletion of the insulin receptor gene from neurons results in a syndrome of hypogonadotrophic hypogonadism in mice in addition to a state of systemic insulin resistance(16). It is therefore possible that insulin resistance at the hypothalamic level contributes to the pathogenesis of this syndrome. The concurrent presence of marked inflammation may contribute to insulin resistance since inflammatory mediators like tumor necrosis factor-α and interleukin-6 may interfere with insulin signal transduction(18). Clearly, further investigation is necessary to define the etiology of this syndrome.

In view of the increasing prevalence of obesity even in younger populations, it would be important to conduct a similar study in the young at the prime of their reproductive years. It is relevant that the prevalence of hypogonadotropic hypogonadism is greater than 50% in patients with type 2 diabetes aged between 18 and 35 years(19).

We also found that SHBG concentrations are negatively related to BMI and positively to age. SHBG concentrations decrease with insulin resistance and low SHBG concentrations are predictive of future development of type 2 diabetes(20). While this is well established in previous studies, the pathophysiological mechanisms behind these associations are not known and need to be explored in future studies.

One of the limitations of our study is that we could not differentiate between type 1 and type 2 DM in our study subjects. The presence of DM was recorded by a physician. Since more than 90% of diabetics have type 2 diabetes, and this number is even higher in those above 45 years of age, this issue is not likely to affect the overall conclusions of this study. We have previously shown that the prevalence of hypogonadism in type 1 DM is markedly lower than that in type 2 DM(5). Only 17 patients were on insulin monotherapy and thus they may have type 1 diabetes. Excluding these men did not change the results of the study.

It is well known that there is a significant day-to-day variability in hormone concentrations, especially T. Like most epidemiologic or cross-sectional studies, the T concentrations in HIM study were measured only once. In view of the variability in T concentrations, this is a limitation.

However, it is not likely that the prevalence of low T concentrations would have altered following repeated measurements since the probability of T concentrations rising or falling with repeated measurements is statistically equal. The issue of repeated measurements is important in the context of diagnosing hypogonadism clinically in the context of a single patient. The fact that our study included a moderately large number of participants also helps to diminish the effect of hormonal variability on study effects and the relationship with BMI.

We did not have a validated questionnaire for erectile dysfunction and symptoms of hypogonadism. Therefore we cannot comment on the frequency of symptomatic hypogonadism in our study. It has been shown in the past that a high percentage of DM men with low testosterone concentrations have symptomatic hypogonadism(3; 21). Lower testosterone concentration are inversely related to visceral adiposity(3; 22). However, waist circumference or body composition imaging was not available in our study. Another limitation of our study is that the subjects were not required to be fasting when providing the blood samples. It has recently been shown that oral glucose load of 75 grams can acutely lower total T concentrations by 25%(23). However, in a prior analysis of these data, no difference was found in total T concentrations drawn between 8am-10am versus 10am-12pm(7).

In conclusion, 40% of obese non-DM men, above the age of 45 years, have subnormal FT concentrations; 26% of normal weight non-DM and 44% of normal weight DM had subnormal FT concentrations. The combination of obesity and DM increases the prevalence of subnormal FT concentrations to 50%. Thus, both obesity and DM appear to exert independent effects on the prevalence of low FT concentrations in addition to age. In view of the high rates of prevalence of subnormal FT in patients with obesity or DM, the concentrations of FT should be measured in these populations especially when these conditions occur concomitantly.

A**CKNOWLEDGEMENTS**

The data presented in the manuscript is from Hypogonadism In Males (HIM) study which was conducted by Solvay Pharmaceuticals, Inc. We are thankful to Dr. Alan Forrest, PharmD and Dr. Qusai Al-share, PhD, State University of New York at Buffalo for their valuable help in statistical analysis. PD is supported by grants from the NIH (R01DK069805-01A1 and R01DK075877-01A2) and the American Diabetes Association (708CR13). He is also supported by grants from Sanofi-Aventis, Merck, Solvay, Glaxo Smith Kline and Amylin Corporation.

