Association between serum bioavailable testosterone concentration and the ratio of glycated albumin to glycated hemoglobin in men with type 2 diabetes

Michiaki Fukui, MD¹, Muhei Tanaka, MD¹,
Goji Hasegawa, MD¹, Toshikazu Yoshikawa MD², Naoto Nakamura, MD¹

¹Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine,
Graduate School of Medical Science, 465 Kajii-cho,
Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan
²Department of Inflammation and Immunology, Kyoto Prefectural University of Medicine,
Graduate School of Medical Science, 465 Kajii-cho,
Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

Running title: Testosterone and ratio of GA to HbA1c

Corresponding author:
Michiaki Fukui, MD
Department of Endocrinology and Metabolism
Kyoto Prefectural University of Medicine
Graduate School of Medical Science
465 Kajii-cho, Kawaramachi-Hirokoji
Kamigyo-ku, Kyoto 602-8566, Japan
sayarinapm@hotmail.com

Received for publication 29 September 2007 and accepted in revised form 15 November 2007.
ABSTRACT

Objective: Testosterone stimulates erythropoiesis and thus glycated hemoglobin (HbA1c) values may be relatively low in male diabetic patients with hypogonadism. We therefore investigated relationships between serum bioavailable testosterone concentration and the ratio of glycated albumin (GA) to HbA1c as well as between serum bioavailable testosterone and hemoglobin concentrations in men with type 2 diabetes.

Research Design and Methods: The above relationships were investigated in 222 consecutive men with type 2 diabetes. We also investigated how the ratio of GA to HbA1c is related to other variables such as age, BMI, and degree of diabetic microangiopathy.

Results: Mean ratio of GA to HbA1c was 2.94±0.38. Serum bioavailable testosterone concentration correlated positively with hemoglobin concentration (r=0.368, P<0.0001), and negatively with the ratio of GA to HbA1c (r=-0.278, P<0.0001). Multiple regression analyses identified serum bioavailable testosterone concentration (β=0.187, P=0.0062), age (β=-0.204, P=0.0075), BMI (β=0.151, P=0.0302), systolic blood pressure (β=0.173, P=0.0090), and plasma total cholesterol (β=0.155, P=0.0141) as independent determinants of hemoglobin concentration, moreover, serum bioavailable testosterone concentration (β=0.155, P=0.0381), and plasma total cholesterol (β=0.170, P=0.0144) as independent determinants of the ratio of GA to HbA1c.

Conclusions: Serum bioavailable testosterone concentration correlated positively with hemoglobin concentration and negatively with the ratio of GA to HbA1c in men with type 2 diabetes, which may lead to underestimation of HbA1c in hypogonadal men with type 2 diabetes.
Testosterone stimulates erythropoiesis, while testosterone replacement therapy increases hemoglobin concentration (1,2). One might suspect that declining serum testosterone concentrations during aging could compromise erythropoiesis. Accordingly, men with hypogonadism as well as those treated with antiandrogenic drugs frequently have anemia (3,4). Since the influence of serum bioavailable testosterone on hemoglobin concentration has not been investigated in men with type 2 diabetes, we presently examined this relationship and its possible implications for management of diabetes.

Measurements of glycated hemoglobin (HbA1c) and glycated albumin (GA) have been used clinically to monitor glycemic control in patients with diabetes mellitus. HbA1c represents an integrated measurement of blood glucose during the preceding 2 months while serum GA, a shorter-term marker, reflects glycemic control over approximately the preceding 2 weeks (5-7). GA is not influenced by a number of physiologic and pathologic conditions that affect HbA1c levels, such as anemia and genetic hemoglobin abnormalities (8,9).

The considerations above raise the possibility that HbA1c levels might be relatively low in male diabetic patients with hypogonadism. To our knowledge, relationships between serum endogenous androgens and the ratio of GA to HbA1c (GA/HbA1c) have not been explored in men with type 2 diabetes. We therefore investigated the effect of serum bioavailable testosterone concentration on GA/HbA1c in these patients.

