The Metabolic Syndrome Is Frequent in Klinefelter's Syndrome and Is Associated With Abdominal Obesity and Hypogonadism

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OBJECTIVE — Klinefelter's syndrome is associated with an increased prevalence of diabetes, but the pathogenesis is unknown. Accordingly, the aim of this study was to investigate measures of insulin sensitivity, the metabolic syndrome, and sex hormones in patients with Klinefelter's syndrome and an age-matched control group.

RESEARCH DESIGN AN METHODS — In a cross-sectional study, we examined 71 patients with Klinefelter's syndrome, of whom 35 received testosterone treatment, and 71 control subjects. Body composition was evaluated using dual-energy X-ray absorptiometry scans. Fasting blood samples were analyzed for sex hormones, plasma glucose, insulin, C-reactive protein (CRP), and adipocytokines. We analyzed differences between patients with untreated Klinefelter's syndrome and control subjects and subsequently analyzed differences between testosterone-treated and untreated Klinefelter's syndrome patients.

RESULTS — Of the patients with Klinefelter's syndrome, 44% had metabolic syndrome (according to National Cholesterol Education Program/Adult Treatment Panel III criteria) compared with 10% of control subjects. Insulin sensitivity (assessed by homeostasis model assessment 2 modeling), androgen, and HDL cholesterol levels were significantly decreased, whereas total fat mass and LDL cholesterol, triglyceride, CRP, leptin, and fructosamine levels were significantly increased in untreated Klinefelter's syndrome patients. In treated Klinefelter's syndrome patients, LDL cholesterol and adiponectin were significantly decreased, whereas no difference in body composition was found in comparison with untreated Klinefelter's syndrome patients. Multivariate analyses showed that truncal fat was the major determinant of metabolic syndrome and insulin sensitivity.

CONCLUSIONS — The prevalence of metabolic syndrome was greatly increased, whereas insulin sensitivity was decreased in Klinefelter's syndrome. Both correlated with truncal obesity. Hypogonadism in Klinefelter's syndrome may cause an unfavorable change in body composition, primarily through increased truncal fat and decreased muscle mass. Testosterone treatment in Klinefelter's syndrome only partly corrected the unfavorable changes observed in untreated Klinefelter's syndrome, perhaps due to insufficient testosterone doses.

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Klinefelter's syndrome is the most common sex chromosome disorder, with a prevalence of 1 in 660 men (1), and is a frequent cause of hypogonadism and infertility. It is caused by the presence of extra X chromosomes, the most common karyotype being 47,XXY. The phenotype is variable, but the most constant finding is small hyalinized testes, hypergonadotropic hypogonadism, infertility, eunuchoid body proportion, increased height, and learning disabilities (2).

Previously Klinefelter's syndrome was associated with an increased risk of diabetes, but this association has not been further investigated (3–5). Epidemiological studies on mortality (6) and morbidity in Klinefelter's syndrome (7) have shown an increased risk of dying from diabetes or being admitted to the hospital with diabetes.

Hypogonadism is common in Klinefelter's syndrome and has been found to be an independent risk factor for development of abdominal adiposity in men with normal chromosomes (8). Hypogonadism is also associated with metabolic syndrome and type 2 diabetes (9–11). Experimental induction of hypogonadism and subsequent testosterone substitution showed a dose-dependent change in body composition with changes in fat-free mass being inversely related to increasing testosterone doses (12). Testosterone treatment of middle-aged abdominally obese men decreased the amount of intra-abdominal fat and increased insulin sensitivity (13). Thus, hypogonadism may lead to abdominal adiposity, thereby increasing the risk of metabolic syndrome and development of type 2 diabetes. In a cross-sectional study of adult patients

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Abbreviations: ATPIII, Adult Treatment Panel III; BFtr, truncal fat; CRP, C-reactive protein; DEXA, dual-energy X-ray absorptiometry; FPG, fasting plasma glucose; FSH, follicle-stimulating hormone; HOMA, homeostasis model assessment; HOMA2%S, HOMA of insulin sensitivity; IMAT, intermuscular adipose tissue; LBM, lean body mass; LH, luteinizing hormone; NCEP, National Cholesterol Education Program; SHBG, sex hormone–binding globulin; SMM, skeletal muscle mass; TBF, total body fat.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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with Klinefelter's syndrome and an age-matched control group, we investigated the impact of hypogonadism on body composition and other components of the metabolic syndrome, including insulin sensitivity.

