The associations of endogenous testosterone and sex hormone-binding globulin with glycosylated hemoglobin levels, in community dwelling men.

The Tromsø Study

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SUMMARY

Objectives: Low levels of endogenous testosterone have been associated with increased risk of cardiovascular disease and atherosclerosis in men. Long-term hyperglycemia, as measured by glycosylated hemoglobin (HbA1c), is related to cardiovascular mortality, and HbA1c across its normal range is also positively related to coronary heart and cardiovascular disease mortality in men. We therefore undertook an analysis of the cross-sectional associations of total testosterone and SHBG levels with HbA1c levels, in a general population of 1419 men aged 25-84.

Methods: Total testosterone, sex hormone-binding globulin (SHBG) and HbA1c were measured by immuno-assay. Partial correlation and multiple regression analyses were used to estimate the associations between total testosterone and SHBG with HbA1c levels. Analyses of variance and covariance were used to compare men with or without diabetes.

Results: In age-adjusted partial correlation HbA1c was inversely associated with total testosterone (p < 0.01) and SHBG (p < 0.001). HbA1c was positively associated with body mass index (BMI) and waist circumference (WC) (p < 0.001). In multiple regression analyses total testosterone, SHBG, age, number of cigarettes smoked, BMI and WC were independently associated with HbA1c levels. Men with a history of diabetes had lower levels of total testosterone in age-adjusted analyses (p < 0.05) and lower levels of SHBG in both age- and WC-adjusted analyses (p < 0.001 and p < 0.01, respectively).

Conclusion: Lower levels of total testosterone and SHBG were associated with increased HbA1c levels and diabetes independent of concomitant variations in obesity and body fat distribution.

Key-words: Cross-sectional · Glycosylated hemoglobin · SHBG · Testosterone · Waist circumference.

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RÉSUMÉ

Associations entre testostérone endogène, sex hormone-binding globulin et taux d’hémoglobine glyquée, chez des hommes vivant en communauté. L’étude Tromso

Objectifs : Des taux bas de testostérone endogène ont été associés à un risque accru de maladies cardiovasculaires et d’athérosclérose chez l’homme. L’hyperglycémie à long terme, mesurée par l’hémoglobine glyquée (HbA1c), est liée à la mortalité cardiovasculaire, et l’HbA1c dans sa zone de normalité est également corrélée positivement à la mortalité coronarienne et cardiovasculaire chez l’homme. Aussi nous avons entrepris une analyse transversale de l’association des taux de testostérone totale et de SHBG avec les taux d’HbA1c, dans une population générale de 1 419 hommes âgés de 25 à 84 ans.

Méthodes : La testostérone totale, la sex hormone-binding globulin (SHBG) et l’HbA1c ont été mesurées par immuno-assay. Une analyse de corrélation partielle et de régression multiple a été utilisée pour estimer les associations entre testostérone totale et SHBG avec l’HbA1c. Les analyses de variance et covariance ont été utilisées pour comparer les hommes avec ou sans diabète.

Résultats : En corrélation partielle ajustée pour l’âge, l’HbA1c était inversement associée avec la testostérone totale (p < 0.01) et la SHBG (p < 0.001). L’HbA1c était positivement associée avec l’indice de masse corporelle (BMI) et le tour de taille (WC) (p < 0.001). En analyse de régression multiple, testostérone totale, SHBG, âge, nombre de cigarettes fumées, BMI et WC étaient indépendamment associés avec les taux d’HbA1c. Les hommes avec un antécédent de diabète avaient des taux plus bas de testostérone totale dans une analyse ajustée pour l’âge (p < 0.05) et des taux plus bas de SHBG dans des analyses ajustées pour l’âge et le WC (p < 0.001 et p < 0.01, respectivement).

Conclusion : Des taux plus bas de testostérone totale et de SHBG sont associés avec des taux augmenté d’HbA1c et avec le diabète, indépendamment de variations concomitantes de l’obésité et de la distribution des graisses corporelles.

Mots-clés : Transversale · Hémoglobine glyquée · SHBG · Testostérone · Tour de taille.