REFERENCES

1. 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* 89:5462-5468, 2004

2. Dhindsa S, Bhatia V, Dhindsa G, Chaudhuri A, Gollapudi GM, Dandona P: The effects of hypogonadism on body composition and bone mineral density in type 2 diabetic patients. *Diabetes Care* 30:1860-1861, 2007

3. Kapoor D, Aldred H, Clark S, Channer KS, Jones TH: Clinical and Biochemical Assessment of Hypogonadism in Men With Type 2 Diabetes: Correlations with bioavailable testosterone and visceral adiposity. *Diabetes Care* 30:911-917, 2007

4. Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, Zajac JD, Jerums G: Low testosterone levels are common and associated with insulin resistance in men with diabetes. *J Clin Endocrinol Metab* 93:1834-1840, 2008

5. Tomar R, Dhindsa S, Chaudhuri A, Mohanty P, Garg R, Dandona P: Contrasting testosterone concentrations in type 1 and type 2 diabetes. *Diabetes Care* 29:1120-1122, 2006

6. Glass AR, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL: Low serum testosterone and sex-hormone-binding-globulin in massively obese men. *J Clin Endocrinol Metab* 45:1211-1219, 1977

7. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C: Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *Int J Clin Pract* 60:762-769, 2006

8. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM: Testosterone therapy in adult men with androgen deficiency syndromes: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 91:1995-2010, 2006

9. Vermeulen A, Verdonck L, Kaufman JM: A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84:3666-3672, 1999

10. Vermeulen A, Kaufman JM, Deslypere JP, Thomas G: Attenuated luteinizing hormone (LH) pulse amplitude but normal LH pulse frequency, and its relation to plasma androgens in hypogonadism of obese men. *J Clin Endocrinol Metab* 76:1140-1146, 1993

11. Zumoff B, Strain GW, Miller LK, Rosner W, Senie R, Seres DS, Rosenfeld RS: Plasma free and non-sex-hormone-binding-globulin-bound testosterone are decreased in obese men in proportion to their degree of obesity. *J Clin Endocrinol Metab* 71:929-931, 1990

12. Giagulli VA, Kaufman JM, Vermeulen A: Pathogenesis of the decreased androgen levels in obese men. *J Clin Endocrinol Metab* 79:997-1000, 1994

13. Hofstra J, Loves S, van Wageningen B, Ruinemans-Koerts J, Jansen I, de Boer H: High prevalence of hypogonadotropic hypogonadism in men referred for obesity treatment. *Neth J Med* 66:103-109, 2008

14. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM: Prevalence of overweight and obesity in the United States, 1999-2004. *Jama* 295:1549-1555, 2006

15. Selvin E, Feinleib M, Zhang L, Rohrmann S, Rifai N, Nelson WG, Dobs A, Basaria S, Golden SH, Platz EA: Androgens and Diabetes in Men: Results from the Third National Health and Nutrition Examination Survey (NHANES III). 2007, p. 234-238

16. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122-2125, 2000

17. Jensen TK, Andersson AM, Jorgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE: Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril* 82:863-870, 2004
18. Dandona P, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 25:4-7, 2004
19. Chandel A, Dhindsa S, Topiwala S, Chaudhuri A, Dandona P: Testosterone concentration in young patients with diabetes. *Diabetes Care* 31:2013-2017, 2008
20. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, Buring JE, Gaziano JM, Liu S: Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med* 361:1152-1163, 2009
21. Kapoor D, Clarke S, Channer KS, Jones TH: Erectile dysfunction is associated with low bioactive testosterone levels and visceral adiposity in men with type 2 diabetes. *Int J Androl* 30:500-507, 2007
22. Nielsen TL, Hagen C, Wraae K, Brixen K, Petersen PH, Haug E, Larsen R, Andersen M: Visceral and subcutaneous adipose tissue assessed by magnetic resonance imaging in relation to circulating androgens, sex hormone-binding globulin, and luteinizing hormone in young men. *J Clin Endocrinol Metab* 92:2696-2705, 2007
23. Caronia L, Dwyer A, Hayden D, Pitteloud N, Hayes F: Abrupt Decrease in Testosterone Following an Oral Glucose Load in Men. *Abstract The Endocrine Society* OR42-2, 2009

