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Bioactive androgens and glucuronidated androgen metabolites are associated with subcutaneous and ectopic skeletal muscle adiposity among older black men

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ABSTRACT

Aging is associated with declining serum levels of androgenic hormones and with increased skeletal muscle fat infiltration, an emerging risk factor for type 2 diabetes mellitus (T2DM). Androgens regulate fat mass and glucose homeostasis, but the effect of androgenic hormones on skeletal muscle fat infiltration is largely unknown. Thus, the aim of the current study was to examine the association of serum androgens and their precursors and metabolites with skeletal muscle fat infiltration and T2DM in a black male population group at high risk of T2DM. Serum androgens, estrogens, and androgen precursors and metabolites were measured using mass spectrometry; and calf skeletal muscle fat distribution (subcutaneous and intermuscular fat; skeletal muscle density) was measured using quantitative computed tomography in 472 Afro-Caribbean men 65 years and older. Bioactive androgens, testosterone, free testosterone, and dihydrotestosterone were associated with less skeletal muscle fat infiltration (r = –0.14 to –0.18, $P < .05$) and increased skeletal muscle density (r = 0.10 to 0.14, $P < .05$), independent of total adiposity. In addition, glucuronidated androgen metabolites were associated with less subcutaneous fat (r = –0.11 to –0.15, $P < .05$). Multivariate logistic regression analysis identified an increased level of 3α-diol-3 glucuronide (odds ratio = 1.38, $P < .01$) and a decreased level of dihydrotestosterone (odds ratio = 0.66, $P < .01$) to be significantly associated with T2DM. Our findings suggest that, in elderly black men, independent of total adiposity, bioactive androgens and glucuronidated androgen metabolites may play previously unrecognized role in skeletal muscle fat distribution. Longitudinal studies are needed to further evaluate

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the relationship between androgens and androgen metabolites with changes in skeletal muscle fat distribution with aging and the incidence of T2DM.

1. Introduction

Black populations have a greater propensity to develop obesity and type 2 diabetes mellitus (T2DM) than other ethnic groups [1]. Although high rates of T2DM in blacks are often attributed to their high rates of obesity, differences in total and central obesity fail to explain differences in T2DM rates [2-8], especially among men [1]. Emerging evidence indicates that insulin resistance and T2DM may be a result of the imbalance of fat distribution in nonadipose tissues, such as skeletal muscle [9]; but the nature of these relationships remains to be clarified, and causality continues to be debated. There is also some evidence that ectopic skeletal muscle fat infiltration, or myosteatosis [9], increases with aging and is greater in black than in white men at all levels of total adiposity [10].

Androgens decline with aging [11] and play an important role in the regulation of overall body fat mass and fat distribution [12], as well as glucose and insulin homeostasis [13]. Although the inverse association of androgens with general and central adiposity [14] and with T2DM [15] is well documented in white men, studies have not assessed relationships with myosteatosis, an emerging risk factor for T2DM, or the relationship of androgen precursors and metabolites with adiposity and T2DM, particularly among black men. Androgens are secreted primarily from the testes, but can also be synthesized and degraded in peripheral target tissues, in the same tissues in which they exert their action. This somewhat limits the use of serum levels of androgens, suggesting that androgen metabolites may reflect intracellular androgen levels beyond serum androgen levels [16] and may be better markers of androgen activity in peripheral tissues [17].

The aim of the current study is to examine the association of serum androgens and their precursors and metabolites with myosteatosis and T2DM in a unique older Afro-Caribbean male population, with low levels of total adiposity, but high risk of T2DM [10]. We hypothesized that higher levels of androgenic hormones would be associated with less skeletal muscle fat infiltration and with lower T2DM risk, independent of total adiposity.

2. Materials and methods

2.1. Study population

Between 1997 and 2003, 3170 previously unscreened men were recruited for a population-based prostate cancer screening study on the Caribbean Island of Tobago, Trinidad and Tobago [18]. To be eligible, men had to be ambulatory, noninstitutionalized, and not terminally ill. Recruitment for the survey was accomplished by flyers, public service announcements, posters, informing health care workers at local hospital and health centers, and word of mouth. Approximately 60% of all age-eligible men on the island participated, and participation was similar across the island parishes. All men were invited to participate in a follow-up clinic examination between 2004 and 2007, and 2031 men in the cohort (70% of survivors) and 451 new participants completed the visit. There were 618 Afro-Caribbean (all 4 grandparents of African ancestry) men 65 years and older who completed the follow-up visit. Serum sex steroid hormone measurements were completed in a random subset of 500 men 65 years and older. Four hundred seventy-two men had complete data on anthropometrics, peripheral quantitative computed tomography (pQCT) measured skeletal muscle composition, demographic information, and medical history. Written informed consent was obtained from every participant, using forms and procedures approved by the Tobago Ministry of Health and Social Services and University of Pittsburgh Institutional Review Boards.

