

# The Use of a Sensitive Equilibrium Dialysis Method for the Measurement of Free Testosterone Levels in Healthy, Cycling Women and in Human Immunodeficiency Virus-Infected Women\*

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## ABSTRACT

Measurements of total and free testosterone levels in women have lacked precision and accuracy because of limited assay sensitivity. The paucity of normative data on total and free testosterone levels in healthy women has confounded interpretation of androgen levels in women with human immunodeficiency virus (HIV) infection and other disease states. Therefore, the objectives of this study were to develop sensitive assays for the measurement of the low total and free testosterone levels in women to define the range for these hormones during the normal menstrual cycle and assess the total and free testosterone levels in HIV-infected women.

By using a larger volume of serum, increasing the incubation time, and reducing the antibody concentration, the sensitivity of the total testosterone assay was increased to 0.008 nmol/L, and that of the free testosterone assay was increased to 2 pmol/L. The mean percent free testosterone was  $1.0 \pm 0.1\%$  of the total testosterone. Serum total and free testosterone levels in the follicular and luteal phases were not significantly different, but both demonstrated a modest preovulatory increase, 3 days before the LH peak. Serum total  $[0.50 \pm 0.32 (14.60 \pm$

$9.22) \text{ vs. } 1.2 \pm 0.7 \text{ nmol/L } (34.3 \pm 21.0 \text{ ng/dL); } P < 0.0001]$  and free testosterone levels  $(5.56 \pm 2.70 (1.58 \pm 0.80) \text{ vs. } 12.8 \pm 5.5 \text{ pmol/L } (3.4 \pm 1.7 \text{ pg/mL); } P < 0.0001)$  were significantly lower in HIV-infected women ( $n = 37$ ) than in healthy women ( $n = 34$ ). Serum total and free testosterone levels were also significantly lower in HIV-infected women who were menstruating normally. There were no significant differences in serum total and free testosterone levels between those who had lost weight and those who had not. Testosterone levels correlated inversely with plasma HIV ribonucleic acid copy number. Serum FSH, but not LH, levels were significantly higher in HIV-infected women than in controls.

Using assays with sufficient sensitivity, we defined the range for total and free testosterone levels during the normal menstrual cycle. Serum total and free testosterone levels are lower in HIV-infected women and correlate inversely with plasma HIV ribonucleic acid levels. The hypothesis that androgen deficiency contributes to wasting in HIV-infected women remains to be tested. (*J Clin Endocrinol Metab* 83: 1312–1318, 1998)

THE PHYSIOLOGICAL role of testosterone in maintaining lean body mass in women remains unclear (1). The data on total testosterone levels in women are scarce because the existing assays, designed for the measurement of serum testosterone levels in men, lack the sensitivity required for precise measurement of the low levels prevalent in women.

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The measurement of free testosterone levels in women has been particularly problematic because these levels are at or below the assay sensitivity; furthermore, normative data for free testosterone levels during the menstrual cycle are not available. Consequently, it has been difficult to interpret total and free testosterone levels in women with human immunodeficiency virus (HIV) infection and other clinical disorders.

Hypogonadism is a common occurrence in the course of HIV infection in men (2–13) and may contribute to sexual dysfunction (11), osteopenia, decreased energy and sense of well-being, and suboptimal muscle mass and function (4). Low testosterone levels in HIV-infected men correlate with weight loss (13), decreased lean body mass and exercise capacity (4), and disease progression (14). These observations have led to speculation that androgen replacement of HIV-infected men may prevent or reverse weight loss and depletion of body cell mass and improve functional perfor-

mance (15–21). It is not known whether androgens play a similar role in preserving lean tissue in HIV-infected women. A recent report (22) found lower total and free testosterone levels in HIV-infected women; however, a tracer analog method was used for the measurement of free testosterone (Coat-a-Tube method, Diagnostics Products Systems, Los Angeles, CA) (23). The validity of this method has been debated (24, 25), and the biological nature of the testosterone fraction measured by this method remains unclear.