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A large body of evidence supports the cross-sectional associations of lower total and free testosterone with increased insulin concentrations and insulin resistance in men [1-4]. Lower levels of endogenous testosterone have also been found in men with impaired glucose tolerance diabetes (IGT) and type-2 diabetes [5, 6]. In addition, low levels of testosterone have been reported to predict insulin resistance and incident type 2 diabetes in older men [7, 8]. Lower levels of testosterone have also been found to be associated with increased risk of cardiovascular disease by some [9, 10] but not by all authors [11]. Low levels of testosterone were recently reported to be associated with atherosclerosis in men [12]. Hyperglycemia, as measured by glycosylated hemoglobin (HbA1c), is related to cardiovascular disease mortality, and HbA1c across its normal range is also positively related to the incidence of death from coronary heart and cardiovascular disease in men [13]. Associations between testosterone levels and HbA1c have previously not been reported in population-based studies.

We therefore undertook an analysis of the cross-sectional association between total and free testosterone and SHBG levels and HbA1c levels; in a general population of 1419 men aged 25-84.

Methods

The fourth Tromsø survey consisted of 2 screening visits 4 to 12 weeks apart that have previously been described in more detail [14]. All participants completed two self-administered questionnaires, checked by trained nurses, including information about smoking habits, physical activity, alcohol consumption and medical history. From this questionnaire a physical activity score was computed adding together the hours of easy and vigorous physical activity, with the hours of vigorous activity receiving double weight. The alcohol intake of beer, wine and hard liquor consumed during a two week period was also scored, assuming an equal amount of alcohol in one glass of each type.

A representative subgroup of 1419 men, aged 25-84 years, from the second screening visit had blood samples drawn for future analysis of both sex hormones and HbA1c in addition to the general examination.

Blood was drawn by venipuncture from non-fasting men between 08.00-16.00 h. Serum samples were stored at -70°C, until they were first thawed for analyses of sex hormones in 2001.

Determination of total testosterone and SHBG was performed on Immulite 2000 (Diagnostic Product Corp. Los Angeles, CA, USA). Free testosterone values were calculated from total testosterone and SHBG using a fixed albumin according to Vermeulen et al. [15]. The intra- and interassay coefficients of variation (CV) for total testosterone were 3.5% and 5%, respectively at a concentration above 1.0 nmol/l and 12% and 20%, respectively in the range 0.1-1.0 nmol/l. For SHBG the intra- and interassay CVs were 3.5% and 6% and limit of detection was 1.0 nmol/l. HbA1c was measured from the hemolysate by a latex-enhanced turbidimetric immunoassay Unimate 3 HbA1c, on a Cobras Mira plus (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). The CV was 3.2% at the lower end of the range (mean 5.3%) and 5.1% at the upper end of the range (mean 11.4%).

Height, weight, and waist circumference were measured in standing subjects wearing light clothing without shoes; waist was measured at the umbilical line according to a written protocol. Body mass index (BMI) (kg/m²) was calculated. Waist circumference (WC) alone was used as an integrated measure of obesity and fat distribution, on the basis of studies suggesting that WC, as opposed to WHR, correlates better with visceral body fat measured by computed tomography or magnetic resonance imaging [16].

Partial correlation adjusted for age was performed between sex hormones, HbA1c, anthropometric measures and lifestyle factors. Multiple linear regression models were used to determine the independent contribution of total and free testosterone, SHBG, BMI, smoking, alcohol consumption, and physical activity to HbA1c level. Analysis of covariance was used to calculate adjusted means of sex hormones and SHBG stratified by HbA1c or diabetic status; age, BMI and/or WC were used as continuous variables and as covariates in the model. HbA1c levels were slightly skewed and required log-transformation before statistical analyses. All statistical tests were two-tailed with statistical significance defined as p < 0.05. The data were analyzed using the SPSS statistical package for Windows version 11.0 (SPSS Inc, Chicago, IL, USA).

Ethics

The Tromsø Regional ethics committee approved the study, and all participants gave written informed consent.

Results

The men were 60.5 ± 10.0 years old, with a mean BMI 26.1 ± 3.4 kg/m², and a waist circumference 95.5 ± 9.3 cm. Approximately two-thirds (67.7%) were non-smokers, about one-third (32.8%) reported drinking less then one alcoholic beverage in a two-week period, and only 10.4% exercised vigorously ≥ 3 times/week. Their mean HbA1c level was 5.5 ± 0.7%, total testosterone 13.0 ± 5.0 nmol/l, free testosterone 203 ± 75 pmol/l and SHBG 51.5 ± 22 nmol/l. A history of diabetes was reported by 45 men (3.2%).