2.2. Hormone assays

Blood samples were obtained in the morning (between 7:00 AM and 9:00 AM) by venipuncture after a 12-hour fast. Whole blood was drawn into sterile red top (serum) tubes and stood at room temperature for a minimum of 20 minutes to clot before centrifugation, and the serum was aliquoted into 1.0-mL cryovials and stored at –80°C until assays were completed. Hormone assays were completed using a validated and highly specific gas chromatography/liquid chromatography/mass spectrometry technique to measure fasting serum levels of unconjugated sex steroid precursors (dehydroepiandrosterone [DHEA], dehydroepiandrosterone sulfate [DHEA-S], androstenedione [4-DIONE], androst-5-ene-3β, 17β-diol [5-DIOL]), sex steroids (testosterone [T], dihydrotestosterone [DHT], estrone [E1], estradiol [E2]), and conjugated androgen metabolites (androstane-3α,17β-diol-3-glucuronide [3G], androstane-3α,17β-diol-17-glucuronide [17G], androsterone-glucuronide [ADT-G]) under Good Laboratory Practices. Details of the laboratory methods have been described [17,19]. Sex hormone binding globulin (SHBG) assays were completed with an Immulite Analyzer with chemiluminescent substrate (Diagnostic Products, Los Angeles, CA). Free fractions of T and estradiol were calculated using the method described by Sodergard et al [20]. The coefficients of variation (CVs) for all sex hormones are listed in Supplementary Table 1.

2.3. Peripheral quantitative computed tomography

A pQCT scan of the calf was performed using the Stratec XCT-2000 to evaluate skeletal muscle fat and muscle cross-sectional areas. Scans were obtained at 66% of the calf length, proximal to the terminal end of the calf. This site was chosen because it is the region of the lower leg with the largest circumference of the calf with very little variability across individuals [21]. Different tissues in the analyses were

separated according to different density thresholds, using the "soft tissue" algorithm. Based on the calibration, fat, muscle, and cortical bone are measured with mineral equivalent densities of 0, 80, and 1200 mg/cm^3, respectively. Therefore, changes in muscle tissue to fat-like tissue will be detected as a shift in mineral equivalent density of the muscle from 80 to 0 mg/cm^3. Images of the cross-sectional area of skeletal muscle and fat were analyzed using the Stratec analysis software version 5.5D (Orthometrix, White Plains, NY). To maintain consistency, all images were analyzed by a single investigator (CL Gordon). There are 2 fat depots within skeletal muscle: fat infiltration within myocytes (intramyocellular fat) and visible fat within the fascia surrounding skeletal muscle (intermuscular fat). We obtained measures of the total fat area (square millimeters), subcutaneous fat area (square millimeters), intermuscular fat area (square millimeters), skeletal muscle area (square millimeters), and muscle density (milligrams per cubic centimeter). Muscle density is a valid measure of fat infiltration within the skeletal muscle and reflects the fat content of skeletal muscle such that greater fat infiltration is associated with lower muscle density [22]. The CVs for pQCT skeletal muscle composition traits are listed in Supplementary Table 1.

2.4. Other measures

Standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was recorded to the nearest 0.1 kg without shoes on a balance beam scale. Body mass index was calculated from body weight and standing height (kilograms per square meter). Waist circumference was measured at the narrowest point of the waist using an inelastic fiberglass tape. If there was no narrowest point, waist circumference was measured at the umbilicus. Total body fat was measured by dual-energy x-ray absorptiometry (DXA) using a Hologic QDR 4500W densitometer (Hologic, Bedford, MA). Scans were analyzed with QDR software version 8.26a. Information on medical conditions and medication use were also assessed using interviewer-administered questionnaires. No participant used oral corticosteroids. Fasting serum glucose was measured using an enzymatic procedure [23], and insulin was measured using a radioimmunoassay procedure developed by Linco Research [22]. Their CVs are listed in Supplementary Table 1. The degree of insulin resistance was estimated by homeostasis model assessment (HOMA) according to the method described by Matthews et al [24]. *Type 2 diabetes mellitus* was defined as fasting serum glucose of at least 126 mg/dL or currently taking antidiabetic medication. *Obesity* was defined as BMI of at least 30 kg/m^2.

