Androgens and Glucuronidated Androgen Metabolites Are Associated with Metabolic Risk Factors in Men

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Context: Androgens are associated with metabolic risk factors in men. However, the independent impact of androgens and androgen metabolites on metabolic risk factors in men is unclear.

Objective: Our objective was to determine the predictive value of serum levels of androgens and glucuronidated androgen metabolites for metabolic risk factors.

Design and Study Subjects: We conducted a population-based study of two Swedish cohorts (1068 young adult and 1001 elderly men).

Main Outcome Measures: We measured correlation of serum dihydrotestosterone (DHT), testosterone (T), and glucuronidated androgen metabolites with fat mass, fat distribution, serum lipids, and insulin resistance.

Results: Both DHT and T were negatively associated with different measures of fat mass in both cohorts ($P < 0.001$). Further statistical analysis indicated that DHT, but not T, was independently negatively associated with different measures of fat mass and insulin resistance ($P < 0.001$). The glucuronidated androgen metabolite androstane-$3\alpha,17\beta$-diol-17glucuronide (17G) was independently positively associated with fat mass ($P < 0.001$). Most importantly, the 17G to DHT ratio was strongly correlated, not only with fat mass but also with central fat distribution, intrahepatic fat, disturbed lipid profile, insulin resistance, and diabetes, explaining a substantial part of the total variance in total body fat (12% in young adult men, 15% in elderly men), the homeostasis model assessment index (10%), and high-density lipoprotein cholesterol (7%).

Conclusion: Our findings demonstrate that 17-glucuronidation of the DHT metabolite androstane-$3\alpha,17\beta$-diol is strongly associated with several metabolic risk factors in men. Future longitudinal studies are required to determine the possible impact of the 17G to DHT ratio as a metabolic risk factor in men. (*J Clin Endocrinol Metab* 92: 4130–4137, 2007)

S ERUM LEVELS OF testosterone (T) are inversely associated with several metabolic risk factors in men (1). Most previous studies have found that serum T and the more potent androgen dihydrotestosterone (DHT) are negatively associated with body mass index (BMI) and fat mass in men (2–10). However, the independent impact of DHT and T as predictors of metabolic risk factors in men is unclear.

Conjugation of sex steroids with glucuronic acid has been suggested to play a major role in the regulation of the intracellular levels of unconjugated steroids as well as their biological activities in tissues (11–13). Circulating levels of androstane-$3\alpha,17\beta$-diol (3α-diol) glucuronide and androsterone glucuronide (ADTG), the two major 5α-reduced androgen metabolites in men, originate from both the testicular androgen T and the adrenal C_{19} steroids (Fig. 1) (13, 14). Two previous studies, including a rather low number of subjects (n = 80 and 110, respectively), presented conflicting results regarding the association of 3α-diol-glucuronides with fat mass in men (7, 8). 3α-Diol can be glucuronidated at either the 3α-hydroxy position (-3G) or at the 17β-hydroxy position (-17G). In previous studies, serum levels of 3α-diol-glucuronides were analyzed by the RIA technique, which could not separate 3G from 17G. We recently developed a liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method to determine 3α-diol-glucuronides and ADTG (15). Using this technique, we can separately analyze 3G and 17G. Interestingly, the enzymes responsible for glucuronidation of the androgen metabolites, the uridine diphosphate-glu-

TESTOSTERONE

DHT

ADT   **3α-DIOL**

Glucuronidated androgen metabolites
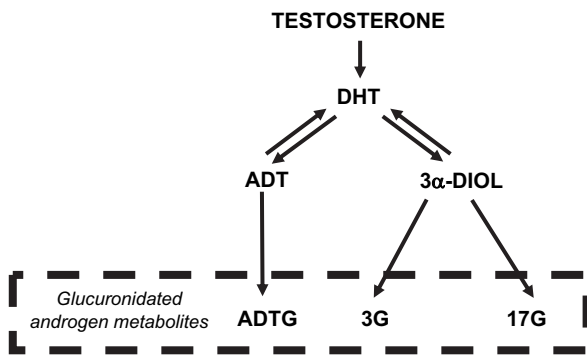
ADTG   **3G**   **17G**

Fig. 1. Metabolism of T and DHT into glucuronidated androgen metabolites. ADT, Androsterone.

curonosyltransferases (UGTs), are believed to display a tissue-specific expression pattern and are suggested to have different specificities for the glucuronidation of the 3 *vs.* 17 position of 3α-diol: UGT2B7 conjugates 3α-diol at the 3 position, whereas both UGT2B15 and UGT2B17 are specific for the 17 position (16). In addition, UGT2B15 is the unique androgen-conjugating enzyme expressed in the adipose tissues, whereas in the prostate, both UGT2B15 and UGT2B17 have been found. The separation of serum 3G from 17G might thus raise the possibility to obtain indirectly an estimate of tissue-specific glucuronidation of 3α-diol.

The aim of the present study was to determine the predictive value of serum levels of androgens and glucuronidated androgen metabolites for metabolic risk factors in men.