LEGENDS

Figure 1: The prevalence of subnormal FT in DM (light shade bars) and non-DM (dark shade bars) men separated into quartiles of age. Quartile 1 (45-52 years) had 408 non-DM and 55 DM men. Quartile 2 (53-59 years) had 378 non-DM and 82 DM men. Quartile 3 (60-68 years) had 326 non-DM and 136 DM men. Quartile 4 (69-91 years) had 339 non-DM and 125 DM men. The prevalence of subnormal FT concentrations was calculated in each quartile for non-DM and DM men. The prevalence was then adjusted to the mean BMI (29.7 kg/m^2) of the whole study population. χ^2 test was used to compare the prevalence among groups. A similar percentage of non-DM and DM men in quartile 1 had subnormal FT (18% vs. 18%, p=0.97). Non-DM men had a lower prevalence of subnormal FT than DM men in the other three quartiles (quartile 2: 22% vs. 41%, p<0.01; quartile 3: 32% vs. 50%, p<0.01 and quartile 4: 58% vs. 67%, p=0.05).

Figure 2 A-D: Inverse relationship of age and BMI with FT in lean (grey circles), overweight (white circles) and obese (triangles) men.

Figure 2A: Inverse relationship of age with FT in non- DM lean (r= -0.53, p<0.001), overweight (r= -0.43, p<0.001) and obese (r= -0.28, p<0.001) men. Thus, while age could explain ~25% of variability (r^2) in FT concentrations in the non-obese, it accounted for a significantly lower variability (8%) in FT in the obese non-DM men (p=0.008).

Figure 2B: Inverse relationship of age with FT in lean (r= -0.57, p<0.001), overweight (r = -0.49, p<0.001) and obese (r= -0.30, p<0.001) DM men. Thus, while age could explain 25-30% of variability (r^2) in FT concentrations in the non-obese, it accounted for a significantly lower variability (9%) in FT in the obese DM men (p=0.05 as compared to non-obese).

Figure 2C: Relationship of FT with BMI in lean (r= 0.01, p=0.9), overweight (r= -0.08, p=0.04) and obese (r= -0.10, p=0.03) non-DM men. Figure 2D: Relationship of FT with BMI in lean (r= 0.13, p=0.5), overweight (r= 0.03, p=0.8) and obese (r= -0.17, p<0.01) DM men.

Table 1: Demographic parameters and mean ± S.D. hormone concentrations of lean (BMI<25 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (BMI ≥30 kg/m^2) non-DM men and DM men.

				lean		overweight		obese	
	All	non-DM	DM	non-DM	DM	non-DM	DM	non-DM	DM
n	1849	1451	398	275	36	687	135	489	227
Age (years)	60.9±10.2	60.2±0.3	63.6±9.8[b]	62.8±11.5*	70.7±10.3*[b]	60.9±10.1*	65.9±9.6*[b]	57.9±9.1	61.0±8.9[b]
BMI (kg/m^2)	29.7± 5.6	29.0±5.2	32.3±6.4[b]	23.0±3.2	23.2±3.2	27.4±3.2	27.7±3.2	34.6±3.2	36.4±3.2[b]
% of men with subnormal FT	35%	33%	45%[b]	26%*	44%[a]	29%*	44%[b]	40%	50% [a]
FT pg/ml	58.1±21.2	58.9±19.5	55.2±20.0[b]	62.0±19.4*	58.8±19.5*	60.9± 19.3*	56.9±19.5* [a]	55.5±19.7	50.7±19.4[a]
FT nmol/L	0.202± 0.074	0.205± 0.068	0.192 ±0.069[b]	0.215± 0.067*	0.204 ±0.068*	0.211 ±0.067*	0.198 ±0.068* [a]	0.193±0.068	0.176 ± 0.067[a]
Total T ng/dl	368.5± 145.3	373.4±140.5	351.5± 144.2[b]	389.5±167.3*	369.7± 149.4*	388.3± 138.0*	356.4± 141.5*[b]	350.2± 132.7	330.4± 135.6 [a]
Total T nmol/L	12.8 ± 5.0	13.0 ± 4.9	12.2 ± 5.0 [b]	13.5 ±5.8*	12.8 ±5.2*	13.5 ±4.8*	12.4 ±4.9*[b]	12.2 ±4.6	11.5±4.7 [a]
BT ng/dl	101.0±49.2	101.8± 44.8	97.5±46.0	106.7±44.1*	96.6±44.5	106.0± 44.2*	101.3± 44.8*	95.6±45.1	89.9±44.4
BT nmol/L	3.5 ± 1.7	3.5 ± 1.6	3.4 ±1.6	3.7 ±1.5*	3.4 ±1.5	3.7 ±1.5*	3.5 ±1.6*	3.3 ± 1.6	3.1±1.5
SHBG (nmol/l)	58.3±29.9	58.1±29.1	59.5±32.7	71.4±27.6*	76.0±27.8*	58.8±27.5*	57.0±27.8	51.8±27.9	53.6±27.5