RESEARCH DESIGN AND METHODS

— A total of 71 patients with Klinefelter's syndrome were recruited from endocrine and fertility clinics. Inclusion criteria were age >18 years, a verified Klinefelter's syndrome karyotype, and signed informed consent. Exclusion criteria were untreated hypothyroidism or hyperthyroidism, present or past malignant diseases, clinical liver disease, or treatment with drugs knowing to interfere with glucose homeostasis or fat metabolism (e.g., glucocorticoids). One of the Klinefelter's syndrome patients had the 49,XXXXY karyotype and was excluded from the analysis. Thirty-five (50%) of the remaining 70 Klinefelter's syndrome patients received testosterone treatment at the time of investigation (intramuscular testosterone injections [$n = 20$], oral testosterone undecanoate [$n = 14$], and mesterolon [$n = 1$]). Because of the inability of some of the Klinefelter's syndrome patients to recall the date of their last injection and because we did not have access to all patients files, we did not have information about the timing of the last injection of testosterone in the treated Klinefelter's syndrome patients. Of the 35 Klinefelter's syndrome patients without testosterone treatment, 9 had received testosterone treatment in the past but not during the last year before examination. A healthy age-matched control group was recruited by advertising for healthy volunteers at the University of Aarhus and at the Blood Bank at the Aarhus University Hospital. None of the healthy control subjects received any kind of steroid therapy.

All patients received oral and written information concerning the study before giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee (# 20010155) and the Danish Data Protection Agency.

All participants were examined in the morning after an overnight fast. Blood was drawn, and serum and plasma were immediately separated and stored at −20°C in multiple vials for later analysis. Body weight was measured (with the participants wearing underwear) to the nearest 0.1 kg, height was measured to the nearest 0.5 cm, BMI was calculated, and waist and hip circumferences were measured. Blood pressure was measured in the sitting position, using a mercury sphygmomanometer.

Whole-body dual-energy X-ray absorptiometry (DEXA) scans were performed on a Hologic 2000/w osteodensitometer (Hologic, Waltham, MA). Total body fat (TBF), lean body mass (LBM), and truncal fat (BFtr) were calculated as percentages. Intermuscular adipose tissue (IMAT)-free skeletal muscle mass (SMM) was then estimated according to a recently developed, magnetic resonance imaging–based, and validated prediction model with minimal variation (14) as

$$\text{IMAT-free SMM} = -0.14 + 1.18 * \text{appendicular LBM (in kilograms)} - 0.03 * \text{age}$$

Because they weighed >130 kg, seven Klinefelter's syndrome patients were not DEXA scanned (weight limit is 130 kg). Another six (four Klinefelter's syndrome patients and two control subjects) were not DEXA scanned because of technical errors. The coefficient of variation (CV) for DEXA scans was <2% from repeated measurements (15).

A maximal oxygen consumption ($V\text{O}_{2max}$) test was performed on a bicycle ergometer using a standardized protocol. The initial workload was increased with 10 W every 30 s until exhaustion. Breath-by-breath gas exchange analysis was performed; maximal oxygen consumption was determined as the highest O_2 consumption achieved during exercise with a calorimeter (Jaeger Oxycon Delta; Erich Jaeger, Hoechberg, Germany), and $V\text{O}_{2max}$ was calculated. Seventy control subjects and 60 Klinefelter's syndrome patients were able to finish the test; the main reason for not finishing the test was leg pain during exercise.

Assays

Plasma glucose levels were measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Serum insulin was determined by a commercial immunological kit (DAKO, Glostrup, Denmark). Androgens, estrogens, sex hormone–binding globulin (SHBG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were analyzed as described (16). We estimated free testosterone by a method described by Bartsch (17), based on measurement of SHBG, total testosterone, and dihydrotestosterone, using the law of mass action and the binding constant of testosterone and dihydrotestosterone to SHBG, including a calculation of testosterone binding to albumin (assuming a constant association constant to albumin). In this system, binding to cortisol-binding globulin is thought to be negligible. The method we used to estimate free testosterone is essentially similar to the method suggested by Vermeulen et al. (18) to be the most reliable and correlates closely with direct measurement of free testosterone by equilibrium dialysis. Plasma lipids and triglycerides were measured using an automated commercially available system (Aeroset; Abbott Diagnostics); CVs were <5%. C-reactive protein (CRP) was measured by an ultrasensitive assay (Diagnostic Products, Los Angeles, CA). Serum adiponectin was determined by a novel in-house time-resolved immunofluorometric assay as previously described (19), leptin was determined by a commercial radioimmunoassay (Linco, St. Louis, MO), and serum fructosamine was analyzed by a commercial colorimetric assay (Horiba ABX Diagnostics, Montpellier, France).

