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Hyperandrogenemia is Common in Asymptomatic Women and is Associated with Increased Metabolic Risk

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Abstract

Objective—Women with metabolic syndrome (MetS) have higher endogenous testosterone (T) levels than unaffected women. We investigated whether hyperandrogenemia (HA) was a marker for increased cardiometabolic risk in reproductively normal premenopausal women.

Methods—We assessed reproductive hormones and metabolic parameters in 198 women with regular menses and no clinical hyperandrogenism (eumenorrheic, EM). HA-EM women were compared to 110 NIH criteria PCOS women.

Results—Twenty-two percent of EM had HA. Non-SHBG bound T was elevated in 68%, followed by elevated total T levels, 43%, and DHEAS levels, 30%. The prevalence of HA increased with BMI category ($P=0.01$): 12% $<25 \text{ kg/m}^2$, 22% $25\text{--}30 \text{ kg/m}^2$, and 31% $\geq 30 \text{ kg/m}^2$. MetS (adjusted odds ratio (AOR) 2.9, CI 1.2–6.9) and dysglycemia risk (AOR 2.7, CI 1.2–5.8) were increased in HA-EM compared to normoandrogenic-EM, adjusted for BMI. SHBG levels were independently associated with these metabolic endpoints ($P<0.001$), whereas androgen levels were not. Cluster analysis confirmed that there was a discrete subset of EM women with HA and metabolic abnormalities.

Conclusions—HA was common in EM women and was associated with increased risk for MetS and dysglycemia. However, low SHBG rather than elevated androgen levels may be the primary predictor of this relationship with metabolic dysfunction.

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Keywords

androgens; polycystic ovary syndrome; obesity; metabolic syndrome

Introduction

Epidemiologic studies in both pre- and postmenopausal women have found higher endogenous testosterone (T) levels in women with metabolic syndrome (MetS) (1, 2, 3). It is well established that polycystic ovary syndrome (PCOS) is associated with MetS (4). However, it is unclear whether unrecognized PCOS accounts for the association between hyperandrogenemia (HA) and MetS in epidemiologic studies. HA is also a common finding in peripubertal girls with obesity, with prevalence rates as high as 60% (5). It has been hypothesized that HA in these girls with obesity is an early marker for PCOS (6). However, no longitudinal studies have been performed to test this hypothesis. Additionally, it is unclear whether metabolic phenotypes differ in hyperandrogenic compared with normoandrogenic peripubertal girls with obesity.

In our clinical studies of PCOS, we always screen potential reproductively normal control women for HA. These women have normal reproductive histories, including regular menses since menarche, and no clinical signs or symptoms of androgen excess. Nevertheless, elevated androgen levels in the PCOS range are a common finding in this population. Herein we report the prospective assessment of HA in a large cohort of women being screened as potential control subjects. We investigated the prevalence and predictors of HA as well as the association of HA with metabolic risk. We also sought to determine whether HA was merely a biomarker for Rotterdam PCOS or an independent predictor of metabolic risk in otherwise reproductively normal women. Unsupervised hierarchical cluster analysis was performed to determine whether there were discrete subsets of reproductively normal women with higher androgens levels and metabolic abnormalities.

Methods

One hundred and ninety-eight women aged 18 – 40 years, with regular menstrual cycles every 27–35 days and no hirsutism (Ferriman-Gallwey score <8) (7) were screened as potential reproductively normal eumenorrheic (EM) control subjects at an academic medical center between 2002–2008. One-hundred and ten women studied during the same time period who fulfilled NIH criteria for PCOS with oligomenorrhea and HA (8) were included for comparison of metabolic and reproductive features with the hyperandrogenic EM (HA-EM) women. To minimize the confounding effect of racial and ethnic differences in insulin sensitivity and secretion, we limited our cohort to Caucasian and Non-Hispanic Black women since Hispanic women are substantially more insulin resistant (9). Potential control participants were recruited by advertisements in local media and on-line. All women were in good health and not taking medications known to alter reproductive hormone levels or glucose homeostasis for at least 1 month before the study. Contraceptive steroids were stopped at least 3 months before the study.