**RESEARCH DESIGN AND METHODS**

**Patients.** Serum bioavailable testosterone concentrations were measured in 222 consecutive men with type 2 diabetes recruited from the outpatient clinic at the Kyoto Prefectural University of Medicine. Relationships between serum bioavailable testosterone and hemoglobin concentrations and between serum bioavailable testosterone concentration and GA/HbA1c were investigated. In addition, we evaluated relationships between GA/HbA1c and age, duration of diabetes, blood pressure, plasma lipid concentration, BMI, waist circumference, severity of diabetic retinopathy, severity of diabetic nephropathy defined by urinary albumin excretion, presence of cardiovascular disease (CVD), smoking status, and current treatment for diabetes.

Blood samples were obtained in the morning. Bioavailable testosterone was separated using precipitation of testosterone bound to globulins with 50% ammonium sulfate, and serum bioavailable testosterone concentrations were measured by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a modification method based on the use of picolinoyl derivatization (10). Intra-assay and interassay coefficients of variation (CV) for serum bioavailable testosterone concentrations at 1pg/mL were 4.73% and 12.94%, respectively. Hemoglobin concentrations were analyzed within 4 hr of blood drawing using a SYMEX XE-2100 autoanalyzer (Sysmex Corporation, Kobe, Japan). HbA1c was measured by high-performance liquid chromatography using an ADAMS-A1c HA-8160 (Arkray, Kyoto, Japan). Interassay CV determined using representative blood samples with
Testosterone and ratio of GA to HbA1c

5.2% and 10.9% HbA1c was 0.63% and 0.45%, respectively. GA was determined by an enzymatic method using albumin-specific proteinase, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan), with a Hitachi 7600 autoanalyzer (Hitachi Instrument Service, Tokyo, Japan). Interassay CV determined using representative serum samples with 17.2% and 26.9% GA was 1.08% and 1.37%, respectively. Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Mean values for biochemical parameters obtained during the preceding year were used for statistical analysis. Urinary albumin and creatinine concentration were determined in early morning spot urine. Urinary albumin excretion was measured with an immunoturbidimetric assay. A mean value for urinary albumin excretion was determined from three urine collections.

Type 2 diabetes was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (11). Retinopathy was graded as no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), or proliferative diabetic retinopathy (PDR). Nephropathy was graded as normoalbuminuria, urinary albumin excretion <30 mg/g Cr; or microalbuminuria, 30-300 mg/g Cr. Sitting blood pressure was measured after a 5-min rest. CVD was defined as a previous myocardial or cerebral infarction based on the clinical history or physical examination. Subjects were classified as nonsmokers, past smokers, or current smokers according to a self-administered questionnaire. Patients were excluded if they had undergone castration for treatment of testicular or prostate cancer, or were taking any medications known to affect sex hormone concentrations (e.g., antiandrogenic agents for prostate cancer). In addition, patients with macroalbuminuria were excluded since advanced diabetic nephropathy can influence hemoglobin concentration. Moreover, patients with malignant disease, liver cirrhosis, thyroid disorders, hematologic disease, or infectious disease were excluded from this study. To minimize effects of diabetic treatment on time-dependent variations of HbA1c and GA levels, we selected patients whose HbA1c levels had been stable for at least the preceding 3 months. Approval for the study was obtained from the local Research Ethics Committee, and informed consent was obtained from all participants.

**Statistical analysis.** Means or frequencies of potential confounding variables were calculated as appropriate. Unpaired Student’s t tests or analyses of variance were conducted to assess statistical significance of differences between groups using Stat View software (version 5.0; SAS Institute, Cary, NC). Because plasma triglyceride concentration showed a skewed distribution, logarithmic (log) transformation was carried out before performing a correlation analysis. Correlations between serum bioavailable testosterone concentration and hemoglobin concentration or GA/HbA1c, as well as between GA/HbA1c and age, duration of diabetes, BMI, or other variables were examined by Pearson’s correlation analyses. All continuous variables are presented as the mean±SD. Multiple linear regression analysis was performed to assess the combined influence of variables on hemoglobin concentration or GA/HbA1c. To examine the effects of various factors on hemoglobin concentration or GA/HbA1c, the following factors were considered as
independent variables: serum bioavailable testosterone concentration, age, duration of diabetes, BMI, systolic blood pressure, plasma total cholesterol concentration, and smoking status. A $P$ value $<0.05$ was considered statistically significant.