Classification of impaired fasting glycemia, diabetes, and metabolic syndrome

We defined metabolic syndrome, in accordance with the definition of the National Cholesterol Education Program (NCEP)/Adult Treatment Panel III (ATPIII), as the presence of three or more of the following criteria: fasting plasma glucose (FPG) >6.1 mmol/l, serum triglyceride >1.7 mmol/l, serum HDL cholesterol <1.0 mmol/l, blood pressure >130/85 mmHg or use of antihypertensive medication, or waist circumference >102 cm (20). A diabetic FPG level was defined as FPG >7 mmol/l; impaired fasting glycemia was defined as FPG between 6.1 and 7.0 mmol/l according to World Health Organization criteria.

Calculation of insulin sensitivity

Insulin sensitivity (%S) and β-cell function were assessed by homeostasis model assessment (HOMA) modeling (21,22), which is based on simultaneously sampled fasting levels of glucose and insulin. The relationship between glucose and insulin in the fasting state reflects the balance between hepatic glucose output and insulin secretion (22). The HOMA2 computer model was downloaded from http://

www.dtu.ox.ac.uk/index.html?maindoc=/homa/download.html.

Statistics

Because the group of testosterone-treated Klinefelter's syndrome patients was very heterogenic with regard to testosterone levels and because we had no valid information on timing of the last intramuscular injection of testosterone, we performed the analyses in two steps. First, we compared the untreated Klinefelter's syndrome patients with the control group and then the untreated Klinefelter's syndrome patients with the testosterone-treated Klinefelter's syndrome patients.

Apart from height, $V_{O_{2max}}$, and the ratio between 17β-estrogen and testosterone, none of the variables were normally distributed, and nonparametric tests were used to test for differences between groups. All results are shown as medians and total range. Spearman correlation analysis was used to describe correlations between variables to select principal-independent variables for later use in regression analyses. Stepwise multivariate regression analysis was used to evaluate the impact of independent variables on the dependent variables (metabolic syndrome [i.e., an individual being classified as having it or not], insulin sensitivity, $V_{O_{2max}}$, and body composition), with inclusion of status (i.e., being a Klinefelter's syndrome patient or a control subject) as a dummy variable. Multivariate analysis was performed on the whole group of participants, including both treated and untreated Klinefelter's syndrome patients as well as the control subjects. The significance levels for entering and for removal of variables from the model were $P < 0.05$ and $P < 0.10$, respectively. Log transformation of variables was used when appropriate. Logistic regression analysis was used to evaluate the impact of variables on the dichotomous variable "metabolic syndrome." All statistics were calculated using intercooled STATA (V8.2; StataCorp, College Station, TX). P values < 0.05 were regarded as significant.

RESULTS

Untreated Klinefelter's syndrome patients versus healthy control subjects

Anthropometry. The two groups were matched by age and height. Weight, BMI, waist, TBF, and BFtr were all significant greater in Klinefelter's syndrome patients, whereas IMAT-free SMM was significantly decreased in Klinefelter's syndrome patients compared with healthy control subjects (Table 1).

Diabetes and the metabolic syndrome. In the Klinefelter's syndrome patients, all the measures of insulin sensitivity and metabolic syndrome except blood pressure were changed in a pathologic direction; fasting serum insulin and fasting plasma glucose were higher among the Klinefelter's syndrome patients, whereas insulin sensitivity (HOMA2%S) was significantly reduced. Total cholesterol, LDL cholesterol, and triglycerides were all significantly increased, and HDL cholesterol was significantly decreased in Klinefelter's syndrome patients. CRP and leptin levels were higher in Klinefelter's syndrome patients, whereas levels of adiponectin and fructosamine were similar between the two groups. With the NCEP/ATPIII criteria, 16 of the 35 Klinefelter's syndrome patients (46%) and 7 control subjects (9.9%) had metabolic syndrome ($P < 0.001$), 3 Klinefelter's syndrome patients (9%), and 1 control subject (1.4%) had diabetic FPG levels ($P = 0.10$), and 6 Klinefelter's syndrome patients (17%) and 2 control subjects (3%) had impaired fasting glycemia ($P = 0.02$).

Sex hormones. Testosterone, free testosterone, and SHBG were significantly reduced, and FSH, LH, and the ratio between 17β-estrogen and testosterone were significantly increased in Klinefelter's syndrome patients. There was no significant difference in 17β-estrogen.

Exercise testing. Klinefelter's syndrome patients had significantly lower maximal oxygen uptake.