Hirsutism was assessed by a study investigator using the modified Ferriman and Gallwey score (10). Blood pressure, waist circumference, weight and height were measured as previously reported (11) and BMI was calculated. Blood pressure data were not available on 6 EM subjects and waist circumference data were missing on 30 EM subjects. A fasting blood sample was collected in the morning for the measurement of glucose, T, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), anti-Müllerian hormone (AMH), and lipid levels. Data were missing for AMH levels in 27 EM women due to insufficient sample. A 75-g oral glucose tolerance test was performed as previously described (12) after a 300-g carbohydrate diet and overnight fast. Non-classical 21-hydroxylase deficiency, hyperprolactinemia and androgen-secreting tumors were excluded using appropriate tests (13).

Ethical Approval

The Institutional Review Board of the Feinberg School of Medicine, Northwestern University, approved this study. Written informed consent was obtained from all subjects prior to participation.

Assays

T and DHEAS were measured by radioimmunoassay as reported (13). In 164 EM women with available sample, T was also measured using the liquid chromatography with tandem mass spectrometry (LC/MS-MS) method to validate the RIA results (14). SHBG was measured by immunoradiometric assay in 104 EM and all PCOS women, and by Immulite in 94 EM women due to a change in core lab methods during the study (11). Subjects with SHBG measured by the different assay methods were compared separately as denoted in the text. Non-SHBG bound or unbound (uT) was calculated from T and SHBG concentrations as reported (15). AMH was measured by enzyme-linked immunosorbent assay (16). Total cholesterol, HDL, triglyceride, glucose and insulin levels were determined as reported previously (12, 17). LDL cholesterol levels were calculated using the Friedewald equation (18).

Data Analyses

HA was defined by an elevated circulating level of T, uT, or DHEAS (11). The thresholds for elevated T, uT, and DHEAS were defined by 2 SD above the mean levels in the nonobese (BMI <25 kg/m²) EM women in the current cohort (n=74): T > 2.08 nmol/L, uT > 0.62 nmol/L, and/or DHEAS >8.23 µmol/L.

ROC curve analysis was performed to establish an AMH threshold predictive of PCOS diagnosis in an independent cohort of 294 women meeting NIH criteria for PCOS (19) and 237 control women of comparable age and BMI with normal androgens and regular menses; ovarian morphology was not assessed. An AMH level of 36.4 pmol/L predicted NIH PCOS with a sensitivity of 80%, and specificity of 81%, with area under the curve of 0.88. About 90% of women with NIH PCOS have polycystic ovarian morphology (PCOM) (20). Accordingly, HA-EM with AMH levels above this threshold were presumed to have PCOM and, therefore, were considered to fulfill Rotterdam PCOS criteria of HA and PCOM (21).

MetS was defined according to the American Heart Association guidelines (22) as the presence of three or more of the following features: 1) central obesity, defined by waist circumference >88 cm or BMI ≥ 30 kg/m²; 2) total triglyceride levels ≥ 1.69 mmol/L; 3) HDL cholesterol level <1.29 mmol/L; 4) systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg; and 5) fasting plasma glucose ≥ 5.55 mmol/L. Dysglycemia was defined by the presence of impaired fasting glucose (IFG, G0 ≥ 5.55 mg/dl) and/or impaired glucose tolerance (IGT, post-challenge glucose ≥ 7.77 mmol/L) (23).

The prevalence of HA was compared after stratification by BMI category: <25 kg/m², normal; $25\text{--}30$ kg/m², overweight; and ≥ 30 kg/m², obese. Chi square test was used to analyze categorical variables. Paired t-tests and Pearson correlation were performed to compare T levels determined by RIA assay to those measured by LC/MS-MS. Analysis of covariance (ANCOVA) was used as indicated for 2-group comparisons of continuous variables to adjust for the potentially confounding effects of total adiposity as estimated by BMI (22, 23) and body fat distribution as estimated by waist circumference (22, 23). Linear regression was used to assess the relationship between BMI and waist circumference in EM women. Logistic regression adjusting for BMI or waist circumference was used to calculate adjusted odds ratios (AOR) for MetS and dysglycemia in HA-EM compared with normoandrogenic EM women (NA-EM). Logistic regression was also performed to assess risk of MetS and dysglycemia as predicted by BMI, waist circumference, fasting insulin (I0) and T as well as SHBG, before and after adjusting for I0, since insulin is a major regulator of SHBG synthesis (24).