In age-adjusted partial correlation analysis HbA1c was inversely associated with total testosterone (r = -0.09; p=0.001) and SHBG (r = -0.10; p < 0.001), but not with free testosterone. HbA1c was positively associated with BMI and WC (r = 0.12; p < 0.001), and number of cigarettes smoked (r = 0.05; p = 0.037) but no significant associations were found.
with neither alcohol consumption nor with physical activity. Total and free testosterone were inversely associated with BMI (r = -0.30; p < 0.001 and r = -0.07; p = 0.008, respectively) and WC (r = -0.33; p < 0.001 and r = -0.09; p < 0.001, respectively). SHBG was also inversely associated with BMI and WC (r = -0.42; p < 0.001 and r = -0.43; p < 0.001, respectively).

In multiple regression analyses total testosterone, SHBG, age, number of cigarettes smoked, BMI, and WC were independently associated with HbA1c levels (Table I). When men with a history of diabetes and men with HbA1c > 6.5% were excluded from the multiple regression analyses only age, number of cigarettes smoked, BMI, and WC were independently associated with HbA1c levels (data not shown).

Table II shows that men with a history of diabetes and men with HbA1c levels > 6.5% (indicating diabetes) have lower levels of total testosterone in age-adjusted analyses and also lower levels of SHBG when adjusted for both age and WC.

Table I
Multiple regression analyses with HbA1c as dependent variable. The table shows the association between HbA1c and testosterone (left) and SHBG (right) with addition of age (model 1), age and smoking (model 2), age, smoking and BMI (model 3), and age, smoking, and waist circumference (model 4).

| Dependent variable | HbA1c* |  |  |  |  |  |
|--------------------|--------|  |  |  |  |  |
| **Model 1**        |        |  |  |  |  |  |
| Total testosterone | -0.09  | < 0.001 |  |  |  |  |
| Age                | 0.19   | < 0.001 |  |  |  |  |
| Adjusted R²        | 0.045  |  |  |  |  |  |
| **Model 2**        |        |  |  |  |  |  |
| Total testosterone | -0.10  | < 0.001 |  |  |  |  |
| Age                | 0.20   | < 0.001 |  |  |  |  |
| Smoking            | 0.07   | 0.008 |  |  |  |  |
| Adjusted R²        | 0.049  |  |  |  |  |  |
| **Model 3**        |        |  |  |  |  |  |
| Total testosterone | -0.07  | 0.015 |  |  |  |  |
| Age                | 0.21   | < 0.001 |  |  |  |  |
| Smoking            | 0.08   | 0.004 |  |  |  |  |
| BMI                | 0.11   | < 0.001 |  |  |  |  |
| Adjusted R²        | 0.060  |  |  |  |  |  |
| **Model 4**        |        |  |  |  |  |  |
| Total testosterone | -0.07  | 0.016 |  |  |  |  |
| Age                | 0.20   | < 0.001 |  |  |  |  |
| Smoking            | 0.07   | 0.008 |  |  |  |  |
| Waist circumference | 0.10   | < 0.001 |  |  |  |  |
| Adjusted R²        | 0.056  |  |  |  |  |  |

* Log transformed values; † Standardized beta coefficient.

Table II
Mean (SD), age and age- and waist circumference-adjusted hormone levels in men with or without a known history of diabetes. Men with HbA1c level > 6.5% were included in the diabetes group.

<table>
<thead>
<tr>
<th></th>
<th>Diabetes (and/or HbA1c &gt; 6.5%)</th>
<th>No diabetes</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>55</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>11.2 (5.4)</td>
<td>13.2 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>11.4</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Age- and WC-adjusted</td>
<td>12.2</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>188 (72)</td>
<td>204 (75)</td>
<td>204</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>204</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>Age- and WC-adjusted</td>
<td>207</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>43.8 (21.4)</td>
<td>51.8 (22.0)</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>39.7</td>
<td>52.0</td>
<td></td>
</tr>
<tr>
<td>Age- and WC-adjusted</td>
<td>43.7</td>
<td>51.8</td>
<td></td>
</tr>
</tbody>
</table>

WC = waist circumference; * p < 0.05; † p < 0.01; and ‡ p < 0.001.
Table III shows that men with total testosterone levels in the lowest quintile compared to men with testosterone levels in the highest quintile have higher HbA1c levels. The difference was clearly significant in both age-adjusted (p < 0.01) and age- and WC-adjusted analyses (p < 0.05).

