Relationship of androgens to body composition, energy and substrate metabolism and aerobic capacity in healthy, young women

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1. Introduction

The role of testosterone in the regulation of body composition, physical capacity, and energy and substrate metabolism in women remains undefined. Current knowledge regarding the effects of testosterone stems primarily from clinical disease processes that result in testosterone excess or deficiency, most notably polycystic ovarian syndrome (PCOS).\textsuperscript{1} Characterized by androgen and estrogen excess, PCOS is associated with obesity, insulin resistance and increased cardiovascular morbidity. While studies in patients with PCOS provide insight into derangements of physiologic and/or metabolic function related to hyperandrogenemia, these data may not adequately reflect the effects of androgens within the normal physiological range. Perhaps more importantly, additional confounding factors (e.g., obesity and hyperinsulinemia in PCOS) complicate the ability to relate physiologic and metabolic sequelae to the unique effects of testosterone. Contrary to the detrimental effects suggested from studies in PCOS, recent studies from our laboratory in post-menopausal women have shown that higher levels of androgens, within the physiological range, may have beneficial effects on body composition, metabolic function and aerobic fitness \cite{1}. Understanding the role of testosterone in the regulation of adiposity and metabolic function in premenopausal women may provide valuable insight into the nature of changes in metabolic and cardiovascular risk in pathological conditions (e.g., PCOS), as well as clinical interventions that modify androgen levels (e.g., oral contraceptives, or testosterone replacement for sexual dysfunction).

The primary goal of this study, therefore, was to evaluate the relationship of circulating androgens and androgen precursors as potential determinants of total and regional body composition,
energy and substrate metabolism and aerobic fitness in premeno-
pausal women. We recruited healthy, non-obese, glucose tolerant, 
cycling females with no history or clinical evidence of endocrine 
pathology for studies evaluating the effect of ovarian steroid hor-
mones on metabolic function [2–5]. These criteria were chosen to 
avoid the confounding affects of obesity, insulin resistance, and 
hyperandrogenemia. Equally as important, we utilized validated 
techniques that were optimized to measure the low circulating 
androgen levels in this population.

2. Experimental

2.1. Subjects

Thirty healthy, young cycling women were recruited in conjunc-
tion with studies designed to evaluate the effects of ovarian 
suppression with gonadotropin hormone releasing agonist 
(GnRHa) on metabolic and physiologic function [2–5]. Women 
were selected who were non-obese (BMI < 30 kg/m²; mean ± SE, 
23.3 ± 0.5 kg/m²), had a stable body weight (±2 kg) for 6 months 
prior to study, were healthy based on medical history, physical 
exam and routine blood tests, were glucose tolerant (glucose 
<7.77 mmol/L 2 h following 75 g oral glucose load), had no history 
of tobacco use, and were not on any medication that could affect 
glucose metabolism or ovarian/reproductive function. None of 
the volunteers had clinical or biochemical evidence of hyperandro-
genemia, a history of polycystic ovary disease, or had been exposed to 
any form of hormone-based contraceptive therapy for at least 
6 months prior to study. Women reported having at least 2 sponta-
neous cycles in the 3 months prior to recruitment and a cycle 
length of between 25 and 32 days. The nature, purpose and possi-
bile risks of the study were explained to each subject before she 
gave written consent to participate. The experimental protocol 
was approved by the Committee on Human Research at the Uni-
versity of Vermont. The current report represents an analysis of 
baseline GnRHa data to evaluate the potential relationship of circu-
latating testosterone levels to body composition, energy and sub-
strate metabolism and aerobic capacity. All data were collected 
in the mid-luteal phase of the subject’s menstrual cycle.

2.2. Body composition

Body mass was measured on a metabolic scale (Scale-Tronix, 
Inc., Wheaton, IL). Fat mass, lean body mass, abdominal adiposity 
and bone mineral mass were each measured by dual energy X-ray 
absorptiometry using a GE Lunar Prodigy densitometer (GE 
Lunar Co., Madison, WI). Abdominal adiposity was measured 
between the L1 and L4 vertebral bodies using the Region of Interest 
option of the software following the general approach of Glickman 
et al. [6] with minor modifications, as described previously [2]. This 
measurement will heretofore be designated as “abdominal fat” 
measured in kg. Visceral and subcutaneous adiposity were 
assessed by computed tomography (CT) with a Phillips Billiance 40 
or 64 CT scanner (Phillips Medical Systems, Cleveland, OH) as de-
scribed previously [7]. Visceral and subcutaneous adiposity were 
available on 16 out of the 30 subjects because this measurement 
was not performed in one of the aforementioned trials.