2.5. Statistical analyses

The distributions of all the continuous measures were first assessed for departures from normality. Dehydroepiandrosterone sulfate, DHT, androsterone (ADT), 3G, 17G, and intermuscular fat were log transformed, whereas subcutaneous fat was square root transformed before statistical analysis. The association between serum sex hormones and adiposity and T2DM-related measures was determined using Spearman correlation analysis. Spearman correlations among all sex steroid variables were calculated and are shown in Supplementary Table 2. Analyses of insulin, glucose, and HOMA traits were conducted after excluding individuals with fasting serum glucose of at least 126 mg/dL or those currently taking antidiabetic medication because we were interested in associations among normoglycemic individuals. Because of their presumed relationships with the components of skeletal muscle composition and our focus on skeletal muscle fat infiltration independent of age, body size, total adiposity, and skeletal muscle, age, height, DXA total body fat, and skeletal muscle were evaluated as covariates. Logistic regression was used to examine the relationships between sex hormones and adiposity measures with T2DM, which were presented in age-adjusted, age- and BMI-adjusted, and age- and waist-adjusted models. The odds ratio (OR) and 95% confidence intervals (CIs) are presented by 1–standard deviation increase for all continuous variables. All variables with P value < .05 in age-adjusted models were further considered for inclusion in the multiple linear regression analysis using a backward procedure to determine the independent associations with T2DM. We examined multicollinearity before the multiple linear regression analysis by assessing the variance inflation factor. We included one total or central adiposity measure (BMI, waist, or DXA total body fat percentage) with no multicollinearity along with sex hormones and pQCT skeletal muscle composition variables in the backward logistic regression model. The model with the best goodness-of-fit is presented as the final model. The Statistical Analysis System (SAS, version 9.1.2; SAS Institute, Cary, NC) and the Statistical Package for the Social Sciences (SPSS, version 17; SPSS, Chicago, IL) were used for statistical analysis.

3. Results

3.1. Characteristics of participants

The mean age of the men was 72 ± 6 years (range, 65-92 years; Table 1). On average, participants were moderately overweight as measured by BMI (mean BMI, 26.8 ± 4.1 kg/m^2), but had relatively low total body fat (mean DXA total body fat percent, 21.7% ± 5.7%). Approximately 19% of the men were obese, and 32% had T2DM.

3.2. Association of sex steroid hormones with adiposity, skeletal muscle composition, and T2DM-related measures among older black men

Sex hormone binding globulin, androgen precursors 4-DIONE and 5-DIOL, all bioactive androgens (T, free testosterone [FT], and DHT), and the androgen metabolite ADT were inversely associated with BMI, waist circumference, DXA total body fat percentage, and pQCT total fat area in the calf (r = –0.11 to –0.42, all P < .05, Table 2). All 3 bioactive androgens were negatively associated with intermuscular fat (r = –0.14 to –0.18, P < .01, Table 2), whereas FT, DHT, ADT, and 17G were positively associated with muscle density (r = 0.10 to 0.17, P < .05, Table 2). The glucuronidated androgen metabolites, ADT-G, 3G, and 17G, were inversely associated with subcutaneous

Table 1 – Selected characteristics of older black men (mean ± SD)