RESULTS

Characteristics of the 222 men with type 2 diabetes enrolled in this study are shown in Table 1. The mean value of GA/HbA1c was $2.94\pm0.38$.

Relationships between GA/HbA1c and other variables are shown in Table 2. Age, duration of diabetes, and plasma HDL-cholesterol concentration were positively associated with GA/HbA1c, while BMI, waist circumference, systolic blood pressure, diastolic blood pressure, plasma total cholesterol concentration, and log (plasma triglyceride concentration) were negatively associated with GA/HbA1c. A positive correlation was found between serum bioavailable testosterone and hemoglobin concentrations ($r=0.368$, $P<0.0001$; Fig. 1A), while a negative correlation was found between serum bioavailable testosterone concentration and GA/HbA1c ($r=-0.278$, $P<0.0001$; Fig. 1B). No significant correlation was found between serum bioavailable testosterone and albumin concentrations ($r=0.147$, $P=0.0625$). Multiple regression analyses identified serum bioavailable testosterone concentration ($\beta=0.187$, $P=0.0062$), age ($\beta=-0.204$, $P=0.0075$), BMI ($\beta=0.151$, $P=0.0302$), systolic blood pressure ($\beta=0.173$, $P=0.0090$), and plasma total cholesterol ($\beta=0.155$, $P=0.0141$) as independent determinants of hemoglobin concentration, moreover, serum bioavailable testosterone concentration ($\beta=-0.155$, $P=0.0381$), and plasma total cholesterol ($\beta=-0.170$, $P=0.0144$) as independent determinants of GA/HbA1c (Table 3).

GA/HbA1c did not differ between patients with normoalbuminuria and microalbuminuria (2.94±0.40 vs. 2.97±0.35, $P=0.5162$). GA/HbA1c was higher in patients with PDR (3.16±0.25, $P=0.0128$) or SDR (3.10±0.28, $P=0.0142$) than that in patients with NDR (2.91±0.40). GA/HbA1c did not differ between patients with and without CVD (2.97±0.37 vs. 2.95±0.38, $P=0.7238$). GA/HbA1c was higher in patients treated with than without insulin (3.15±0.45 vs. 2.87±0.32, $P<0.0001$). GA/HbA1c did not differ between patients treated with and without statin (2.88±0.37 vs. 2.96±0.38, $P=0.1821$) or angiotensin II receptor blocker or angiotensin converting enzyme inhibitor (2.95±0.36 vs. 2.94±0.39, $P=0.7533$). GA/HbA1c was higher in patients treated with than without antiplatelet agent (3.05±0.46 vs. 2.92±0.35, $P=0.0416$). GA/HbA1c did not differ according to smoking status (2.90±0.27 vs. 2.93±0.41 vs. 3.04±0.46 for current smokers, past smokers, and nonsmokers, respectively).

CONCLUSIONS

Serum bioavailable testosterone concentration correlated positively with hemoglobin concentration in men with type 2 diabetes, which is compatible with previous findings in the general population. Serum bioavailable testosterone concentration correlated negatively with GA/HbA1c. Multiple regression analysis also identified serum bioavailable testosterone as a determinant of hemoglobin concentration or GA/HbA1c.

GA and HbA1c have a tendency to vary in parallel, although the normal range of variation of GA/HbA1c has not yet been determined. GA and HbA1c are equivalent measures of glycemic control in general, although the former is short
Testosterone and ratio of GA to HbA1c
term and the latter is long term marker. In
certain diabetic patients in whom HbA1c
measurement proves insufficient for
clinical management (8,9), measurement
of serum GA represents an alternative
assessment of glycemic control. For
example, anemia is an important factor
affecting HbA1c levels. Thus, changes in
the GA/HbA1c would indicate artefactual
change, which make GA/HbA1c to have a
clinical value.