Testosterone-treated Klinefelter's syndrome patients versus untreated Klinefelter's syndrome patients

Anthropometry. We found no difference in any anthropometric measures between the groups, although TBF ($P = 0.08$) and BFtr ($P = 0.11$) tended to be lower in the testosterone-treated Klinefelter's syndrome group (Table 1).

Diabetes and the metabolic syndrome. A significantly lower level of LDL cholesterol was found in the testosterone-treated Klinefelter's syndrome group. Total cholesterol, fasting glucose CRP, and leptin levels tended to be lower in the testosterone-treated Klinefelter's syndrome group, whereas no difference in HOMA2%S or frequency of diabetes or metabolic syndrome was found. Adiponectin was, however, significantly lower in the testosterone-treated Klinefelter's syndrome group.

Sex hormones. FSH and LH were significantly lower, whereas 17β-estrogen was significantly higher in the testosterone-treated Klinefelter's syndrome group. No differences in testosterone, free testosterone, SHBG, or 17β-estrogen-to-testosterone ratio were found between the groups.

Exercise testing. No difference was found between the groups.

All participants

We then studied all participants in univariate regression and multiple regression models. We first studied Klinefelter's syndrome patients and control subjects separately in univariate analyses, and subsequently combined treated and untreated Klinefelter's syndrome patients and control subjects in multivariate models in an attempt to identify factors contributing to the observed differences in insulin sensitivity, BFtr, SMM, $V_{O_{2max}}$, and the dichotomous variable metabolic syndrome between groups.

Associations between sex hormones and variables related to the metabolic syndrome

HOMA2%S correlated significantly with testosterone (Klinefelter's syndrome $r = 0.31$, $P = 0.01$; control $r = 0.28$, $P = 0.02$) and BFtr (Klinefelter's syndrome $r = -0.70$, $P < 0.0001$; control $r = -0.52$, $P < 0.0001$) (Fig. 1). It also correlated with SHBG, CRP, adiponectin, BMI, IMAT-free SMM, TBF, and $V_{O_{2max}}$ (results not shown).

BFtr correlated significantly with leptin (Klinefelter's syndrome $r = 0.89$, $P < 0.0001$; control $r = 0.84$, $P < 0.0001$), testosterone (Klinefelter's syndrome $r = -0.43$, $P = 0.0007$; control $r = -0.43$, $P = 0.0002$), and CRP (Klinefelter's syndrome $r = 0.47$, $P = 0.0002$; control $r = 0.29$, $P = 0.02$) (Fig. 1). It also correlated with free testosterone, SHBG, age, adiponectin, and $V_{O_{2max}}$ (results not shown).

$V_{O_{2max}}$ correlated significantly with age (Klinefelter's syndrome $r = -0.30$, $P = 0.03$; control $r = -0.59$, $P < 0.0001$) and IMAT-free SMM (Klinefelter's syndrome $r = 0.49$, $P = 0.0003$; control $r = 0.36$, $P = 0.003$) (Fig. 1). It also correlated with free testosterone, CRP, leptin, and BFtr (results not shown).

Table 1—*Number and age of participants, median (range) of anthropometric data, DEXA-derived body composition data, sex hormones, measures of the metabolic syndrome, insulin sensitivity and frequency of metabolic syndrome and diabetes in untreated Klinefelter's syndrome (U-KS) patients, testosterone-treated Klinefelter's syndrome patients (T-KS), and normal men*