Subjects and Methods

Study subjects: young adult men

The Gothenburg Osteoporosis and Obesity Determinants (GOOD; 18.9 ± 0.5 yr of age, n = 1068) study was initiated to determine both environmental and genetic factors involved in the regulation of bone and fat mass (Table 1) (17). Study subjects were randomly identified using national population registers, contacted by telephone, and asked to participate. Subjects had to be older than 18 and younger than 20 yr of age and willing to participate in the study. There were no other exclusion criteria.

Study subjects: elderly men

Subjects from the Gothenburg part of The Osteoporotic Fractures in Men Study (MrOS) Sweden (men aged 69–81 yr, n = 1001) were randomly identified using national population registers, contacted, and asked to participate (Table 1) (18). Information about prevalent diabetes mellitus was obtained through questionnaires. To be eligible for the study, the subjects had to be able to walk without aids. There were no other exclusion criteria. Informed consent was obtained from all study participants in both MrOS and GOOD.

Dual x-ray absorptiometry (DXA)

Fat mass and lean mass were assessed using the Lunar Prodigy DXA (young adult men; GE Lunar Corp., Madison, WI) or Hologic QDR 4500/A-Delphi (elderly men; Hologic, Waltham, MA).

Peripheral computer tomography (CT) analyses in the distal arm and leg (GOOD)

A peripheral CT device (XCT-2000; Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to scan the distal leg and distal arm of the nondominant leg and arm, respectively. The sc cross-sectional ad-

ipose tissue area was measured using a scan through the diaphysis of the radius and tibia.

Abdominal CT analyses (GOOD)

A CT technique was used to measure the cross-sectional adipose areas of the abdomen (n = 343). Adipose tissue areas were determined with the subject in a recumbent position with a General Electric High-Speed Advantage CT system, version RP2 (GE Medical Systems, Milwaukee, WI) (19, 20). Adipose tissue areas were measured using one scan at the fourth lumbar vertebra level.

Assessment of sex hormones in serum

Serum levels of T, DHT, 3G, 17G, and ADTG were analyzed as previously described (15, 21). Briefly, the validated gas chromatography-mass spectrometry (GC-MS) system was used for the analysis of T [limit of detection 0.05 ng/ml, interassay coefficient of variation (CV) 3.4%] and DHT (limit of detection 0.01 ng/ml, interassay CV 4.1%). The GC-MS uses a 50% phenyl-methylpolysiloxane capillary column with helium as carrier gas. The analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. In addition to the mass detected for the analyte, the retention time of the analyte, which corresponds to the retention time and mass of the synthetic standard, ensures the specificity of the method. Free T was calculated as previously described, taking the concentrations of total T and SHBG into account and assuming a fixed albumin concentration of 43 g/liter (22, 23). ADTG (limit of detection 2.00 ng/ml, interassay CV 3.7%), 3G (limit of detection 0.50 ng/ml, interassay CV 10.7%), and 17G (limit of detection 0.50 ng/ml, interassay CV 5.3%) were analyzed by a validated LC-MS/MS method. The system uses a 4-mm particle size Synergy Hydro-RP column at a flow rate of 1.0 ml/min and the analytes are detected using a Sciex API3000 triple quadrupole mass spectrometer equipped with TurboIonSpray. The retention times of ADTG, 3G, and 17G correspond to those of the synthetic standards and the multiple reaction monitoring used to detect the analytes further ensure the specificity of the method. The highly specific GC-MS and LC-MS/MS techniques avoid the cross-reactivity seen using immunoassays and can reliably identify androgens and androgen metabolites.

Serum/plasma levels of leptin, insulin, glucose, and lipids

Leptin was analyzed in serum samples using a commercially available kit (Diagnostic Systems Laboratories Inc., Webster, TX; interassay CV 5.3%). Fasting serum insulin was measured with an immunometric method based on chemiluminescence technology on a ADVIA Centaur (Bayer AB, Solna, Sweden; interassay CV < 10%). Fasting plasma glucose was quantitated by an enzymatic method on a Modular (Roche, Gothenburg, Sweden; interassay CV < 4%). The homeostasis model assessment (HOMA) index was calculated as the product of the fasting serum insulin level (microunits per milliliter) and the fasting plasma glucose level (millimoles per liter), divided by 22.5. Serum lipid analysis was performed on a Konelab 20 autoanalyzer (Thermo Electron Corp., Vantaa, Finland). Total cholesterol and triglyceride levels were determined in human fasting serum by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein (Apo) B-containing lipoproteins with magnesium sulfate and dextran sulfate. Low-density lipoprotein (LDL) was calculated using Friedewald's formula. ApoB and ApoA1 were determined by immunoprecipitation enhanced by polyethylene glycol at 340 nm. Interassay CVs were less than 5% for all Konelab analyses.