Testosterone concentrations and prevalence of subnormal FT in DM and non-DM columns were adjusted to the mean age (60.9 years), BMI (29.7 kg/m^2) and SHBG concentration (58.4 nmol/L) of the whole population. Testosterone concentrations and prevalence of subnormal FT in the lean, overweight and obese columns were adjusted only for age and SHBG.

*p<0.05 vs obese men in the same group (DM or non-DM)

[a]p<0.05 vs non-DM men

[b]p<0.001 vs non-DM men

Normal ranges: Total T (300-1000 ng/dl), FT (50-280 pg/ml), BT (90-285 ng/dl) and SHBG (20-60 nmol/l).

To convert into nmol/l, total T and BT were divided by 28.8 and FT by 288.

Figure 1

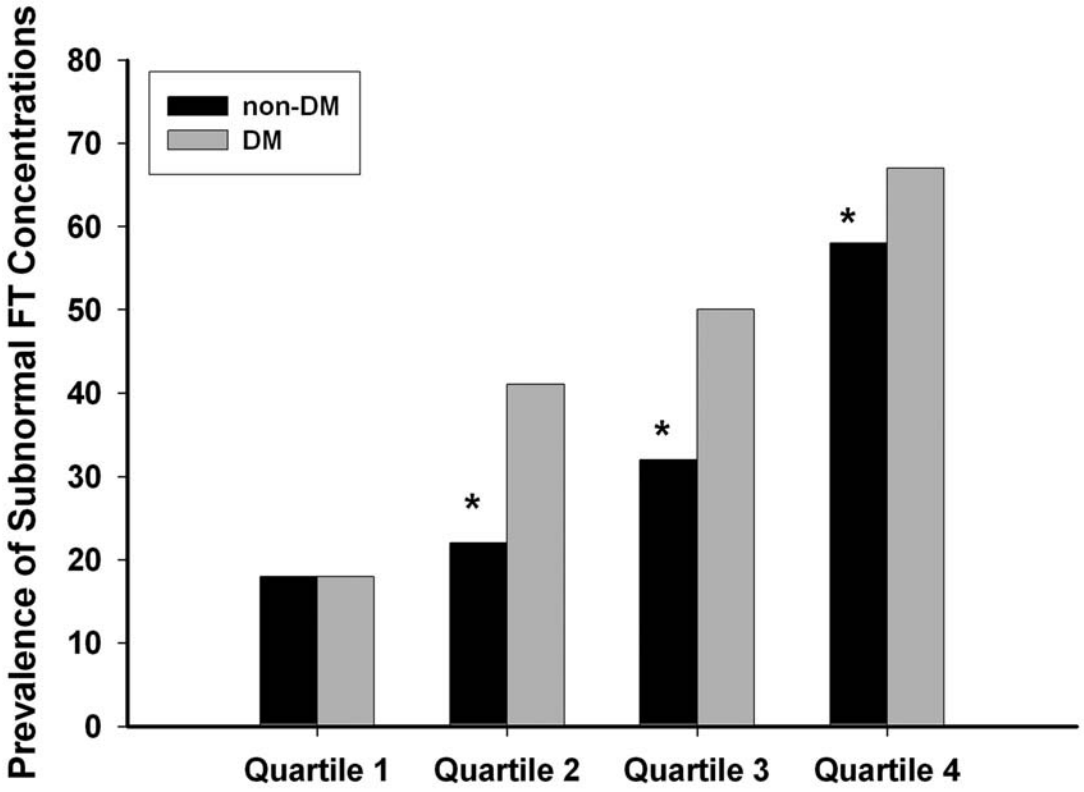
Prevalence of Subnormal FT Concentrations
80
70
60
50
40
30
20
10
0
non-DM
DM
*
*
*
Quartile 1
Quartile 2
Quartile 3
Quartile 4