N	472
Age (y)	72.2 ± 6.0
BMI (kg/m^2)	26.8 ± 4.1
Waist circumference (cm)	92.8 ± 10.4
DXA total body fat (%)	21.7 ± 5.7
Calf skeletal muscle composition	
Total fat (mm^2)	1819 ± 890
Subcutaneous fat (mm^2)	1237 ± 660
Intermuscular fat (mm^2)	402 ± 420
Skeletal muscle density (mg/cm^3)	78.0 ± 3.7
Skeletal muscle (mm^2)	6833 ± 1227
T2DM-related measures	
Glucose (mg/dL)	111 ± 41
Insulin (μU/mL)	11.5 ± 5.4
HOMA-IR	3.2 ± 2.1
Medical conditions	
Obese (%)	18.6%
T2DM (%)	32.2%
Serum sex steroid hormones	
SHBG (nmol/L)	53.0 ± 19.5
DHEA (ng/mL)	1.5 ± 0.9
DHEA-S (μg/mL)	1.0 ± 0.6
4-DIONE (ng/mL)	0.6 ± 0.2
5-DIOL (pg/mL)	607.3 ± 315.6
T (ng/mL)	4.6 ± 2.0
FT (ng/dL)	8.7 ± 3.25
DHT (pg/mL)	476.5 ± 245.8
ADT (pg/mL)	177.3 ± 90.0
ADT-G (ng/mL)	31.7 ± 17.4
3G (ng/mL)	1.3 ± 0.9
17G (ng/mL)	2.7 ± 1.5
E1 (pg/mL)	42.2 ± 15.5
E2 (pg/mL)	25.1 ± 9.4

fat (r = –0.11 to –0.15, P < .05, Table 2). We also found that SHBG was positively associated with skeletal muscle (r = 0.10, P < .05, Table 2), whereas ADT was negatively associated with skeletal muscle (r = –0.16, P < .01, Table 2). All findings related to skeletal muscle composition were independent of age, height, and total adiposity. We found no association between serum estrogen concentrations and androgen precursors DHEA and DHEA-S with any adiposity measures (data not shown). None of the androgens or estrogens was associated with fasting glucose levels (data not shown). SHBG, T, and DHT were negatively correlated with insulin (r = –0.21, r = –0.16, and r = –0.15, respectively; all P < .05) and the HOMA index (data not shown) after adjusting for age and BMI.

3.3. *The relationship of sex steroid hormones, adiposity, and skeletal muscle composition measures with T2DM among older black men*

We further quantified the relationship of serum sex steroid hormones, adiposity, and skeletal muscle composition measures with T2DM, adjusting for age, age and BMI, or age and waist circumference (Table 3). Serum concentrations of 5-DIOL and all 3 bioactive androgens (T, FT, and DHT) were inversely associated with T2DM, whereas serum levels of ADT-G and 3G were positively associated with T2DM in all models. Subcutaneous fat and skeletal muscle were inversely associated with T2DM, whereas intermuscular fat was positively associated with T2DM only in the age-adjusted model.

We further tested the independent associations of these measures with T2DM using backward multivariate logistic regression analysis. The model with the best goodness-of-fit is presented as the final model (Table 3). Dihydrotestosterone, subcutaneous fat, and skeletal muscle remained inversely correlated with T2DM, whereas 3G and waist circumference remained positively correlated with T2DM.

4. Discussion

We examined the association of serum androgens and their precursors and metabolites with ectopic skeletal muscle fat infiltration and indices of T2DM risk in a black population at

Table 2 – Correlation of serum levels of androgens with adiposity and skeletal muscle composition in black men

		Androgen precursors		Bioactive androgens			Androgen metabolites			
	SHBG	4-DIONE	5-DIOL	T	FT	DHT	ADT	ADT-G	3G	17G
BMI (kg/m^2) [a]	–0.42 *	–0.14 †	–0.14 †	–0.35 ‡	–0.21 ‡	–0.37 ‡	–0.20 ‡	0.07	0.07	0.17 †
Waist (cm) [a]	–0.41 ‡	–0.13 *	–0.15 †	–0.38 ‡	–0.25 ‡	–0.36 ‡	–0.18 †	0.09	0.13 *	0.16 †
DXA total body fat (%)	–0.33 ‡	–0.17 †	–0.14 †	–0.33 ‡	–0.22 ‡	–0.32 ‡	–0.22 †	0.07	0.07	0.18 †
Total fat (mm^2) [b]	–0.22 ‡	–0.13 †	–0.11 †	–0.28 ‡	–0.23 ‡	–0.20 ‡	–0.17 †	–0.04	–0.03	0.01
Subcutaneous fat (mm^2) [c]	0.06	0.02	0.03	0.01	–0.01	0.08	0.04	–0.11 *	–0.15 †	–0.14 †
Intermuscular fat (mm^2) [c]	–0.08	–0.06	–0.10	–0.18 †	–0.14 †	–0.15 †	–0.08	0.04	0.03	–0.09
Skeletal muscle density (mg/cm^3) [d]	–0.06	0.05	0.02	0.10	0.14 *	0.10 *	0.14 *	0.11	0.03	0.17 †
Skeletal muscle (mm^2) [b]	0.10 *	–0.05	0.05	–0.06	–0.02	–0.06	–0.16 †	–0.01	–0.02	0.04

Values are Spearman correlation coefficients.
* P < .05.
† P < .01.
‡ P < .001.
[a] Adjusted for age.
[b] Adjusted for age and height.
[c] Adjusted for age, height, and DXA total body fat.
[d] Adjusted for age, height, DXA total body fat, and pQCT skeletal muscle area.