Statistical analysis

Associations among variables were examined with Pearson's correlation. The 17G to DHT ratio was computed using the nontransformed values of both 17G and DHT, followed by transformation of the ratio when used in association analyses. The independent predictive value of DHT and 17G for parameters reflecting fat mass, insulin resistance, and serum lipids was calculated using linear regression analyses including age, DHT, and 17G as covariates. Standardized β-values are given. Odds ratios (ORs) were calculated using logistic regression and used to determine the predictive value of the 17G to DHT ratio (per sD) for over-

TABLE 1. Anthropometrics, body composition, and serum/plasma parameters in young adult and elderly men

Variable	Young adult (n = 1068)		Elderly (n = 1001)	
	Means ± SD	Median (IQR)	Means ± SD	Median (IQR)
Age (yr)	18.9 ± 0.5	18.9 (18.4–19.3)	75.3 ± 3.2[a]	75.3 (72.9–78.6)
Height (cm)	181.4 ± 6.7	181.5 (176.8–186.0)	175.7 ± 6.4[a]	175.4 (171.6–180.0)
Weight (kg)	73.8 ± 11.9	71.9 (66.0–79.5)	80.9 ± 12.2[a]	80.2 (72.1–88.4)
BMI (kg/m^2)	22.4 ± 3.2	21.9 (20.3–23.8)	26.2 ± 3.5[a]	25.9 (23.8–28.3)
Overweight (%)	16.1		60.6[a]	
Obese (%)	3.6		12.5[a]	
Diabetes (%)	ND		11.1	
Serum/plasma parameters				
Leptin (ng/ml)	7.7 ± 8.5	5.2 (3.3–8.7)	22.6 ± 21.0[a]	15.9 (9.4–28.0)
Insulin (μU/ml)	ND	ND	10.6 ± 11.2	7.9 (5.2–13.0)
Glucose (mmol/liter)	ND	ND	5.8 ± 1.5	5.4 (5.0–6.0)
HOMA index	ND	ND	2.9 ± 4.0	2.0 (1.2–3.3)
Total cholesterol (mmol/liter)	ND	ND	5.43 ± 1.05	5.42 (4.71–6.09)
Triglycerides (mmol/liter)	ND	ND	1.46 ± 0.78	1.26 (0.97–1.71)
HDL (mmol/liter)	ND	ND	1.26 ± 0.35	1.22 (1.00–1.47)
LDL (mmol/liter)	ND	ND	3.51 ± 0.96	3.48 (2.86–4.11)
ApoA1 (g/liter)	ND	ND	1.61 ± 0.32	1.57 (1.39–1.80)
ApoB (g/liter)	ND	ND	1.06 ± 0.24	1.06 (0.90–1.21)
ApoB/ApoA1	ND	ND	0.68 ± 0.18	0.66 (0.55–0.79)
T (ng/ml)	4.69 ± 1.52	4.55 (3.66–5.53)	4.43 ± 1.87[a]	4.31 (3.26–5.57)
Free T (ng/ml)	0.126 ± 0.041	0.122 (0.098–0.150)	0.077 ± 0.033[a]	0.075 (0.057–0.093)
DHT (ng/ml)	0.31 ± 0.11	0.29 (0.23–0.37)	0.35 ± 0.19[a]	0.33 (0.23–0.46)
ADTG (ng/ml)	61.2 ± 35.4	51.8 (40.6–70.2)	33.3 ± 67.1[a]	25.4 (17.0–36.8)
3G (ng/ml)	1.52 ± 0.92	1.32 (0.96–1.78)	1.50 ± 2.24	1.18 (0.79–1.69)
17G (ng/ml)	4.03 ± 2.08	3.63 (2.54–5.15)	2.64 ± 2.47[a]	2.22 (1.35–3.40)
17G to DHT ratio	14.4 ± 8.3	12.5 (8.6–18.2)	9.8 ± 12.9[a]	6.7 (4.1–11.4)
SHBG (nmol/liter)	20.4 ± 7.4	19.7 (15.0–24.6)	47.3 ± 24.7[a]	42.7 (30.5–57.2)
DXA[b]				
Total body lean (kg)	57.4 ± 6.2	57.1 (53.1–61.5)	59.3 ± 6.7	59.1 (54.5–63.7)
Total body fat (kg)	13.4 ± 8.0	11.3 (8.0–16.0)	18.5 ± 5.7	18.0 (14.5–22.2)
Total body fat (%)	17.2 ± 7.4	15.8 (11.9–21.1)	23.4 ± 5.0	23.6 (20.0–26.6)
Arm fat (kg)	0.56 ± 0.41	0.44 (0.28–0.68)	1.06 ± 0.37	0.99 (0.81–1.26)
Leg fat (kg)	2.48 ± 1.43	2.10 (1.53–3.08)	2.87 ± 0.94	2.75 (2.21–3.41)
Trunk fat (kg)	6.76 ± 4.31	5.59 (3.89–8.15)	9.67 ± 3.56	9.53 (7.11–11.93)
Central fat distribution (%)	49.8 ± 4.5	49.8 (46.6–53.0)	51.3 ± 6.2	52.0 (47.4–55.6)
Peripheral sc fat (CT)				
Distal arm (cm^2)	7.0 ± 3.1	6.3 (4.9–8.2)	ND	ND
Distal leg (cm^2)	13.6 ± 5.0	12.8 (10.2–16.2)	ND	ND
Abdominal fat (CT) (n = 343)				
Subcutaneous fat abdomen (cm^2)	107 ± 87	80 (54–127)	ND	ND
Deep sc fat abdomen (cm^2)	50.0 ± 51.8	33.0 (18.1–60.1)	ND	ND
Intraabdominal fat (cm^2)	36.2 ± 18.6	32.7 (23.7–45.3)	ND	ND
Intraperitoneal fat (cm^2)	22.8 ± 12.3	20.5 (14.3–28.8)	ND	ND
Retroperitoneal fat (cm^2)	13.3 ± 8.1	11.4 (7.6–17.2)	ND	ND
Liver attenuation (HU)	59.4 ± 4.4	60.0 (58.0–62.0)	ND	ND

Values are given as means ± SD and median [interquartile range (IQR)]. HU, Hounsfield units; ND, not determined.