Unsupervised hierarchical cluster analysis was performed to agnostically assess whether there were subgroups of EM women. Six traits, BMI, T, DHEAS, I0, fasting glucose (G0), and SHBG, were included in this analysis. Quantitative trait values were log-normalized and adjusted for age, assay site and assay method. To ensure equal scaling, an inverse normal transformation was applied to each trait. Normalized trait residuals were clustered using unsupervised, agglomerative, hierarchical clustering according to a generalization of Ward's minimum variance method on Manhattan distances between trait values (25, 26). Cluster stability was assessed by computing the mean Jaccard coefficient from a repeated nonparametric bootstrap resampling ($n=1000$) of the dissimilarity matrix (27). Jaccard coefficients below 0.5 indicate that a cluster does not capture any discernable pattern within the data, while a mean coefficient above 0.6 indicates that the cluster reflects a real pattern within the data (27). Traits were ranked by their importance in defining the clusters according to the relative separation of cluster trait distributions, as determined by Kruskal-Wallis tests between clusters for each trait. More important traits were indicated by lower corresponding P-values. Cluster analysis was performed using R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). All other statistical analyses were performed with SAS 9.4 (SAS Institute, Inc., Cary, NC). Data are reported as the mean $\pm$ SD with the level of alpha set at 0.05.

Results

HA is common in EM and associated with metabolic abnormalities

Forty-four women (22%) were HA and 154 were NA (78%). T levels measured by RIA and LC/MS-MS were significantly correlated ($R=0.47$, $P<0.0001$) and did not differ by paired t -test ($P=0.74$). The assignment of EM to the HA subgroup differed for only one woman when T was measured by LC/MS-MS. Among the HA-EM women, uT was the most commonly elevated androgen, 68%, followed by elevated total T levels, 43%, and DHEAS levels, 30%. Four women had elevated total T but normal uT and DHEAS levels. Among the EM women, the prevalence of HA was associated with increasing BMI category: 12% in women with normal BMI ($<25 \text{ kg/m}^2$), 22% in women with BMI in the overweight range ($25\text{--}30 \text{ kg/m}^2$), and 31% in women with BMI in the obese range ($>30 \text{ kg/m}^2$, $P=0.01$, Figure 1).

HA-EM and NA-EM were of comparable age, but BMI was higher in HA-EM ($P=0.01$, Table 1). Accordingly, all analyses were adjusted for BMI. T ($P<0.0001$), uT ($P<0.0001$), DHEAS ($P<0.0001$) and AMH ($P=0.003$) levels were higher, while SHBG levels were lower in HA-EM compared with NA-EM ($P=0.02$ [immunoradiometric] and $P=0.008$ [Immulite]). Triglyceride levels were higher in HA-EM compared with NA-EM ($P=0.009$). Blood pressure, G0, cholesterol, HDL and LDL levels did not differ. I0 ($P=0.05$), post-challenge insulin levels ($P=0.002$) and post-challenge glucose levels ($P=0.05$) were higher in HA-EM compared with NA-EM. Waist circumference was increased in HA-EM compared to NA-EM ($P=0.003$, t -test, unadjusted for BMI). When analyses were adjusted for waist circumference rather than BMI, the significant findings were unchanged with the exception of I0 levels, which were no longer statistically significant ($P=0.11$, Supplemental Table 1).

The adjusted odds of MetS (AOR 2.9, [1.2–6.9], $P=0.02$) and dysglycemia (AOR 2.7, [1.2–5.8], $P=0.01$) were increased in HA-EM compared with NA-EM (Figure 2), independent of BMI. When the logistic regression analysis was adjusted for waist circumference rather than BMI, the increased risk for dysglycemia persisted (AOR 2.6, [1.1–5.9], $P=0.02$), while there was a nonsignificant trend toward increased risk for MetS in HA-EM (AOR 2.3 [0.9–6.0], $P=0.09$). This finding was likely due to the substantial reduction in sample size because of missing waist measurements since BMI and waist circumference were highly correlated in our EM women ($R^2=0.73$, $P<0.0001$).