Our results suggest that we may
underestimate HbA1c level in male
diabetic patients with hypogonadism
because of a negative association between
serum bioavailable testosterone
concentration and GA/HbA1c that arises
partly from a positive association between
serum bioavailable testosterone and
hemoglobin concentrations. Men with
diabetes have significantly lower plasma
concentrations of endogenous androgen
than nondiabetic men (12-15); endogenous androgen concentrations also
decline with advancing age (16). Diabetic
men with poor control or advanced age
should be paid close attention to the
evaluation of HbA1c levels.

GA/HbA1c was negatively associated
with BMI or waist circumference in the
present study. Koga et al. (17) made a
similar observation, suggesting that
increased serum albumin turnover in
obese subjects could keep serum GA low
relative to plasma glucose concentrations.
We additionally found age to be positively
associated with GA/HbA1c and systolic
blood pressure or plasma total cholesterol
concentration to be negatively associated
with GA/HbA1c. Although the reasons for
these associations are not clear, possible
explanations may be that age (r=-0.390,
P<0.0001) is negatively associated with
hemoglobin while systolic blood pressure
(r=0.253, P=0.0002) or plasma total
cholesterol concentration (r=0.258,
P<0.0001) is positively associated with
hemoglobin concentration. Cholesterol is
a precursor of testosterone, and serum
bioavailable testosterone concentration is
positively associated with plasma total
cholesterol concentration (r=0.194,
P=0.0039). GA/HbA1c was higher
according to progression of diabetic
retinopathy. Finally, ongoing treatment
for diabetic patients such as insulin or
antiplatelet agent was associated with
GA/HbA1c.

We excluded patients with
macroalbuminuria in this study since
advanced diabetic nephropathy can
influence hemoglobin concentration.
MacIsaac RJ et al. (18) demonstrated that
patients with type 2 diabetes can progress
to a significant degree of renal impairment
while remaining normoalbuminuric.
Certainly, estimated glomerular filtration
rate (eGFR) were below 60 ml/min per
1.73 m\(^2\) in 42 of 222 patients in this study.
However, a significant correlation
between serum bioavailable testosterone
and hemoglobin concentrations (r=0.343,
P<0.0001), or between serum bioavailable
testosterone concentration and GA/HbA1c
(r=−0.292, P<0.0001) persisted even after
excluding patients with eGFR below 60
ml/min per 1.73 m\(^2\).

Limitation of our study include that we
cannot have control groups of non-
diabetic men with and without
hypogonadism and /or hypogonadal men
before and after testosterone replacement
to see whether GA/HbA1c is different,
and varies in these populations.

To our knowledge, this is the first study
to investigate the relationship between
serum bioavailable testosterone
concentration and GA/HbA1c as well as
the relationship between serum
bioavailable testosterone and hemoglobin
concentrations in men with type 2
diabetes. The results of this study have
raised new issues concerning the pathophysiologic characteristics of anemia in male diabetic patients with hypogonadism. The mechanism through which testosterone stimulates erythropoiesis is unclear. Testosterone enhances proliferation of erythroid burst-forming units and colony-forming units by stimulating specific nuclear receptors (19). Medras et al. (20) demonstrated the significant decrease of aldolase and pyruvate kinase activity in erythrocytes in men with hypogonadism, which may decrease the lifespan of erythrocyte. Moreover, Solomon et al. (21) reported that androgen therapy prolongs red cell survival as well as increases red cell production. We suggest that low serum bioavailable testosterone concentration could be an underrecognized contributor to anemia, while a low serum bioavailable testosterone concentration could affect GA/HbA1c, and thus cause spuriously low HbA1c values. Our findings should be confirmed in larger populations and in other clinical settings before they can be considered for clinical applications.