[a] $P < 0.05$ *vs.* young adult men; central fat distribution is calculated as trunk fat/total body fat × 100.

[b] The young adult and elderly men were investigated by different DXA equipment (young adult: Lunar Prodigy; GE Lunar Corp.; elderly: Hologic QDR 4500/A-Delphi, Hologic) and therefore, the fat and lean data cannot be directly compared between the young adult and elderly men.

weight, obesity, insulin resistance, and diabetes. For the analyses of the independent associations of T and DHT with fat parameters, DHT residuals and T residuals were calculated. DHT residuals were derived from linear regression analyses with DHT as dependent and T as independent parameter, whereas T residuals were derived from linear regression analyses with T as dependent and DHT as independent parameter.

Results

The general characteristics of the young adult (mean 19 yr of age) and elderly (mean 75 yr of age) cohorts are given in Table 1. The elderly cohort had higher body weight (+10%), BMI (+17%), and serum levels of leptin (+194%) than the young adult cohort (Table 1). Serum levels of ADTG (−46%),

17G (−34%), and free T (−39%) were clearly reduced, whereas serum levels of T (−6%) were slightly reduced in the elderly cohort, compared with the young adult cohort. In contrast, serum DHT levels were slightly higher in the elderly cohort (+13%).

Young adult men

The two bioactive androgens DHT and T were both negatively associated with body weight, BMI, serum leptin, and all measures of total body fat as well as region-specific fat deposits (Table 2). Linear regression analyses including both T and DHT indicated that DHT, but not T, was indepen-

TABLE 2. Correlation analyses of serum levels of bioactive androgens and glucuronidated androgen metabolites *vs.* parameters reflecting body composition and serum/plasma parameters in young adult men

	Bioactive androgens			Glucuronidated androgen metabolites			Ratio 17G/DHT
	T	Free T	DHT	ADTG	3G	17G	
Height	−0.03	−0.06[a]	−0.01	−0.01	−0.02	−0.02	−0.02
Weight	−0.17[a]	−0.10[a]	−0.21[a]	0.01	0.07[a]	0.11[a]	0.25[a]
BMI	−0.18[a]	−0.08[a]	−0.23[a]	0.01	0.09[a]	0.14[a]	0.29[a]
Leptin	−0.16[a]	−0.07[a]	−0.23[a]	0.04	0.12[a]	0.15[a]	0.29[a]
DXA							
Total body lean	−0.05	−0.05	−0.06[a]	−0.05	−0.03	−0.04	0.00
Total body fat	−0.20[a]	−0.09[a]	−0.23[a]	0.06[a]	0.13[a]	0.21[a]	0.35[a]
Total body fat (%)	−0.18[a]	−0.08[a]	−0.21[a]	0.07[a]	0.14[a]	0.22[a]	0.35[a]
Arm fat	−0.20[a]	−0.09[a]	−0.25[a]	0.06	0.12[a]	0.20[a]	0.36[a]
Leg fat	−0.17[a]	−0.08[a]	−0.22[a]	0.04	0.11[a]	0.18[a]	0.32[a]
Trunk fat	−0.20[a]	−0.09[a]	−0.23[a]	0.07[a]	0.14[a]	0.22[a]	0.36[a]
Central fat distribution	−0.11[a]	−0.04	−0.07[a]	0.08[a]	0.13[a]	0.16[a]	0.20[a]
Peripheral sc fat (CT)							
Distal arm	−0.17[a]	−0.07[a]	−0.26[a]	0.03	0.09[a]	0.16[a]	0.32[a]
Distal leg	−0.14[a]	−0.08[a]	−0.20[a]	−0.01	0.03	0.09[a]	0.22[a]
Abdominal fat (CT)							
Subcutaneous fat abdomen	−0.21[a]	−0.10	−0.25[a]	0.08	0.16[a]	0.19[a]	0.35[a]
Deep sc fat abdomen	−0.21[a]	−0.10	−0.25[a]	0.08	0.19[a]	0.19[a]	0.34[a]
Intraabdominal fat	−0.19[a]	−0.09	−0.26[a]	0.09	0.18[a]	0.21[a]	0.37[a]
Intraperitoneal fat	−0.17[a]	−0.09	−0.22[a]	0.05	0.15[a]	0.19[a]	0.32[a]
Retroperitoneal fat	−0.18[a]	−0.07	−0.23[a]	0.14[a]	0.19[a]	0.19[a]	0.33[a]
Liver attenuation	0.11[a]	0.05	0.12[a]	−0.04	−0.07	−0.15[a]	−0.22[a]

Pearson's correlation coefficients are shown in young adult men (n = 1068). All variables, except height and total body lean mass, are log transformed. Central fat distribution is calculated as trunk fat/total body fat × 100.