2.3. Peak aerobic capacity (peak VO2)

Peak VO2 was measured during a graded, treadmill test to voli-
tional fatigue. Briefly, a comfortable initial walking speed was 
found for each volunteer and was maintained throughout the test. 
The grade was increased 2.5% every two minutes until volitional fa-
tigue. Peak VO2 was defined as the highest 30-s average VO2 value 
measured during the last 2 min of the test.

2.4. Oral glucose tolerance test

A 75-g OGTT was performed after an overnight fast (at 08:00 h). 
Blood samples were collected at 0, 60, 90 and 120 min for analysis 
of glucose levels via the glucose oxidase technique (YSI Life Sci-
ences, Yellow Springs, OH). Glucose AUC was determined using 
the trapezoid method.

2.5. Indirect calorimetry

Resting energy expenditure and substrate oxidation were 
performed using the ventilated hood technique (DeltaTrac, Yorba 
Linda, CA), as described [5]. The subject was gently awakened (at 
06:30 h), allowed to void if necessary, returned to bed and placed 
under the hood for 30 min. Carbon dioxide production and oxygen 
consumption were utilized to calculate the respiratory quotient.

2.6. Hormone measurements

Serum levels of testosterone, androstenedione (A4A), dehydro-
epiandrosterone (DHEA), and DHEA-sulfate (DHEA-S) were mea-
sured by radioimmunoassay. Prior to measurement, steroids 
were extracted from serum with hexane: ethyl acetate (3:2). 
Androstenedione, DHEA and testosterone were then separated by 
Celite column partition chromatography using increasing concen-
trations of toluene in trimethylpentane. Dehydroepiandrosterone 
sulfate (DHEA-S) and sex-hormone binding globulin (SHBG) were 
measured by direct chemiluminescent immunoassays using the

### Table 1

| Physical, metabolic, and functional characteristics of the study population. |
|---|---|---|
| Mean ± SE | Range |
| Age (year) | 27.3 ± 0.8 | 21–36 |
| Weight (kg) | 64.3 ± 1.7 | 49.0–82.7 |
| Height (cm) | 166.1 ± 1.5 | 151.0–180.8 |
| Fat mass (kg) | 20.1 ± 1.2 | 7.0–37.1 |
| Body fat (%) | 31.6 ± 1.4 | 15.2–46.9 |
| Lean body mass (kg) | 42.1 ± 0.9 | 31.2–52.7 |
| Appendicular skeletal muscle mass (kg) | 18.5 ± 0.5 | 13.8–24.0 |
| Abdominal fat (kg)* | 1.7 ± 0.2 | 0.4–4.2 |
| Visceral fat (cm²)* | 32.6 ± 3.7 | 32.0–91.0 |
| Subcutaneous fat (cm²)* | 256.7 ± 23.1 | 79.0–413.0 |
| Glucose AUC (<10⁻¹ mmol/L min⁻¹) | 0.59 ± 0.14 | 0.44–0.77 |
| VO2 max (ml/kg lean body mass min⁻¹) | 39.5 ± 1.3 | 23.8–50.1 |
| Resting energy expenditure (kcal/day) | 1358 ± 28 | 1100–1690 |
| Respiratory quotient | 0.84 ± 0.01 | 0.73–0.92 |

| Table 2

| Hormonal characteristics of the study population. |
|---|---|---|
| Mean ± SE | Range |
| Total testosterone (ng/dl) | 32.8 ± 1.6 | 8.5–53.5 |
| Free testosterone (pg/ml) | 5.9 ± 0.3 | 1.8–8.9 |
| DHEA (ng/ml) | 6.4 ± 0.5 | 1.2–13.4 |
| DHEA-S (µg/ml) | 133.1 ± 11.0 | 22.6–314.0 |
| Androstenedione (pg/ml) | 1.4 ± 0.1 | 0.4–2.2 |
| SHBG (nmol/l) | 52.7 ± 3.1 | 15.0–111.0 |

AUC, Area under the curve; VO2 max, peak aerobic capacity; n = 30 for all data with the 
exception of visceral and subcutaneous fat measurements, where n = 16. 
* DEXA measurement. 
+ CT measurement.

DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone-sulfate; SHBG, 
sex hormone binding globulin.
that was not normally distributed (e.g., DHEA) was log10 transformed and normality was reassessed. All variables with skewed distributions were normally distributed following log10 transformation. For correlation analyses, all relationships were statistically adjusted for age, and insulin sensitivity data were adjusted for lean body mass using analysis of covariance. Relationships between variables were assessed by Pearson correlation coefficients. All data are presented as means ± SE.

### 2.7. Statistical analysis

Prior to statistical analysis, the normality of the distribution of variables was assessed using the Shapiro Wilks test. Any variable that was not normally distributed (e.g., DHEA) was log_{10} transformed and normality was reassessed. All variables with skewed distributions were normally distributed following log_{10} transformation. For correlation analyses, all relationships were statistically adjusted for age, and insulin sensitivity data were adjusted for lean body mass using analysis of covariance. Relationships between variables were assessed by Pearson correlation coefficients. All data are presented as means ± SE.

### 3. Results

Baseline physical characteristics and steroid hormone levels of the 30 participants are outlined in Tables 1 and 2. Based on these data, it is apparent that subjects were non-obese, glucose tolerant, and of average fitness level. Subjects had no evidence of biochemical hyperandrogenemia, according to testosterone and free testosterone levels. The androgen precursors DHEA, DHEA-S, and Δ^{3}A were also within normal ranges.

Correlations between circulating androgen levels and morphological, physiological, and metabolic variables. Table 3. Higher serum testosterone levels correlated with greater fat mass (r = 0.377, p = 0.04; Fig. 1). Log-transformed DHEA correlated negatively with visceral adiposity as measured by CT (r = −0.569, p = 0.02). None of the serum androgens, nor SHBG, correlated with appendicular skeletal muscle mass, abdominal adiposity, VO_{2} max, or insulin sensitivity. Because of the well-known relationship of testosterone to adiposity, we also evaluated the relationship of testosterone and other steroids to physiological factors that presage weight gain: resting energy expenditure, substrate oxidation and insulin sensitivity (as reflected by glucose AUC during the oral glucose tolerance test) [9]. No relationship of any androgen or SHBG was found to resting energy expenditure or substrate oxidation.

### 4. Discussion

This study evaluated healthy, non-obese, pre-menopausal women with no history of hyperandrogenemia, hyperinsulinemia, or hirsutism to evaluate the association of endogenous androgen levels to body composition, energy and substrate metabolism and aerobic fitness. We found that higher serum testosterone levels, within the normal physiologic range, correlated with greater fat mass, but were not related to abdominal adiposity or other metabolic and functional indices. Although cause–effect cannot be discerned from these associations, further analysis revealed no association between greater testosterone levels and metabolic predictors of weight gain, such as reduced resting energy expenditure or fat oxidation, or increased insulin sensitivity [9], arguing against androgens as effectors of fat accumulation. Additionally, we found that serum levels of the sex-steroid precursor DHEA negatively correlated with visceral adiposity. No other androgen or precursor was related to adiposity or other metabolic and function indices. A particular strength of our study is the careful selection of volunteers to permit the evaluation of these associations without the confounding effects of hyperinsulinemia, hyperandrogenemia, PCOS, or obesity. Equally important is our use of validated hormonal assays that permit the precise assessment of low circulating androgen levels evident in this population.

This study was spurred by our recent work in healthy, non-obese, post-menopausal women, which showed that increasing endogenous androgen levels, within the normal physiologic range, correlated with body composition, metabolic function, and physical function. Specifically, in non-obese post-menopausal women,
higher serum testosterone levels were related to greater maximal aerobic capacity and reduced fat mass, while androgen precursors and metabolites were positively associated with insulin sensitivity [1]. These prior results align with a number of studies in post-menopausal women demonstrating a favorable relationship between physiologic androgen levels and body morphometry, insulin sensitivity, and functional capacity [10–14]. Why these relationships differ between pre- and post-menopausal women, however, is not clear. We hypothesize that differences relate to the complexities of the pre- and post-menopausal hormonal environments; more specifically, to higher levels of ovarian estrogens in pre-menopausal women. That is, the effects of androgens on these variables may be overshadowed by stronger regulatory effects of estrogens.