Table 3 – The association of serum sex hormone levels with prevalent T2DM in black men

	Age-adjusted ORs (95% CI)	Age- and BMI-adjusted ORs (95% CI)	Age- and waist-adjusted ORs (95% CI)	Multivariate ORs (95% CI)
Sex steroid hormone				
SHBG (nmol/L)	**0.72 (0.57-0.89)**	**0.77 (0.60-0.98)**	0.80 (0.62-1.03)	–
DHEA (ng/mL)	0.98 (0.95-1.00)	0.81 (0.65-1.00)	0.82 (0.66-1.02)	–
DHEA-S (μg/mL)	1.04 (0.85-1.28)	1.05 (0.85-1.29)	1.06 (0.86-1.30)	–
4-DIONE (ng/mL)	0.96 (0.78-1.16)	0.97 (0.80-1.19)	0.98 (0.80-1.20)	–
5-DIOL (pg/mL)	**0.72 (0.58-0.89)**	**0.75 (0.60-0.93)**	**0.75 (0.60-0.94)**	–
T (ng/mL)	**0.71 (0.58-0.87)**	**0.75 (0.60-0.93)**	**0.77 (0.62-0.96)**	–
FT (ng/dL)	**0.77 (0.64-0.94)**	**0.80 (0.65-0.98)**	**0.82 (0.66-0.99)**	–
DHT (pg/mL)	**0.66 (0.53-0.82)**	**0.69 (0.55-0.87)**	**0.69 (0.55-0.87)**	**0.66 (0.52-0.84)**
ADT (pg/mL)	**0.81 (0.66-0.99)**	0.84 (0.68-1.03)	0.84 (0.68-1.03)	–
ADT-G (ng/mL)	**1.32 (1.10-1.60)**	**1.32 (1.08-1.60)**	**1.32 (1.08-1.60)**	–
3G (ng/mL)	**1.26 (1.05-1.52)**	**1.27 (1.05-1.54)**	**1.27 (1.04-1.53)**	**1.38 (1.10-1.74)**
17G (ng/mL)	1.03 (0.85-1.25)	1.00 (0.82-1.22)	1.00 (0.82-1.22)	–
E1 (pg/mL)	0.93 (0.76-1.13)	0.92 (0.75-1.12)	0.92 (0.75-1.12)	–
E2 (pg/mL)	0.92 (0.75-1.12)	0.91 (0.74-1.11)	0.90 (0.74-1.10)	–
Total and central adiposity				
BMI (kg/m^2)	**1.27 (1.05-1.54)**	N/A	N/A	–
Waist (cm)	**1.40 (1.14-1.70)**	N/A	N/A	**1.61 (1.20-2.10)**
DXA total body fat (%)	**1.26 (1.03-1.52)**	N/A	N/A	–
Skeletal muscle composition				
Total fat (mm^2)	1.06 (0.88-1.29)	N/A	N/A	–
Subcutaneous fat (mm^2)	**0.90 (0.78-1.00)**	**0.71 (0.55-0.91)**	**0.67 (0.53-0.86)**	**0.68 (0.52-0.88)**
Intermuscular fat (mm^2)	**1.34 (1.10-1.64)**	1.22 (0.97-1.54)	1.18 (0.94-1.49)	–
Skeletal muscle density (mg/cm^3)	0.94 (0.74-1.21)	0.97 (0.75-1.25)	1.02 (0.79-1.31)	–
Skeletal muscle (mm^2)	0.84 (0.68-1.04)	**0.75 (0.60-0.94)**	**0.75 (0.60-0.94)**	**0.78 (0.61-1.00)**

Odds ratio is presented for a 1-SD increase in predictor variable. Significant ORs are highlighted in boldface font. Logistic regression analysis was used to obtain model 1 (age-adjusted ORs), model 2 (age- and BMI-adjusted ORs), and model 3 (age- and waist-adjusted ORs). Backward regression analysis was used to obtain the multivariate ORs.

high risk of T2DM [25]. Our findings suggest that there is an inverse association between fat infiltration in skeletal muscle, fasting insulin and insulin resistance, and bioactive androgens, independent of total adiposity. Moreover, in multivariable models, higher dihydrotestosterone was independently associated with a lower, whereas ADT-G was associated with a higher, prevalence of T2DM. We also confirmed previous work in white men and young black men [14,26-30], and extended the existing literature by reporting that higher levels of bioactive androgens are associated with lower total and central adiposity among older black men.