[a] *P* < 0.05.

dently associated with the different measures of fat mass (*e.g.* total body fat DHT β = −0.20, *P* < 0.001; T β = −0.04, *P* = 0.44; and data not shown). Because DHT was highly correlated to T (r = 0.81, *P* < 0.0001) and it is difficult to evaluate the independent associations of two parameters that are highly correlated, additional statistical analyses were performed to evaluate the possible independent association of DHT with fat mass. For both BMI and total body fat, the DHT residuals were significant predictors in linear regression models including both DHT residuals and T (*P* < 0.001). In contrast, T residuals were not significant predictors of these parameters when included in linear regression models together with DHT, supporting the notion that DHT, but not T, is independently associated with fat parameters. Liver attenuation, which is inversely associated with the amount of fat in the liver, was positively associated with both T and DHT. Neither T nor the glucuronidated androgen metabolites were associated with height or lean mass (Table 2).

The glucuronidated androgen metabolite 17G was strongly associated with most fat deposits as well as with central fat distribution (Table 2). Inclusion of the two different forms of 3α-diol glucuronides as covariates in the same linear regression model demonstrated that 17G, but not 3G, was independently associated with these different measures of fat mass and distribution (*e.g.* total body fat 17G β = 0.19, *P* < 0.001; 3G β = 0.03, *P* = 0.31; and data not shown). 17G was moderately correlated with liver attenuation (Table 2).

Elderly men

Similarly as seen in the young adult men, serum levels of both DHT and T were negatively associated with body weight, BMI, serum leptin, and total and regional fat deposits (Table 3). Again, linear regression analyses including both T

and DHT indicated that DHT, but not T, was independently associated with the different measures of fat mass (*e.g.* total body fat DHT β = −0.21, *P* < 0.001; T β = −0.03, *P* = 0.57; and data not shown). DHT in the elderly men was also highly correlated to T (r = 0.80, *P* < 0.0001). For both BMI and total body fat, the DHT residuals were significant predictors in linear regression models including both DHT residuals and T (*P* < 0.001). In contrast, T residuals were not significant predictors of these parameters when included in linear regression models together with DHT, supporting the notion that DHT, but not T, is independently associated with fat parameters.

A significant positive association was found between 17G and body weight, BMI, serum leptin, most fat mass deposits, and central fat distribution in the elderly men (Table 3). Inclusion of 3G and 17G in the same linear regression model demonstrated that 17G, but not 3G, was independently associated with all the different measures of fat mass and central fat distribution (*e.g.* total body fat 17G β = 0.13, *P* = 0.001; 3G β = −0.01, *P* = 0.78; and data not shown).

Analyses of fasting serum demonstrated that DHT and T were negatively correlated and 17G was positively correlated with serum insulin and the HOMA index (Table 3). Linear regression analyses including both T and DHT indicated that DHT, but not T, was independently associated with the HOMA index (DHT β = −0.13, *P* = 0.038; T β = −0.03, *P* = 0.60), and this was supported by the fact that the DHT residuals were significant predictors in linear regression models including both DHT residuals and T (*P* = 0.004), whereas T residuals were not significant predictors of the HOMA index when included in models together with DHT. Serum lipid measurements demonstrated that 17G was strongly negatively associated with HDL cholesterol and ApoA1 and

positively with triglycerides (Table 3). Inclusion of ADTG, 3G, and 17G as covariates in the same linear regression model showed an independent association between 17G, but not 3G or ADTG, and these different lipid measurements (*e.g.* HDL 17G $\beta = -0.21$, $P < 0.001$; 3G $\beta = -0.06$, $P = 0.20$; ADTG $\beta = 0.06$, $P = 0.25$; and data not shown).

The 17G to DHT ratio is associated with fat mass and metabolic risk factors

Univariate correlation analyses demonstrated that DHT explained 5.4 and 5.6% of the total variance in total body fat in young adult and elderly men, respectively, whereas 4.4 and 1.5% of the variance was explained by 17G in young adult and elderly men, respectively (Tables 2 and 3). When both DHT and 17G were included in the same linear regression model, a substantial part of the variance in total body fat (14% in both young adult and elderly men) was explained by the model, accounting for a larger part of the total variance than the sum of what DHT and 17G accounted for when evaluated separately. For BMI, DHT explained 4.4 and 4.0% of the total variance in young adult and elderly men, respectively, whereas 2.0 and 2.6% of the variance was explained by 17G in young adult and elderly men, respectively (Tables 2 and 3). When both DHT and 17G were included in the same linear regression model, 10.1 and 14.0% of the variance in BMI in young adult and elderly men, respectively, was explained, again accounting for a larger part of the total variance than the sum of what DHT and 17G accounted for when evaluated separately. The regression analyses, including both DHT and 17G, demonstrated that DHT was independently and negatively associated with body weight, BMI, all the different fat deposits, central fat distribution, and intrahepatic fat, whereas 17G was independently and positively associated with all these parameters in both young adult (BMI DHT $\beta = -0.30$, $P < 0.001$; 17G $\beta = 0.23$, $P < 0.001$; total body fat DHT $\beta = -0.32$, $P < 0.001$; 17G $\beta = 0.30$, $P < 0.001$; and data not shown) and elderly men (BMI DHT $\beta = -0.41$, $P < 0.001$; 17G $\beta = 0.37$, $P < 0.001$; total body fat DHT $\beta = -0.42$, $P < 0.001$; 17G $\beta = 0.34$, $P < 0.001$; and data not shown).