				P value	
	U-KS	T-KS	Control subjects	U-KS vs. control subjects*	U-KS vs. T-KS*
n	35	35	71		
Age (years)	35.0 (19.0–66.2)	38.7 (19.3–62.3)	36.4 (19.2–68.0)	0.95	0.82
Height (cm)	183.6 ± 9.16	185.4 ± 8.02	181.1 ± 5.67	0.09†	0.39†
Weight (kg)	92.5 (56.8–183)	85.0 (65.8–150)	82.5 (60.1–127.5)	0.0008	0.56
BMI (kg/m^2)	27.3 (20.0–60.6)	25.1 (18.1–54.7)	24.9 (19.0–36.9)	0.008	0.37
Waist (cm)	109 (81–179)	104 (75.0–175)	92 (76–133)	<0.0001	0.27
$V_{O_{2max}}$ (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$)	30.6 ± 7.13	31.0 ± 9.9	44.1 ± 10.8	<0.0001†	0.83†
BFtr (%)	34.0 (10.1–49.3)	23.2 (2.45–56.2)	17.6 (3.22–43.7)	<0.0001	0.11
Body fat (%)	28.8 (12.5–42.4)	22.2 (7.88–45.0)	18.4 (6.16–37.3)	<0.0001	0.08
IMAT-free SMM (kg)	31.9 ± 4.02	32.6 ± 4.15	34.2 ± 4.40	0.01†	0.50†
Testosterone (T) (nmol/l)	12.7 (0.75–37.3)	14.0 (1.88–72.2)	21.8 (10.6–55.5)	<0.0001	0.19
Free testosterone (nmol/l)	0.33 (0.01–0.66)	0.33 (0.06–2.60)	0.53 (0.17–1.37)	<0.0001	0.15
SHBG (nmol/l)	31 (16–79)	29 (13–87)	36 (17–99)	0.04	0.38
17β-estradiol (E_2) (pmol/l)	77 (40–140)	89 (44–290)	81 (40–210)	0.82	0.04
E_2/T ratio	10.3 ± 2.06	8.68 ± 1.44	3.78 ± 0.18	<0.0001	0.52
FSH (IU/l)	27.7 (1.90–49.2)	14.3 (0.2–49.3)	3.3 (0.6–46)	<0.0001	0.01
LH (IU/l)	17.2 (1.0–27.4)	9.1 (0.60–22.2)	3.4 (1.4–22.3)	<0.0001	0.0001
Triglycerides (mmol/l)	1.48 (0.41–40.2)	1.64 (0.63–5.61)	0.82 (0.40–2.46)	0.0001	0.72
Total cholesterol (mmol/l)	5.84 (3.70–13.5)	5.16 (3.78–7.36)	4.66 (3.34–7.38)	0.002	0.07
LDL cholesterol (mmol/l)	3.75 (2.0–5.14)	3.30 (2.15–4.74)	3.0 (1.68–5.49)	0.004	0.04
HDL cholesterol (mmol/l)	1.01 (0.52–2.20)	0.95 (0.61–1.92)	1.29 (0.77–2.55)	0.0001	0.33
Plasma glucose (mmol/l)	5.5 (4.8–10.2)	5.3 (4.0–10.4)	5.1 (4.2–8.2)	<0.0001	0.08
Serum insulin (pmol/l)	66.5 (15–270)	54 (13–420)	36 (9–136)	0.0001	0.89
HOMA2-B (%)	88.1 (39.7–240)	94.8 (44.0–291)	73.8 (33.1–180)	0.10	0.28
HOMA2%S (%)	68.4 (17.7–301)	84.8 (11.7–359)	128 (34.4–514)	<0.0001	0.70
CRP (mg/dl)	0.21 (0.03–2.74)	0.17 (0.02–1.83)	0.11 (0.02–1.99)	0.0005	0.10
Leptin (ng/ml)	14.0 (2.41–116)	8.44 (1.51–75.2)	3.07 (1.24–17.0)	<0.0001	0.08
Fructosamine (μmol/l)	274 (230–373)	279 (237–386)	271 (230–380)	0.17	0.26
Adiponectin (mg/l)	9.10 (3.78–18.1)	6.97 (3.0–24.2)	7.37 (3.14–22.2)	0.31	0.05
Metabolic syndrome (%)	46 (16/35)	49 (17/35)	10 (7/71)	<0.001‡	0.73‡
Diabetes (%)	9 (3/35)	11 (4/35)	1 (1/71)	0.10§	0.71§
Impaired fasting glycemia (%)	17 (6/35)	20 (7/35)	3 (2/71)	0.02§	0.72§

Data are medians (total range) or means ± SD. *Mann-Whitney rank-sum test. †Student's *t* test. ‡χ^2 test. §Fisher's exact test. HOMA2-B, β-cell function estimated by HOMA2 modeling.