Cluster analysis identifies a discrete subgroup with HA and metabolic abnormalities

Cluster analysis identified two distinct clusters of EM women (Figure 3). Cluster B ($n=80$, mean Jaccard score 0.78, Table 2) was characterized by higher T, DHEAS, G0, I0, and BMI and lower SHBG levels than cluster A ($n=118$, mean Jaccard score 0.72). The Jaccard scores greater than 0.6 indicated that both clusters reflected real patterns within the data (27). Comparison of P values associated with each trait suggested the following relative importance of each trait in defining the distinct clusters: 1) BMI, 2) I0, 3) SHBG, 4) G0, 5) T, 6) DHEAS. The prevalence of HA-EM women defined by elevated T, uT, or DHEAS levels was significantly higher in Cluster B (40%) compared with Cluster A (10%, $P<0.0001$, Figure 3).

Predictors of cardiometabolic risk

The odds of MetS and dysglycemia were strongly negatively associated with SHBG levels (MetS OR 0.008 [0.001–0.049], $P<0.0001$, dysglycemia OR 0.097 [0.024–0.386], $P=0.001$) but not associated with T or DHEAS levels. After adjusting for I0, the negative association of MetS and dysglycemia risk with SHBG levels persisted (MetS OR 0.029 [0.004–0.196], $P=0.0003$, dysglycemia OR 0.231 [0.055–0.975], $P=0.05$). As expected, increases in BMI and I0 levels were also associated with increased risk for MetS (BMI OR 5.3 [2.8–10.2], $P<0.0001$; I0 OR 3.7 [2.3–6.0], $P<0.0001$) and dysglycemia (BMI OR 1.7 [1.0–2.8], $P=0.03$; I0 OR 39.5 [6.5–238.8], $P<0.0001$).

Do HA-EM have evidence for Rotterdam PCOS?

Sixteen HA-EM women had high AMH levels (36%), suggesting these women had PCOM and, thus, fulfilled Rotterdam PCOS criteria (21). In contrast, the prevalence of high AMH was significantly lower in NA-EM women at 17% ($P=0.005$). After excluding HA-EM women with elevated AMH (i.e. those with presumptive Rotterdam PCOS), the increased odds of MetS persisted (AOR 2.9 [1.1–7.2], $P=0.02$), while the increased risk for dysglycemia only trended towards significance (AOR 2.3 [0.9–5.4], $P=0.07$).

Reproductive and metabolic traits were compared in HA-EM and PCOS to assess whether there were any distinctive features associated with these phenotypes (Table 1). HA-EM and PCOS were of comparable age, but BMI was lower in HA-EM ($P=0.01$), therefore, all analyses were adjusted for BMI. T and uT levels were lower in HA-EM women compared with PCOS ($P<0.0001$ and $P=0.01$, respectively), while SHBG levels did not differ. DHEAS ($P=0.003$) were higher and AMH ($P=0.0002$) levels lower in HA-EM compared to PCOS. Total cholesterol ($P=0.04$) and LDL ($P=0.002$) levels were higher, and HDL ($P=0.05$) levels were lower in PCOS, while triglyceride levels did not differ. Fasting and post-challenge glucose levels did not differ, while fasting and post-challenge insulin levels were lower in HA-EM compared with PCOS ($P=0.008$ and $P=0.04$, respectively).

Discussion

We found that HA was common among asymptomatic, reproductive-age EM women. Further, the prevalence of HA was positively associated with increasing BMI, reaching prevalence rates as high as 31% among women with obesity. However, the risk for MetS and dysglycemia was increased in HA-EM compared with NA-EM, independent of BMI. The risk of MetS also persisted after exclusion of HA-EM women with probable Rotterdam PCOS indicated by high AMH levels. Lower SHBG levels, independent of fasting insulin levels, were strongly associated with MetS and dysglycemia, while total T and DHEAS levels were not. These findings suggest that SHBG rather than androgens was associated with metabolic risk in women. Accordingly, HA resulting from increased uT may be a marker for metabolic risk due to low SHBG rather than increased T.