Because DHT was negatively and 17G was positively associated with fat mass and 17G is the 17-glucuronidated form of the DHT metabolite 3α-diol (Fig. 1), we next investigated the predictive role of the ratio between 17G and DHT for different fat parameters. The 17G to DHT ratio was rather strongly positively correlated with all fat deposits investigated (r = 0.22–0.42, Tables 2 and 3, and Fig. 2A), explaining a substantial part of the variance in total body fat (12% in the young adult men, 15% in the elderly men). Interestingly, the 17G to DHT ratio was also significantly associated with a central fat distribution (Tables 2 and 3), explaining 4 and 12% of the variance in young adult and elderly men, respectively. In addition, the 17G to DHT ratio was positively associated with intrahepatic fat as indicated by a negative association between the 17G to DHT ratio and liver attenuation (Table 2), and it explained 4.8% of the variance in liver attenuation.

Importantly, the 17G to DHT ratio was rather strongly associated with the HOMA index, explaining as much as 10% of the variance in this parameter (Table 3 and Fig. 2B). The 17G to DHT ratio remained independently correlated with the HOMA index after adjustment for age and percent total body fat ($\beta = 0.18$, $P < 0.001$).

TABLE 3. Correlation analyses of serum levels of bioactive androgens and glucuronidated androgen metabolites *vs.* parameters reflecting body composition and serum/plasma parameters in elderly men

	Bioactive androgens			Glucuronidated androgen metabolites			Ratio 17G to DHT
	T	Free T	DHT	ADTG	3G	17G	
Height	0.00	0.02	−0.01	0.00	0.02	−0.01	0.00
Weight	−0.13[a]	−0.05	−0.18[a]	0.04	0.09[a]	0.13[a]	0.35[a]
BMI	−0.15[a]	−0.07[a]	−0.20[a]	0.04	0.09[a]	0.16[a]	0.39[a]
DXA							
Total body lean	−0.00	0.06	−0.06	0.06	0.10[a]	0.13[a]	0.21[a]
Total body fat	−0.21[a]	−0.15[a]	−0.23[a]	0.02	0.05	0.12[a]	0.39[a]
Total body fat %	−0.23[a]	−0.20[a]	−0.24[a]	0.00	0.02	0.09[a]	0.35[a]
Arm fat	−0.22[a]	−0.17[a]	−0.24[a]	−0.01	0.01	0.07[a]	0.34[a]
Leg fat	−0.20[a]	−0.19[a]	−0.23[a]	−0.05	−0.03	−0.01	0.24[a]
Trunk fat	−0.18[a]	−0.11[a]	−0.20[a]	0.06	0.10[a]	0.18[a]	0.42[a]
Central fat distribution	−0.04	0.05	−0.05	0.14[a]	0.18[a]	0.27[a]	0.34[a]
Serum/plasma parameters							
Leptin	−0.23[a]	−0.16[a]	−0.25[a]	−0.00	0.04	0.06	0.35[a]
Insulin	−0.15[a]	−0.09[a]	−0.16[a]	0.03	0.02	0.11[a]	0.30[a]
Glucose	−0.04	−0.00	−0.08[a]	0.04	0.06	0.10[a]	0.20[a]
HOMA index	−0.14[a]	−0.09[a]	−0.16[a]	0.03	0.03	0.12[a]	0.32[a]
Total cholesterol	0.03	0.00	0.07[a]	0.06[a]	0.07[a]	0.04	−0.03
Triglycerides	−0.10[a]	−0.06	−0.10[a]	0.09[a]	0.15[a]	0.17[a]	0.29[a]
HDL	0.04	−0.04	0.05	−0.12[a]	−0.12[a]	−0.20[a]	−0.27[a]
LDL	0.04	0.03	0.08[a]	0.09[a]	0.08[a]	0.06[a]	−0.03
ApoA1	0.04	−0.03	0.07[a]	−0.07[a]	−0.05	−0.11[a]	−0.20[a]
ApoB	0.01	0.00	0.04	0.08[a]	0.07[a]	0.08[a]	0.03
ApoB/ApoA1	−0.02	0.02	−0.01	0.11[a]	0.10[a]	0.14[a]	0.16[a]