The data regarding androgenic hormone effects on fat infiltration in skeletal muscle are very limited. Previous study among 54 healthy young eugonadal white men has reported that T administration decreases ectopic skeletal muscle fat infiltration [31]. Our results raise the possibility that lower androgenic hormone levels may promote increased fat infiltration in skeletal muscle and insulin resistance. However, it is also possible that increased skeletal muscle fat infiltration may have an effect on androgen metabolism and/or androgen production; and such hypotheses should be tested in future longitudinal studies in this population. The presence of androgens and androgen receptors in fat is well established [32], and an inverse association between serum T concentrations and total and central body fat in men is well known [14,29,33].

With the exception of FT in our study, all bioactive androgens were inversely associated with fasting insulin and insulin resistance. In addition, DHT was independently associated with a decreased prevalence of T2DM. A meta-analysis of 43 studies and 6427 men and several large studies in African American men suggest a strong link between low T levels and hyperglycemia, insulin resistance, and T2DM [13,15,34]. Whether insulin and insulin resistance impair T synthesis, or reduced T increases hyperinsulinemia and insulin resistance is still uncertain. A few longitudinal studies, such as the Massachusetts Male Aging Study [35] and the Rancho Bernardo Study [36], have reported a positive association between low baseline bioactive androgens and future onset of T2DM among men; but additional longitudinal studies are needed in large multiethnic cohorts. Although epidemiological evidence suggests a consistent association between hyperglycemia, insulin resistance, T2DM, and low T levels, the mechanism behind these associations are still unclear. It is possible that the Leydig cell dysfunction, caused by insulin resistance mediated changes in the production of hormones and cytokines locally in the target tissue and in adipose tissue, is responsible for low T levels observed in insulin resistance and T2DM [37]. Androgens may also influence insulin resistance via activation of peroxisome proliferator-activated receptor–α [38] and by reducing serum levels of tumor necrosis factor–α [39]. Nonetheless, some have suggested that androgen deficiency is a consequence of a poor metabolic status in diseases such as obesity and T2DM rather than a direct cause of these conditions [40]. It is possible that the relationship between lower androgen levels and insulin resistance is mediated by total and central adiposity as was previously shown in nondiabetic white men [41]. However, in

our analyses, we found that the association between DHT and T2DM was independent of overall adiposity, waist circumference, and subcutaneous fat.

We further found associations between androgen metabolites and adiposity. In particular, 17G and, to some extent, 3G were positively related to total and central adiposity and inversely to subcutaneous fat. Furthermore, 17G was associated with greater skeletal muscle density. There are limited data on the association of androgen metabolites and adiposity. The role and importance of glucuronidated metabolites are still controversial. Previous studies have reported a positive association of 3G with total and visceral fat in white men [42], an increase in 3G levels with weight gain [43], and a decrease in 3G levels with weight loss in young white men [44]. However, comparison of our results with these previous studies is difficult because glucuronidated androgen metabolites were derived using different methods, such as radioimmunoassay, which could not separate 3G and 17G. Using liquid chromatography–tandem mass spectrometry, Vandeput et al [30] found a positive association between 17G and central adiposity in white men. Our work extends these observations to black men. Vadenput et al [30] also reported no association of 3G with body fat in white men and hypothesized that fat tissue is mainly involved in 17-glucuronidation of 3-diol, whereas 3-glucuronidation of 3-diol is mainly dependent on glucuronidation in other tissues. The physiological role and importance of glucuronidated metabolites are still controversial. Increased fat may lead to a greater capacity for 17-glucuronidation of 3-diol and, subsequently, to increased levels of 17G. However, it is possible that glucuronidated metabolites are directly involved in the regulation of fat tissue; but a probable causal link and cellular mechanisms remain to be tested and established in future studies.