In order to independently assess whether there were subtypes of EM women, we performed unsupervised hierarchical cluster analysis (25, 26, 27). We found a distinct subgroup of EM women characterized by higher BMI, G0, I0, T, DHEAS and lower SHBG levels. The mean

Jaccard coefficient above 0.6 indicated that this cluster reflected a real pattern within the data (27). The cluster analysis suggested that women with HA and metabolic abnormalities were a discrete subgroup rather than the tail of a normal distribution of T levels. This subgroup was enriched with HA-EM women defined by elevated T, uT or DHEAS levels, representing 40% of the cluster, which supports the use of androgen levels to identify EM women with metabolic risk. Of the traits measured, BMI, IO and SHBG were the strongest drivers of the distinct clusters, suggesting that these were the key traits defining the subtypes. Indeed, these traits were all independently associated with MetS and dysglycemia, supporting their importance in the association of HA and metabolic risk.

It is well-established that women with PCOS are insulin resistant (28) and have increased risk for MetS (29) and type 2 diabetes (17). Women with the NIH PCOS phenotype of HA and oligomenorrhea have the greatest metabolic risk (30), while metabolic risk in the non-NIH Rotterdam phenotypes is much more modest (31). We used AMH levels as a proxy for ultrasonography to assess ovarian morphology (32, 33) to determine whether our HA-EM women fulfilled criteria for non-NIH Rotterdam PCOS. We established AMH thresholds in our own population of PCOS women diagnosed by NIH criteria. Thirty-six percent of HA-EM had evidence for Rotterdam PCOS defined by HA and elevated AMH (8). Increased risk for MetS persisted after excluding these women suggesting that it was independent of PCOS diagnosis.

We compared HA-EM women to women with NIH PCOS in order to identify distinguishing features of their metabolic and reproductive phenotypes. Total and bioavailable T levels were lower, while DHEAS levels were higher in HA-EM. These findings suggested that there was a greater contribution of adrenal relative to ovarian HA in HA-EM (34). Further, HA-EM had lower fasting and post-challenge insulin levels compared with PCOS (4), suggesting that they were more insulin sensitive. However, SHBG levels were similar in HA-EM and PCOS women, despite lower levels of T and insulin in HA-EM; both of the latter hormones are negative regulators of SHBG production (35, 36). It is unclear what additional factors contributed to the lower SHBG levels in HA-EM.

There is an extensive literature on the association between increased endogenous total and free T levels and cardiometabolic risk (1, 2, 3, 37, 38, 39, 40). In premenopausal women across a range of BMI from normal weight to obese, low SHBG and elevated free T levels were associated with larger abdominal adipocytes and increased waist circumference, as well as increased fasting glucose and insulin levels (38). In contrast, these associations were not observed with total T levels (38). We performed regression analyses to explore putative determinants of MetS and dysglycemia in our cohort. SHBG levels were independently associated with MetS and dysglycemia risk whereas the androgens, T and DHEAS, were not. This finding is consistent with these earlier studies (38, 39), and suggests low SHBG rather than HA played a causal role in metabolic risk. Indeed, HA may be a consequence of low SHBG, which results in increased non-SHBG bound T (uT), the leading cause of HA in our cohort, present in 68% of HA-EM women. The cluster analysis supported a more important role for SHBG compared to T in defining the HA cluster. In addition, the finding that low SHBG levels were a distinctive feature of HA-EM women, not accounted for by insulin or androgen levels, supports a possible causal role for SHBG in metabolic

dysfunction. Moreover, this hypothesis is consistent with Mendelian randomization studies, which have provided genetic evidence that SHBG is pathogenically related to the development of type 2 diabetes and to PCOS (41, 42).