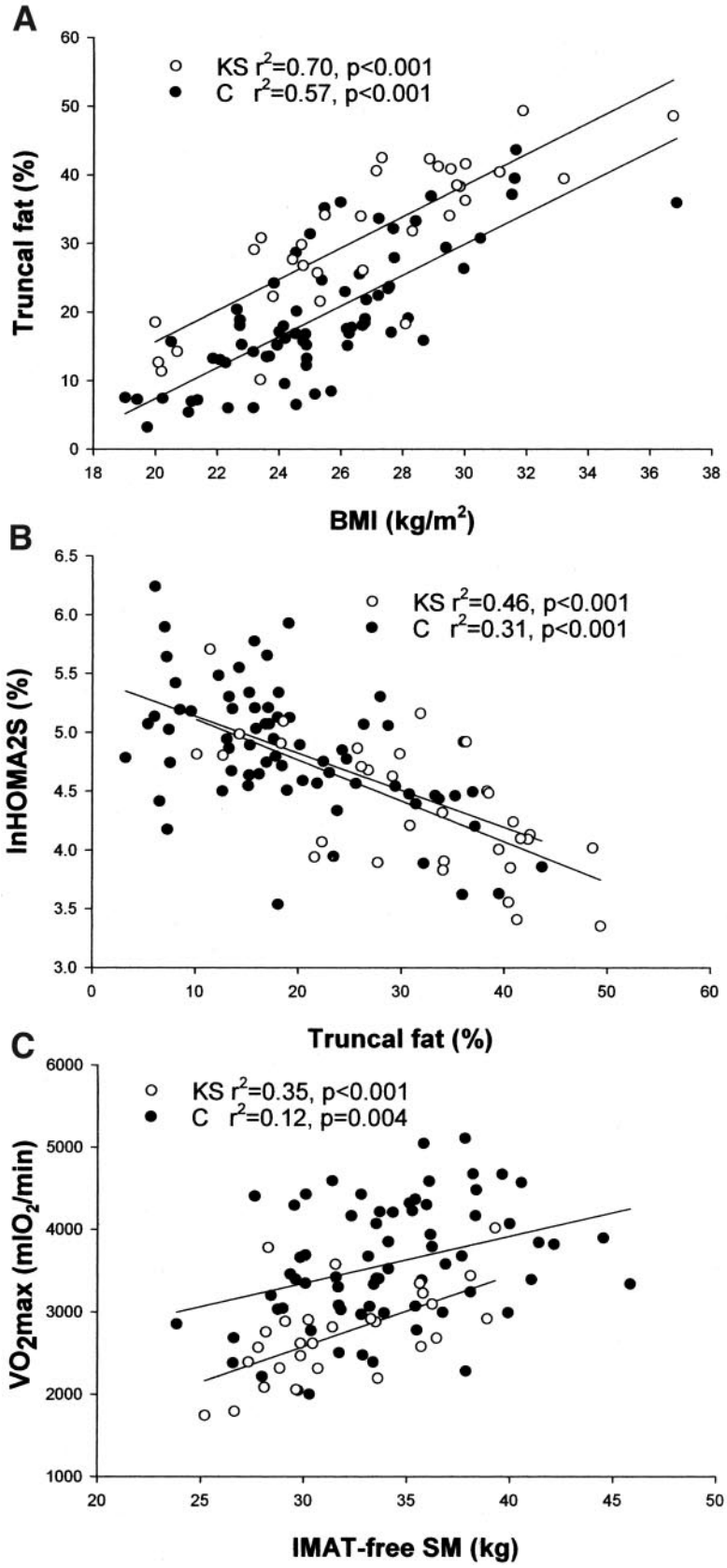

Figure 1—A*: BFtr in correlation with BMI. Klinefelter's syndrome patients (KS, ○) have more BFtr (~8% more) for any value of BMI than control subjects (C, ●). (No formal testing for differences between the two regression lines was performed, but CIs for interception with the y-axis were not overlapping, indicating a significant difference.)* B*: Insulin sensitivity (lnHOMA2%S) in relation to truncal fat. Klinefelter's syndrome patients (○) and control subjects (●) show the same negative correlation between insulin sensitivity and BFtr.* C*:* Vo_{2max} *in relation to IMAT-free SMM. Klinefelter's syndrome patients (○) have less maximal oxygen uptake* (Vo_{2max}) *for any value of IMAT-free SMM than control subjects (●).*

Multivariate models to predict independent variables of the metabolic syndrome

BFtr ($r = -0.57$, $P < 0.0001$) and SHBG ($r = 0.23$, $P = 0.001$) were the only independent variables accounting for 48% of the variance in HOMA2%S, whereas status (i.e., having Klinefelter's syndrome or not), testosterone, CRP, adiponectin, leptin, and IMAT-free SMM did not enter the model. In contrast, in a similar analysis, Klinefelter's syndrome ($r = -0.42$, $P < 0.0001$) along with IMAT-free SMM ($r = 0.31$, $P < 0.0001$), BFtr ($r = -0.20$, $P = 0.005$), and age ($r = -0.30$, $P < 0.0001$) accounted for 56% of the variance of $V_{O_{2max}}$.