Pearson's correlation coefficients are shown in elderly men (n = 1001). All variables, except height and total body lean mass, are log transformed. Subjects with known diabetes mellitus were excluded from the correlation analyses regarding insulin, glucose, and HOMA index. Central fat distribution is calculated as trunk fat/total body fat × 100.
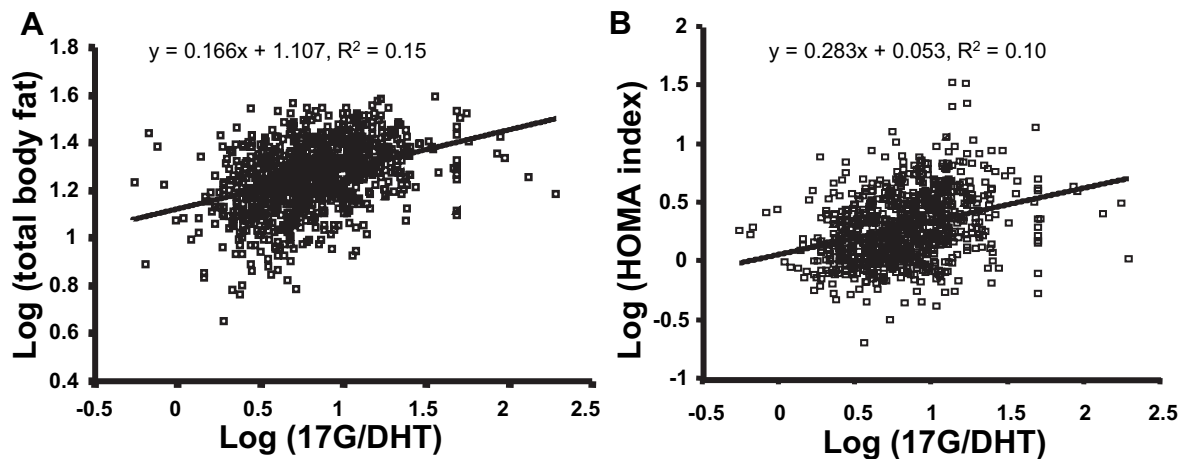
[a] $P < 0.05$.

FIG. 2. The 17G to DHT ratio is a positive predictor of total body fat and the HOMA index in men. Scatter plots of the associations between the 17G to DHT ratio and total body fat as determined by DXA (A) and the HOMA index in elderly men (B) are displayed. R^2 for the linear correlations are given in the figure ($P < 0.001$ for both total body fat and the HOMA index). Subjects with known diabetes mellitus were excluded from the correlation analyses regarding the HOMA index.

In the elderly men, the 17G to DHT ratio was positively associated with serum triglycerides and negatively with HDL cholesterol and ApoA1 (Table 3), explaining 7.3% of the variance in HDL cholesterol.

To quantify further the impact of the 17G to DHT ratio on overweight, obesity, and insulin resistance, ORs were computed by logistic regression analyses, demonstrating that the 17G to DHT ratio was strongly and independently associated with overweight and obesity in both the young adult and the elderly men (OR 2.06–4.45 per SD increase, Table 4). Furthermore, in elderly men, the 17G to DHT ratio was positively associated with insulin resistance both unadjusted and adjusted for BMI (Table 4) or percent body fat (data not shown). Finally, the impact of the 17G to DHT ratio for prevalent diabetes was investigated, demonstrating that it was associated with diabetes ($P < 0.001$, Table 5).

Discussion

It is known that T is negatively associated with BMI and fat mass in men (2–9). Although it is difficult to evaluate the independent associations of the two highly correlated parameters DHT and T with fat parameters, the statistical analyses in the present study, including two large cohorts, in-

dicate that DHT, but not T, is independently associated with fat mass. We also show that the glucuronidated androgen metabolite 17G is specifically and positively correlated with fat mass, whereas 3G and ADTG have no predictive value. Most importantly, we demonstrate that the 17G to DHT ratio is rather strongly associated with not only fat mass but also several other metabolic risk factors in men.

T and the more potent androgen DHT are the two bioactive androgens in men. In correlation analyses, both serum T and serum DHT were clear negative correlates of BMI and several different measures of fat mass in both young adult and elderly men. The negative association between T and fat mass confirms several previous studies (2–9), and a negative association between serum DHT and fat mass has previously been reported in a study by Couillard *et al.* (10). Our additional statistical analyses indicated that DHT, but not T, was independently associated with fat parameters and insulin resistance.

Because UGTs and steroid aldoketoreductases such as 3α-hydroxysteroid dehydrogenase, involved in the inactivation of androgens, are expressed in adipose tissue (24–26), one may speculate that DHT is metabolized to glucuronidated androgen metabolites in adipose tissue and that these diffuse into the general circulation in which they can be measured (11, 14, 27). Because the UGT2B15 enzyme is highly and

TABLE 4. The impact of the 17G to DHT ratio on overweight, obesity, and insulin resistance

	Young adults, 17G to DHT (per SD)	Elderly, 17G to DHT (per SD)
Overweight OR	2.24 (1.84–2.73)[a]	2.06 (1.76–2.41)[a]
Obesity OR	4.45 (2.87–6.92)[a]	2.09 (1.72–2.54)[a]
IR OR	ND	1.96 (1.65–2.31)[a]
IR OR, BMI adjusted	ND	1.54 (1.30–1.84)[a]

Logistic regression analysis. The 95% confidence interval is shown between *parentheses*. Subjects with known diabetes mellitus were excluded from the logistic regression analyses regarding insulin resistance. The ORs are adjusted for age. Overweight is BMI greater than 25 kg/m²; obesity is BMI greater than 30 kg/m². IR, Insulin resistance defined as HOMA index greater than 2.5; ND, not determined.
[a] $P < 0.001$.