Figure 2a

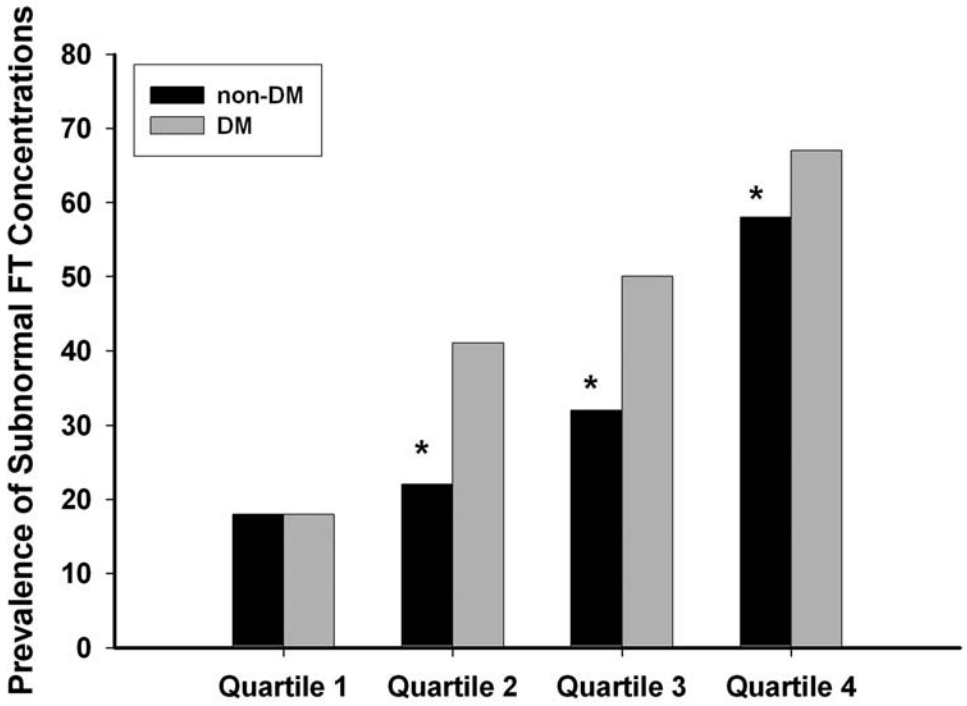

Figure 2b