In a model with BFtr as the dependent variable, leptin ($r = 0.61$, $P < 0.0001$), age ($r = 0.2$, $P < 0.0001$), $V_{O_{2max}}$ ($r = -0.24$, $P < 0.0001$), adiponectin ($r = -0.10$, $P = 0.004$), and SHBG ($r = -0.16$, $P = 0.002$) accounted for 80% of the variance, excluding Klinefelter's syndrome status, testosterone, free testosterone, and CRP from the model. Finally, in a logistic regression model with the dichotomous variable metabolic syndrome as the dependent variable, BFtr was the only independent predictor (odds ratio = 1.23, $P < 0.0001$), excluding testosterone, free testosterone, SHBG, status (Klinefelter's syndrome or not), CRP, leptin, adiponectin, age, and $V_{O_{2max}}$ from the model.

CONCLUSIONS — The main result of the present study is the strikingly increased frequency of the metabolic syndrome in Klinefelter's syndrome, with a high occurrence of increased body fat, waist circumference, insulin resistance, and increased LDL cholesterol and CRP levels, but with apparently normal blood pressure and, paradoxically, a normal level of adiponectin. The strongest predictor of the metabolic syndrome was adiposity and especially BFtr. For any given BMI value, Klinefelter's syndrome patients have higher percentage of BFtr than control subjects, even in the normal range of BMI (Fig. 1*A*). Although Becker et al. (23) in 1966 stated that 50% of their 50 Klinefelter's syndrome patients were obese (but slim during their adolescence), the typical man with Klinefelter's syndrome has always been described as tall and slim, with narrow shoulders and long arms and legs. In contrast to this dogmatic picture, we found a dramatic change in body composition in Klinefelter's syndrome patients compared with normal control subjects.

Hypogonadism in Klinefelter's syndrome is relative rather than absolute. The median total testosterone level was in the low-normal range but was substantially and significantly lower than the testosterone level in the control subjects, similar to previous findings (24), with reciprocally increased levels of LH and FSH (24,25), clearly illustrating that these Klinefelter's syndrome patients are hypogonadal. In contrast to some reports (25–27) but in accordance with others (24), SHBG was significantly lower and 17β-estradiol was normal in Klinefelter's syndrome patients.

Almost half of the Klinefelter's syndrome patients fulfilled the NCEP/ATPIII criteria for the metabolic syndrome, whereas only 10% of the control subjects did, even though no difference in blood pressure was detected. Plasma lipids were increased, except for HDL cholesterol, which was reduced. A prospective study of Japanese-American men showed that those with testosterone levels in the lower quartile had a 2.3-fold increased risk of developing metabolic syndrome (10), somewhat lower than the ~5 times elevated risk in our study. Klinefelter's syndrome patients had a higher frequency of impaired fasting glycemia and diabetes. This corresponds with the report of Nielsen et al. in 1969 (4), who found diabetic results for oral glucose tolerance tests in 39% of their Klinefelter's syndrome patients. Calculation of insulin sensitivity by the HOMA model showed a significant decrease in Klinefelter's syndrome patients but a significant increase in β-cell function, which reflects the fact that the Klinefelter's syndrome patients indeed are insulin resistant and compensate with increased production of insulin. This is partially in contrast to a recent report in which fasting hyperinsulinemia was present but with a nonsignificant decrease in insulin sensitivity (5). The participants in that study were young (22 years) and fairly lean (BMI 24 kg/m^2), and the study may have been underpowered. Pei et al. (3) found that Klinefelter's syndrome patients ($n = 7$) as well as hypogonadotropic hypogonadal patients ($n = 7$) were insulin resistant and hyperinsulinemic as judged by an insulin sensitivity test and oral glucose tolerance test. Nevertheless, we find it quite striking to uncover such a high incidence of the metabolic syndrome.