In conclusion, serum bioavailable testosterone concentration correlated positively with hemoglobin concentration and negatively with GA/HbA1c in men with type 2 diabetes, which may lead to underestimation of HbA1c in hypogonadal men with type 2 diabetes, although we cannot clarify if these findings are applicable to non-diabetic men in this study.
REFERENCES

Tchernof A: Contribution of age and declining androgen levels to features of the metabolic syndrome in men. *Metabolism* 54: 1034-1040, 2005


### TABLE 1. Clinical characteristics of patients with diabetes

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>222</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.5±10.5</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>50.5±12.0</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>13.1±11.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3±3.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.7±8.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.1±1.1</td>
</tr>
<tr>
<td>GA (%)</td>
<td>20.8±4.3</td>
</tr>
<tr>
<td>GA/HbA1c</td>
<td>2.94±0.38</td>
</tr>
<tr>
<td>Hemoglobin (mg/dl)</td>
<td>14.5±1.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>133±15</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>77±10</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.99±0.78</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.98±1.24</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.34±0.39</td>
</tr>
<tr>
<td>Smoking (none/past/current)</td>
<td>44/113/65</td>
</tr>
<tr>
<td>Retinopathy (NDR/SDR/PDR)</td>
<td>174/29/19</td>
</tr>
<tr>
<td>Nephropathy (normo-/microalbuminuria)</td>
<td>149/73</td>
</tr>
<tr>
<td>Cardiovascular disease (-/+</td>
<td>186/36</td>
</tr>
<tr>
<td>Diabetic treatment (Diet/OHA/Insulin)</td>
<td>26/139/57</td>
</tr>
<tr>
<td>Hypertensive treatment (CCB/ARB and/or ACE-I)</td>
<td>62/78</td>
</tr>
<tr>
<td>Hyperlipidemic treatment (statin/fibrate)</td>
<td>48/4</td>
</tr>
<tr>
<td>Antiplatelet agent (-/+</td>
<td>176/46</td>
</tr>
<tr>
<td>Bioavailable testosterone [pg/ml (nmol/l)]</td>
<td>785±365 (2.73±1.27)</td>
</tr>
</tbody>
</table>

GA, glycated albumin; NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; OHA, oral hypoglycemic agents; CCB, calcium channel blocker; ARB, angiotensin II receptor blocker; ACE-I, angiotensin converting enzyme inhibitor.
**TABLE 2. Correlation between GA/HbA1c and other variables.**

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.278</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at onset</td>
<td>0.038</td>
<td>0.5892</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>0.200</td>
<td>0.0037</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.268</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>-0.231</td>
<td>0.0018</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>-0.176</td>
<td>0.0093</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>-0.233</td>
<td>0.0005</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.255</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log (triglyceride)</td>
<td>-0.394</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.150</td>
<td>0.0258</td>
</tr>
</tbody>
</table>

GA, glycated albumin
**TABLE 3. Multiple regression analysis on hemoglobin concentration or GA/HbA1c**

<table>
<thead>
<tr>
<th></th>
<th>Hemoglobin</th>
<th></th>
<th>GA/HbA1c</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\beta)</td>
<td>(P)</td>
<td>(\beta)</td>
<td>(P)</td>
</tr>
<tr>
<td>Bioavailable testosterone</td>
<td>0.187</td>
<td>0.0062</td>
<td>-0.155</td>
<td>0.0381</td>
</tr>
<tr>
<td>Age</td>
<td>-0.204</td>
<td>0.0075</td>
<td>0.088</td>
<td>0.2937</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>-0.108</td>
<td>0.1081</td>
<td>0.090</td>
<td>0.2230</td>
</tr>
<tr>
<td>BMI</td>
<td>0.151</td>
<td>0.0302</td>
<td>-0.129</td>
<td>0.0914</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.173</td>
<td>0.0090</td>
<td>-0.094</td>
<td>0.1965</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.155</td>
<td>0.0141</td>
<td>-0.170</td>
<td>0.0144</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.056</td>
<td>0.3692</td>
<td>-0.055</td>
<td>0.4187</td>
</tr>
</tbody>
</table>
FIGURE LEGEND

Figure 1. Correlations between serum bioavailable testosterone and hemoglobin concentrations (A), and between serum bioavailable testosterone concentration and the ratio of glycated albumin to glycated hemoglobin (GA/HbA1c)(B) in men with type 2 diabetes.
Testosterone and ratio of GA to HbA1c

FIGURE 1

A

\[ r = 0.368 \]
\[ P < 0.0001 \]

B

\[ r = -0.278 \]
\[ P < 0.0001 \]