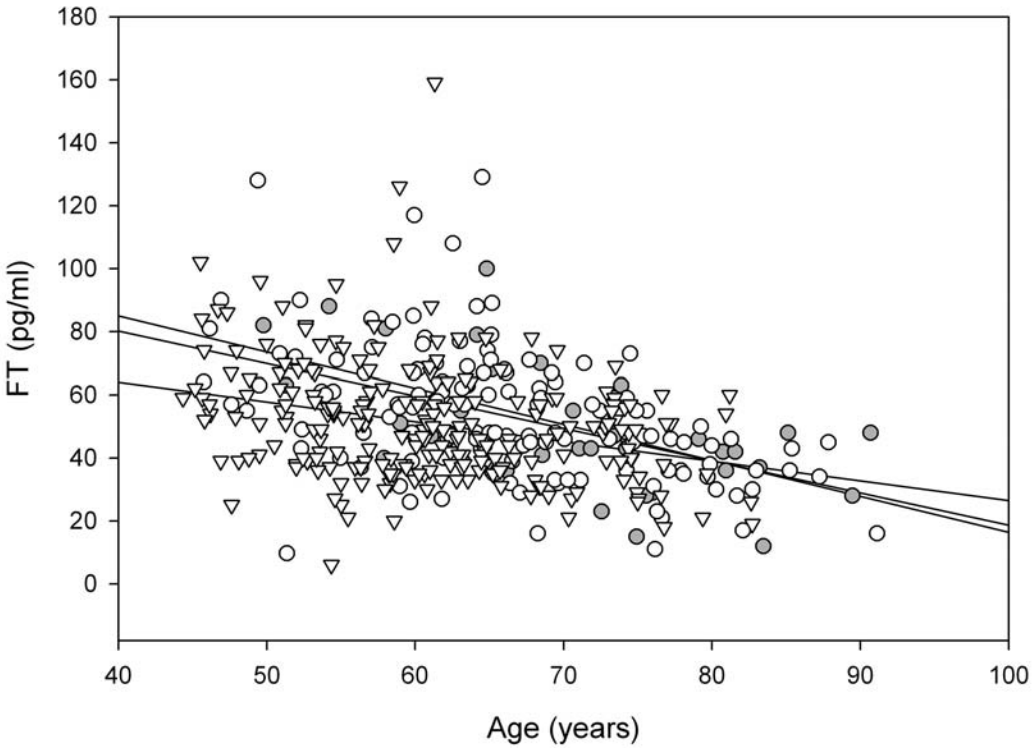

Figure 2c

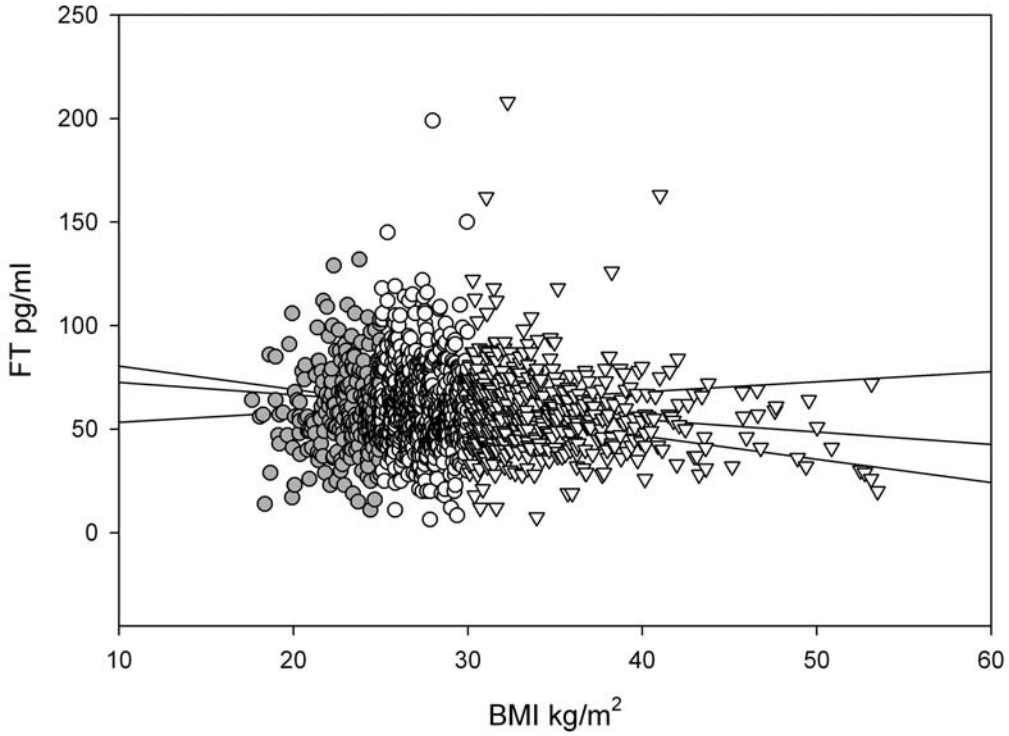

Figure 2d