Discussion

In this large population-based study endogenous total testosterone levels was inversely associated with HbA1c, and lower levels of total testosterone were found in men with diabetes. In addition, higher levels of HbA1c were found in men with the lowest testosterone levels. Even though no previous study has reported on the associations of HbA1c and endogenous sex hormones, studies on fasting glucose or post challenge glucose levels have shown an inverse association with total testosterone which agrees with our findings [4, 6]. High serum glucose concentrations indicate an early or established glucose intolerance, which may result in type 2 diabetes. Cross-sectional studies have shown that increased insulin concentrations, at least partly reflecting insulin resistance, are inversely associated with total testosterone [3, 4, 17], and a reduced concentration of testosterone is an independent risk factor for diabetes [7]. Total body fat or central adiposity can largely modify these associations [18], and low levels of total testosterone and free testosterone have been associated with increasing obesity [19, 20] in agreement with our findings.

If obesity is involved in the causal pathway between testosterone and glucose intolerance, then adjusting for BMI may be considered as an over-adjustment. However, adjusting for BMI reduced, but did not eliminate, the associations between total testosterone and HbA1c. Low levels of testosterone have also been found to predict central obesity [21] and administration of testosterone to centrally obese middle aged men reduced adiposity and decreased insulin resistance [22]. Body fat distribution is strongly associated with both diabetes and cardiovascular disease [23, 24], but adjusting for central adiposity using WC as a surrogate measurement only reduced, but did not eliminate, the association between total testosterone and HbA1c.

The association between SHBG and HbA1c, and the SHBG-diabetes association also remained significant after adjusting for both BMI and WC. Thus, in agreement with previous studies the association between SHBG and metabolic complications was independent of the contribution of central adiposity [25]. Our findings are also in agreement with previous reports in that low concentrations of SHBG are associated with insulin resistance, hyperinsulinemia and glucose intolerance [26-29]. In addition low levels of SHBG are associated with central obesity [1, 30] and have been reported to predict diabetes mellitus type 2 in men [7]. It has been suggested that the inverse relationship between SHBG levels and cardiovascular risk is mediated, to a large, extent by concomitant variation in body fat [31]. If decreasing testosterone levels cause increasing adiposity leading to hyperinsulinemia, then one could still argue that testosterone is modulating the SHBG levels.

Free testosterone was not associated with HbA1c or with diabetes. However, when total testosterone and SHBG decrease in parallel, free testosterone levels may be unchanged.

Of the examined life-style factors only the number of cigarettes smoked was associated with HbA1c as previously reported [32]. However, alcohol consumption and physical activity are not precisely estimated from self-reports and misclassification could have obscured these associations.

Besides being a cross-sectional study there are some limitations connected to this study. Hormone levels were based on a single serum sample, drawn between 08.00-16.00 h. Preferably samples should have been drawn in the morning because of the diurnal variation of total and free testosterone; we observed the expected higher levels of both total and free testosterone in the morning. But, as the diurnal variation is prominent in younger men and diminishes in older men and higher levels of HbA1c is seen in older men, this would only have weakened the described associations. Although hormone analyses were performed using sera that had been frozen for an average of 6.5 years, previous studies have not demonstrated any deterioration in hormone concentration when samples had been frozen and stored in tightly sealed containers for up to 10 years [33, 34]. We did not measure free testosterone, but the calculation we used was recently validated by two different investigators and found to be a reliable index of free testosterone [15, 35].

Protein intake has been reported to be inversely and fiber intake positively associated with SHBG [36]. The diet of our participants could thus possibly have affected the SHBG levels, and consequently influenced the testosterone levels. However, the reported associations were weak.

Unfortunately we did not have information on whether the participants had type 1 or type 2 diabetes. Of the men who reported having diabetes, no one was younger than 53 years, and we believe most of the men had type 2 diabetes. In

<table>
<thead>
<tr>
<th>Total testosterone</th>
<th>Low (≤ 9.0)</th>
<th>High (≥ 16.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>298</td>
<td>285</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.56 (0.83)</td>
<td>5.36 (0.44)</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>5.55</td>
<td>5.37</td>
</tr>
<tr>
<td>Age- and WC-adjusted</td>
<td>5.53</td>
<td>5.39</td>
</tr>
</tbody>
</table>

WC = waist circumference; * p < 0.05 and † p < 0.01.
addition, the individuals with HbA1c > 6.5% who did not report having diabetes are likely to have type 2 diabetes. Any inclusion of men with type 1 diabetes in this study would probably dilute the observed associations, since they would be expected to be leaner and not to have the same metabolic profile as those with glucose intolerance or type 2 diabetes.

In summary, lower levels of testosterone and SHBG were associated with increased HbA1c levels and diabetes. The results support the hypothesis that lower levels of testosterone in men are associated with CHD risk factors, and that this association is independent of obesity and body fat distribution.

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References