Our study has limitations. First, ovarian morphology was not determined by ultrasound but AMH levels were used as a surrogate biomarker for PCOM (32, 33). A limitation of this approach was that the diagnosis of PCOS did not include PCOM, and it is possible some HA-EM women with normal AMH levels did have PCOM. Nevertheless, our AMH threshold for PCOM was similar to those established in women with ultrasonographically determined PCOM (32, 33, 43). Second, waist measurements to assess body fat topography were missing in a substantial number of women. Accordingly, our finding that increased risk for MetS in HA-EM did not persist when adjusting for waist circumference rather than BMI was likely confounded by lack of statistical power due to reduced sample size. This possibility was supported by the fact that BMI and waist circumference were highly correlated in our cohort. Finally, our regression analyses were hypothesis-generating. Future studies using approaches such as Mendelian randomization will be needed to investigate the causal roles of SHBG and androgens in metabolic dysfunction.

Conclusion

In conclusion, we have found that the prevalence of HA among asymptomatic, EM women was high and was associated with increasing BMI. Further, HA-EM had increased risk for MetS and dysglycemia compared to NA-EM, independent of BMI. Cluster analysis supported these findings by identifying a distinct subset of EM women with HA and metabolic abnormalities. SHBG rather than androgen levels appeared to be the key predictor of MetS and dysglycemia. HA, in particular, elevated uT, was a biomarker for metabolic risk in asymptomatic premenopausal women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Study Importance Questions

What is already known about this subject?

- Epidemiologic studies have found higher endogenous testosterone levels in women with metabolic syndrome.
- It is well established that PCOS is associated with metabolic syndrome.
- It is unclear whether hyperandrogenemia is associated with metabolic risk independent of PCOS.

What does your study add?

- Our study found that the prevalence of hyperandrogenemia among asymptomatic, eumenorrheic women was surprisingly high and was associated with increasing BMI. Hyperandrogenemia was associated with increased risk for metabolic syndrome, independent of obesity, even in the absence of PCOS.
- Cluster analysis identified a distinct subset of eumenorrheic women with hyperandrogenemia and metabolic abnormalities. Increased BMI and fasting insulin levels and low SHBG levels were the key drivers of this distinct subset.
- SHBG levels were independently associated with metabolic risk whereas androgen levels were not. Hyperandrogenemia may be a consequence of low SHBG levels rather than a cause of metabolic risk.

Figure 1 –. Prevalence of HA among EM women by BMI category.

The prevalence of HA was 12% in EM women with normal weight (BMI <25 kg/m²), 22% in EM women with BMI in the overweight range (BMI 25–30 kg/m²), and 31% in EM women with BMI in the obese range (BMI ≥ 30 kg/m², P=0.01), suggesting obesity is a significant risk factor for HA even in otherwise asymptomatic women.

Figure 2 –. Increased metabolic risk in HA-EM compared with NA-EM. HA-EM had increased risk for MetS (AOR 2.9 [CI 1.2–6.9]) and dysglycemia (AOR 2.7 [1.2–5.8]) which were independent of differences in BMI. After excluding HA-EM with elevated AMH, who may fulfill Rotterdam criteria for PCOS, the increased risk for MetS (AOR 2.9 [1.1–7.2], but not dysglycemia persisted (data not shown).

A

B

Figure 3 –. Cluster analysis in EM women.

A) Unsupervised cluster analysis identified two distinct clusters within the EM women: Cluster A (grey circles, mean Jaccard score 0.72), and Cluster B (red triangles, mean Jaccard score 0.78). The prevalence of HA (outlined in purple) was significantly higher in Cluster B (40%) compared with Cluster A (10%, $P < 0.0001$). B) Median and 25–75 percentiles for adjusted, normalized trait distributions are pictured. Cluster B was distinguished from Cluster A by higher T, DHEAS, G0, BMI, and I0, and lower SHBG levels. C) Heat map for hierarchical clustering of 198 EM women. The two clusters are shown in the color bar as red and grey, respectively. The distribution of HA samples within these clusters are denoted by darker shades of red and grey in the color bar. Heatmap colors correspond to trait z-scores, as shown in the frequency histogram. BMI, body mass index; SHBG, sex hormone binding globulin; DHEAS, dehydroepiandrosterone sulfate; G0, fasting glucose; I0, fasting insulin.

Table 1.