CRP, a marker of low-grade inflammation and a predictor of cardiovascular disease (28), was significantly increased in Klinefelter's syndrome patients. This is in concert with a cross-sectional study on middle-aged nondiabetic men, in which testosterone, free testosterone, and SHBG had an inverse correlation with CRP (29). Adiponectin has been reported to be inversely correlated to obesity (30); however, in the present study, the level of adiponectin in Klinefelter's syndrome was comparable to that of control subjects, which may be explained by the concomitant hypogonadism that has been shown to increase the level of adiponectin independently of BMI (31). Further, testosterone treatment has been shown to normalize (decrease) adiponectin (31–33), and likewise we found a significantly lower level of adiponectin in treated compared with untreated Klinefelter's syndrome patients. Whether the increased amount of adiponectin may counteract the other risk factors seen in Klinefelter's syndrome (increased CRP, total cholesterol, and decreased HDL cholesterol levels) is unknown. Epidemiological studies on mortality in Klinefelter's syndrome have shown an increased risk of dying from circulatory diseases (34,35) but not ischemic heart disease (34). Leptin is also correlated to the amount of body fat (36), and we found a tremendous increase in the Klinefelter's syndrome patients, probably reflecting their increased TBF. Maximal oxygen uptake was diminished in Klinefelter's syndrome patients, and, in multivariate analysis, it correlated negatively to BFtr, diagnosis of Klinefelter's syndrome, 17β-estradiol, and age but positively correlated to the IMAT-free SMM. Decreased LBM (which partially reflects muscle mass) has been described in hypogonadal states, and testosterone treatment can increase LBM, muscle size (12,37), and strength (12). The effect of hypogonadism on $V_{O_{2max}}$ and, thus, on physical fitness may be operative through several mechanisms; the decrease in muscle mass and increase in fat mass makes physical activity more difficult, and a well-known symptom in hypogonadal states is fatigue, which in turn makes exercise even more difficult. In the multivariate analysis, Klinefelter's syndrome status itself was the strongest (negative) predictor of $V_{O_{2max}}$, followed by SMM. Remarkably, for any given size of SMM, Klinefelter's syndrome patients had a significantly lower $V_{O_{2max}}$ (Fig. 1*C*).

In multivariate analyses, BFtr was the independent variable with the most significant impact on both the metabolic syndrome and measures of insulin sensi-

tivity. When controlling for BFtr, the impact of hypogonadism on the presence of the metabolic syndrome or not and on insulin sensitivity disappeared. This result supports previous findings in type 2 diabetic patients and healthy volunteers by Abate et al. (38) and Tsai et al. (39) who both found that measures of insulin resistance, hepatic glucose output, and insulin secretion were not dependent on sex hormone levels after controlling for upper body obesity.

Because of the cross-sectional design of this study, we cannot determine the order of events that eventually lead to increased incidence of metabolic syndrome in Klinefelter's syndrome. Whether increased TBF precedes the hypogonadal state in Klinefelter's syndrome is speculative and probably not likely, and it seems more plausible that the hypogonadal state and increased TBF are both part of a vicious cycle in Klinefelter's syndrome. However, although the cross-sectional nature precludes most conclusions on causality, the fact that the parameter "Klinefelter's syndrome status" in a multiple linear regression model of $V_{O_{2max}}$ is a significant contributor to the observed differences between Klinefelter's syndrome and control subjects shows that the genotype, i.e., having Klinefelter's syndrome, does explain a part of the observed differences. The consequences of a given genotype materialize long before the present measurements and can be viewed as a stable marker of host susceptibility, enabling one to draw conclusions regarding causality even from a studies with a cross-sectional design (40).

When comparing the group of testosterone-treated with untreated Klinefelter's syndrome patients, we did not find dramatic differences. The only significant differences found were decreases in FSH, LH, LDL cholesterol, and adiponectin and an increase in 17β-estradiol. The amount of body fat tended to be lower as did fasting plasma glucose, total cholesterol, leptin, and CRP. An explanation for this unexpected lack of difference could be the use of inadequate low doses of testosterone, reflected by the lack of difference in testosterone or free testosterone between the treated and untreated groups of Klinefelter's syndrome patients and the inability to suppress FSH and LH to normal values. The higher levels of 17β-estrogen in the testosterone-treated Klinefelter's syndrome group could be caused by an increased aromatase activity. Supporting this hypothesis is the significantly higher ratio between 17β-estrogen and testosterone in the untreated Klinefelter's syndrome group compared with control subjects although the absolute level of 17β-estrogen is not increased. The decrease in adiponectin in the testosterone-treated Klinefelter's syndrome group is a potentially adverse effect of testosterone treatment, but whether this rather negative effect of testosterone treatment is counterbalanced by the concomitant reduction in LDL and total cholesterol, fat mass, and fasting glucose is currently unknown. Although not proven from this or other studies, it seems reasonable that testosterone supplementation should be offered to almost all patients with Klinefelter's syndrome.

In summary, we describe for the first time the severe magnitude of the metabolic syndrome in Klinefelter's syndrome. A number of components of the metabolic syndrome are present Klinefelter's syndrome, but, notably, normal blood pressure was found. Significant truncal obesity was present. Hypogonadism is frequent in Klinefelter's syndrome, and we recommend that all patients with Klinefelter's syndrome should be treated properly with testosterone substitution. However, prospective randomized studies are needed to prove the postulated efficacy of testosterone supplementation in preventing the occurrence of the metabolic syndrome.

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