We confirmed an association between SHBG, adiposity, and insulin resistance [45,46]. Some have hypothesized that the association between bioactive androgens and T2DM is mediated through SHBG [47]. A previous study has shown that insulin may directly inhibit SHBG secretion from hepatoma cells in vitro [48]. A recent study of 40- to 70-year-old predominantly white men examined the association of SHBG and T2DM after adjusting for total and free T levels [47]. Although we found SHBG to be associated with insulin and insulin resistance, after adjusting for waist circumference and DHT androgens in our sample, we found no association between SHBG and T2DM, suggesting that, among black men, the association between SHBG and insulin and insulin resistance may be mediated though central adiposity and DHT.

We found no association between serum estrogens and any of the adiposity- or T2DM-related measures. Some studies suggest that, in obese men, higher estradiol levels, generated by aromatization of androgens in peripheral fat tissues [49], suppress gonadotropic hormone levels and thus testicular T production [50]. Estradiol was positively associated with adiposity [51] and T2DM [15] in men in some studies, whereas, in others, no association between estradiol and obesity was observed [52]. None of these past studies examined the relationships between estrogen levels and skeletal muscle fat infiltration.

The potential mechanisms responsible for androgen effects on fat infiltration in skeletal muscle are unknown and require further investigation. Testosterone inhibits the activity of lipoprotein lipase, the main enzymatic regulator of triglyceride uptake in fat [53], resulting in inhibition of lipid uptake and increased lipid mobilization [54]. Androgens also inhibit the differentiation of human mesenchymal pluripotent stem cells and preadipocytes into adipocytes and stimulate the commitment into the myogenic lines via the androgen receptor [55]. Whether these mechanisms mediate androgenic hormone effects on skeletal muscle fat infiltration is unclear and will require further research.

We found subcutaneous fat to be independently and inversely associated with T2DM. Previous studies have shown that, independent of overall adiposity, lower subcutaneous fat is associated with glucose abnormalities, insulin resistance, and T2DM [25,56]. Some have hypothesized that, in addition to impaired lipid storage and utilization, an overflow of fat storage in the inter- and intramuscular compartments may be due to a defect in the ability of subcutaneous fat to store excess fatty acids [57]. Previous studies have suggested that increased accumulation of skeletal muscle fat may impair the insulin receptor substrate 1/phosphatidylinositol 3-kinase pathway and growth-factor-regulated protein kinase B pathway of insulin signaling [58]. Others have suggested that increased accumulation of lipid intermediates, such as diacylglycerol, long-chain fatty acyl-CoA species, ceramides, and oxidized lipid mediators, due to increased accumulation of fat in myocytes may be responsible for suppressing insulin signaling [59]. Others have proposed that the link between myosteatosis and T2DM may be through impaired secretion of cytokines [60]. However, all previous studies have been cross-sectional; and a causal link between myosteatosis, subcutaneous fat, and T2DM remains to be established in longitudinal studies.

The present study has several potential limitations. First, we assessed the associations between sex hormones and adiposity and T2DM among older black men, which may limit the generalizability of our findings. Second, it is important to emphasize that T2DM was ascertained without an oral glucose tolerance test and that some misclassification was likely to have occurred. Finally, our study was cross-sectional in design; and a longitudinal study would better delineate the effects of sex hormones on fat infiltration in skeletal muscle and T2DM risk.

In conclusion, this report is the first to investigate the association between sex steroid hormones and metabolites with skeletal muscle fat infiltration measured by QCT. Our findings support the notion that bioactive androgens and glucuronidated androgen metabolites may play previously unrecognized role in skeletal muscle fat distribution, suggestive of possibly another link between androgens and T2DM. In addition, our findings suggest an independent association of bioactive androgens, glucuronidated androgen metabolites, and subcutaneous fat around skeletal muscle with T2DM. Therefore, longitudinal studies are critically needed to evaluate the relationship between androgens and metabolites with changes in skeletal muscle fat distribution with aging, and to further test if androgen metabolites and/or skeletal muscle fat distribution is related to the incidence of T2DM in high-risk black male populations. Such studies may provide new insight into the pathophysiology of T2DM and suggest new

therapeutic targets for the treatment and prevention of insulin resistance and T2DM.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2010.12.014.

Conflict of Interest

CL Gordon has received a consulting fee for reading of the pQCT scans. Other authors have nothing to disclose.

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