Baseline Clinical and Biochemical Features in NA-EM and HA-EM

	NA-EM (n=154)	HA-EM (n=44)	PCOS (n=110)	P* NA-EM v HA-EM	P* HA-EM v PCOS
Age (years)	29 ± 6	28 ± 6	27 ± 4	0.41 [†]	0.34 [†]
BMI (kg/m ²)	28.8 ± 8.1	31.5 ± 6.7	35.4 ± 8.0	0.01 [†]	0.01 [†]
Waist circumference (cm)	88 ± 17 (n=129)	97 ± 16 (n=39)	102 ± 18 (n=110)	0.003	0.19 [†]
Race	82% Non-Hispanic White 18% Non- Hispanic Black	91% Non-Hispanic White 9% Non- Hispanic Black	84% Non-Hispanic White 16% Non- Hispanic Black	0.07 [‡]	0.14 [‡]
Total Testosterone - RIA (nmol/L)	1.01 ± 0.38	1.98 ± 0.62	2.81 ± 1.04	<0.0001	<0.0001
Bioavailable Testosterone (nmol/L)	0.28 ± 0.17	0.73 ± 0.35	0.90 ± 0.42	<0.0001	0.01
SHBG – Immunoradiometric (nmol/L)	93 ± 66 (n=87)	62 ± 56 (n=17)	61 ± 34 (n=110)	0.02	0.10
SHBG – Immulite (nmol/L)	60 ± 28 (n=67)	40 ± 20 (n=27)	NA	0.008	NA
DHEAS (μmol/L)	3.67 ± 1.56	6.76 ± 2.98	5.15 ± 2.47	<0.0001	0.003
AMH (pmol/L)	24.4 ± 18.5 (n=132)	44.8 ± 41.8 (n=39)	82.5 ± 56.3 (n=31)	0.003	0.0002
Systolic BP (mmHg)	111 ± 11	115 ± 13	118 ± 11	0.48	0.75
Diastolic BP (mmHg)	69 ± 8	71 ± 9	75 ± 9	0.60	0.12
Total Cholesterol (mmol/L)	3.94 ± 0.78	4.14 ± 0.85	4.64 ± 0.93	0.45	0.04
HDL (mmol/L)	1.29 ± 0.31	1.27 ± 0.36	1.11 ± 0.23	0.83	0.05
LDL (mmol/L)	2.23 ± 0.73	2.28 ± 0.78	2.80 ± 0.78	0.47	0.002
Triglycerides (mmol/L)	0.93 ± 0.47	1.28 ± 0.93	1.58 ± 1.12	0.009	0.38
G0 (mmol/L)	5.05 ± 0.33	5.16 ± 0.39	5.11 ± 0.50	0.60	0.23
G120 (mmol/L)	5.94 ± 1.33	6.44 ± 1.33	7.05 ± 1.78	0.05	0.31
I0 (pmol/L)	90.3 ± 48.6	111.1 ± 55.6	187.5 ± 125.0	0.05	0.0008
I120 (pmol/L)	382.0 ± 298.6	576.4 ± 368.1	1090.4 ± 944.5	0.002	0.04

Data are mean ± SD.

* ANCOVA, adjusted for BMI, unless otherwise noted.

[†] Student's *t*-test.[‡] Chi-square test.

Table 2.

Subtypes Defined by Cluster Analysis

	Cluster A (n=118)	Cluster B (n=80)	P*
BMI (kg/m²)	25.5 ± 5.2	35.2 ± 7.5	2.20E ⁻¹⁶
I0 (pmol/L)	69.5 ± 41.7	125.0 ± 55.6	2.20E ⁻¹⁶
SHBG (nmol/L)	88 ± 57	48 ± 37	1.59E ⁻¹²
G0 (mmol/L)	5.00 ± 0.39	5.27 ± 0.28	3.51E ⁻¹⁰
Total Testosterone (nmol/L)	1.08 ± 0.59	1.46 ± 0.56	2.29E ⁻⁰⁷
DHEAS (μmol/L)	3.70 ± 1.86	5.33 ± 2.64	1.96E ⁻⁰⁶

Data are mean ± SD.

* Student's *t*-test.