TABLE 5. The impact of androgens and glucuronidated androgen metabolites on prevalent diabetes

	Crude	BMI adjusted
T (per SD)	0.85 (0.72–1.00)	0.88 (0.74–1.05)
DHT (per SD)	0.82 (0.69–0.97)[a]	0.86 (0.72–1.02)
ADTG (per SD)	1.07 (0.87–1.31)	1.06 (0.87–1.30)
3G (per SD)	1.19 (0.97–1.44)	1.16 (0.95–1.42)
17G (per SD)	1.23 (0.99–1.52)	1.17 (0.95–1.44)
17G to DHT ratio (per SD)	1.56 (1.29–1.89)[b]	1.48 (1.20–1.82)[b]

Logistic regression analysis. The 95% confidence interval is shown between *parentheses*. Crude means adjusted for age; BMI adjusted means adjusted for age and BMI.
[a] $P < 0.05$.
[b] $P < 0.001$.

rather tissue-specifically expressed in adipose tissue (26) and described to mainly glucuronidate the 17- but not the 3-hydroxy position of 3α-diol *in vitro* (16), one may speculate that serum 17G, but not 3G, is dependent on glucuronidation of 3α-diol in adipose tissue. This notion is supported by the present finding that serum 17G, but not 3G, was independently positively associated with fat mass. Although the present cross-sectional association study was not designed to address the issue of causality, one may therefore speculate that adipose tissue is mainly involved in 17-glucuronidation of 3α-diol, whereas the 3-glucuronidation of 3α-diol is mainly dependent on glucuronidation in other tissues.

Because DHT was independently negatively and 17G was independently positively associated with fat mass and 17G is the 17-glucuronidated form of the DHT metabolite 3α-diol (Fig. 1), the predictive role of the ratio between 17G and DHT for different fat parameters was investigated. This ratio could be regarded as a parameter reflecting the total activity of enzymes involved in the transformation of DHT into 3α-diol and 17-glucuronidation of 3α-diol. In the present study, we found a rather strong positive association between the 17G to DHT ratio and all measures of fat mass, explaining a substantial part of the total variance in several metabolic risk factors, including total body fat, central fat distribution, intrahepatic fat, insulin resistance, and HDL cholesterol in men.

There are two primary hypotheses suggested from this strong positive relationship between the 17G to DHT ratio and the amount of fat mass. First, the amount of fat might be an important determinant of the 17-glucuronidation of 3α-diol, which is an irreversible step in DHT metabolism/inactivation (Fig. 1). Thus, an increase in the amount of fat would result in an increased total capacity for 17-glucuronidation of 3α-diol, which in turn would result in increased levels of 17G and decreased levels of DHT in serum. If this notion is valid, then a substantial part of 17-glucuronidation of 3α-diol is located in adipose tissue. Second, it is possible that androgens/androgen metabolites are directly involved in the regulation of the amount of fat. However, our cross-sectional cohort studies cannot determine the causality of the strong association between the 17G to DHT ratio and fat mass in men.

Results of the present study suggest that the higher glucuronide concentrations in the plasma of obese men reflect increased androgen processing in adipose tissue. In this regard, the presence of large amounts of fat tissue would slightly shift the equilibrium between androgen synthesis and inactivation, leading to reduced circulating levels of the active androgens and higher levels of the specific metabolite 17G.

In elderly men, the 17G to DHT ratio was strongly associated with not only fat mass but also, in a BMI-independent manner, insulin resistance and prevalent diabetes. HDL cholesterol and ApoA1 are established metabolic risk factors for men (28). In the present study, the 17G to DHT ratio was rather strongly negatively associated with both HDL cholesterol and ApoA1, explaining as much as 7.3% of the variance in HDL cholesterol. Further mechanistic studies are required to determine the potential causality of the BMI-

independent associations between the 17G to DHT ratio and both insulin resistance and HDL cholesterol.

The lack of association between T or free T and lean mass, in the present cross-sectional association study, is contradicting some previous studies (29, 30) but is supported by the recent findings from a large subgroup (n = 2486) of the MrOS U.S. study, demonstrating no association between bioavailable T and lean mass in elderly men (31). A limitation of the present study is that we did not explore the possible influence of other covariates such as physical activity and health status.

In conclusion, the present study demonstrates that DHT is negatively and the specific glucuronidated androgen metabolite 17G is positively associated with fat mass, central fat distribution, and insulin resistance in men. Most importantly, the 17G to DHT ratio was rather strongly correlated with fat mass, central fat distribution, intrahepatic fat, disturbed lipid profile, insulin resistance, and diabetes. These findings demonstrate that specifically the formation of the DHT metabolite 3α-diol and the 17-glucuronidation of 3α-diol are strongly associated with several metabolic risk factors in men. Future longitudinal studies are required to determine the possible impact of the 17G to DHT ratio as a metabolic risk factor for diabetes and cardiovascular events in men.

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