To date, our understanding of the relationship between sex steroids, body composition, and metabolic function in pre-menopausal females stems largely from patients with hormone excess, as observed in PCOS. The positive correlation found in the present study between testosterone and total body fat mass in non-obese subjects parallels the well-recognized relationship between excess androgens and adiposity in the PCOS-phenotype [15–17]. In PCOS, whether the rise in androgens precedes increased adiposity and insulin resistance or whether hyperinsulinemia triggers the increase in androgen production remains controversial. Because of the cross-sectional nature of the current study, we are likewise unable to determine whether greater testosterone levels promote increased adiposity or are simply a consequence of greater levels of body fat. However, we attempted to address this issue by assessing the relationship of androgen levels to metabolic predictors of weight gain; namely, reduced resting energy expenditure, decreased fat oxidation or greater insulin sensitivity (as evidenced by glucose tolerance measures). The fact that circulating testosterone levels were not related to any of these variables argues against a causative role for testosterone in promoting greater adiposity. Instead, our data favor the interpretation that increasing amounts of body fat may promote increased circulating testosterone, possibly through an effect on tissue insulin sensitivity [18,19]. Importantly, we cannot discount the possibility that testosterone promotes fat gain via modulation of energy intake, which some animal models have suggested [20,21]. In the present study, we did not assess food intake because of the well-known inaccuracies of this measurement [22]. Acknowledging that we cannot ascribe cause–effect directionality, our data nonetheless argue against a role for testosterone contributing to fat accumulation via effects on a variety of metabolic predictors of weight gain.

Despite the relationship to overall adiposity, circulating testosterone levels did not correlate to abdominal adiposity. Conventional wisdom regarding the effects of testosterone on body fat patterns derives primarily from PCOS, where a hyperandrogenic state is believed to promote greater abdominal adiposity [23]. In agreement with these findings in PCOS, data in male-to-female transsexuals receiving supra-physiologic testosterone therapy over the course of 1–3 years demonstrates an increase in visceral fat deposition [24,25]. However, these data are contradicted by studies showing that suppression of androgenemia with gonadotropin-releasing hormone agonist actually increases visceral adiposity in women with PCOS [26]. Such an effect of testosterone to prevent fat accumulation is consistent with in vitro studies showing an inhibitory effect on pathways of lipid accumulation in adipocytes [27]. Thus, there appears to be a complex relationship between circulating androgen levels and abdominal adiposity that is dependent on the integrity of ovarian function and testosterone levels. Our work adds to this literature by suggesting a minimal role for physiologic testosterone concentrations in regulating abdominal adiposity in non-obese, cycling premenopausal women.

Further contributing to the complexity of the relationship between sex steroids and regional adiposity is the intracrine conversion of steroid precursors to active hormones. Specifically, it is unclear whether circulating concentrations of hormones are comparable to tissue levels, and therefore whether they reflect end-organ exposure to androgens. In this study, we identified a negative relationship between the steroid precursor DHEA and visceral adiposity. DHEA and DHEA-S are inactive steroids which circulate in the highest concentrations of any steroid hormone [28] and are converted to active androgens and estrogens in peripheral target tissues, including adipose depots, depending on the local expression of steroidogenic enzymes [29,30]. In this context, whether the relationships we noted reflect the effects of estrogens or androgens is uncertain. Studies consistently demonstrate an inverse relationship between DHEA and total adiposity [31–33], but the relationship between body fat distribution and DHEA is less clear and may be confounded by the effects of aging on both DHEA levels and adiposity [30]. Our data agree with clinical trials showing that DHEA administration promotes the loss of visceral fat [12], although this result is controversial [34]. Considering the balance of evidence, our data suggest a favorable effect of circulating DHEA levels on visceral adiposity, possibly through the aforementioned androgenic effect on adipocyte physiology [27].

In summary, the association between circulating androgens and adiposity in women is complex. While the relationship of hyperandrogenemia to overall and regional adiposity in women is presumed based on data from PCOS, this interpretation should be made with caution [26]. Our current data suggest that, within the physiologic range, elevated testosterone levels in pre-menopausal women are associated with increased adiposity, but not with regional fat distribution. In fact, the androgen precursor DHEA was negatively related to visceral adiposity. The failure of testosterone and other androgens and their precursors to correlate to metabolic predictors of weight gain suggests that the relationship of testosterone to adiposity is unlikely explained by its effect to alter energy expenditure, fat oxidation or insulin sensitivity. Moreover, unlike post-menopausal women [1], androgens and their precursors did not correlate with other metabolic or physiologic outcomes. Further studies that manipulate circulating androgen levels and/or block their effects are needed to clarify the relationships observed in our study. Additionally, similar studies in post-menopausal women will be needed to discern why circulating androgens show unique, beneficial relationships to adiposity and metabolic parameters following ovarian senescence.

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References