The objective of this study was to develop sensitive assays for precise and accurate measurement of total and free testosterone levels in women and to characterize the changes in these hormones during the normal menstrual cycle in healthy young women. Using the reference range thus generated, we wished to assess whether total and free testosterone levels are lower in HIV-infected women and whether androgen levels correlate with disease severity.

## Materials and Methods

### Healthy women

Thirty-four healthy women, 20–45 yr of age, with regular menstrual cycles served as controls. These women were not using any hormonal contraception. Blood samples were collected daily in the morning throughout the menstrual cycle. We measured LH and progesterone levels to verify the occurrence of ovulation. Although the LH peak indicated the timing of ovulation, an increase in serum progesterone to levels above 10 ng/mL (data not shown) after the LH peak provided further evidence that ovulation had occurred and that the corpus luteum had assumed its normal secretory function. Serum total and free testosterone and sex hormone-binding globulin (SHBG) levels were measured in nine evenly spaced samples during the menstrual cycle, four samples were obtained before the ovulatory peak, one sample was obtained on the day of the ovulatory peak, and four samples were obtained after the LH peak. The samples were taken 3 days apart and thus covered the entire menstrual cycle.

### HIV-infected women

Serum samples from 37 HIV-infected women were collected during a scheduled out-patient visit to the HIV clinic for measurements of plasma HIV ribonucleic acid (RNA) by RT-PCR, CD4<sup>+</sup> CD8<sup>+</sup> cell counts, and LH, FSH, SHBG, and total and free testosterone levels. Demographic data, weight in the preceding 12 months, menstrual history, and current medications were recorded. These patients were heterogeneous in terms of treatment and stage of disease.

The HIV-infected women were 23–50 yr of age. Eight HIV-infected women had lost an average of  $16.1 \pm 15.2$  lb (range, 4–42 lb;  $10.7 \pm 9.3\%$ ; range, 3.4–26.3%) during the preceding year (Table 1); three of these women had lost between 5–10%, and three had lost more than 10% of their usual pre-morbid weight. All other women had either gained weight or had stable weights during the preceding year.

Menstrual histories were available for 31 HIV-infected women; of these, 22 (71%) had normal menstrual cycles, 3 had undergone hysterectomy, and 6 (28%) were amenorrheic. We assessed whether menstrual irregularities were more common in women with weight loss. Of the 8 women who had experienced weight loss, 5 were menstruating regularly, 1 was postmenopausal, 1 was amenorrheic but did not have elevations of LH and FSH, and one had undergone hysterectomy.

### Measurement of free testosterone: standardization of the dialysis procedure

Free testosterone levels were measured by an equilibrium dialysis method (26). Dialysis cells, originally designed by Nelson and Tomei (27) for measuring free T<sub>4</sub> in serum, were used to separate the dialyzable (free) and bound fractions of testosterone. Two hundred microliters of serum in the inner compartment were dialyzed against 2.4 mL dialysis buffer that was designed to approximate the composition of a protein-

**TABLE 1.** Demographic characteristics of HIV-infected women

Patient characteristics	Mean $\pm$ SD	Median	Range
<b>Healthy women</b>			
Age (yr)	31.7 $\pm$ 6.7	32	19–50
Wt (lb)	137.5 $\pm$ 20.4	136.4	100.1–176.0
Body mass index (kg/m <sup>2</sup> )	22.8 $\pm$ 2.7	22.7	17.2–28.4
<b>HIV-infected women</b>			
Age (yr)	39.1 $\pm$ 7.0	39	23–50
Wt, present (lb)	138 $\pm$ 31	139	85–227
Body mass index, (kg/m <sup>2</sup> )	23.7 $\pm$ 5.3	21.5	14.3–33.7
Wt, previous (lb)	137 $\pm$ 30	136	88–232
CD4 count ( $\times 10^9/L$ )	302 $\pm$ 290	191	0–1,069
CD8 count ( $\times 10^9/L$ )	813 $\pm$ 529	754	29–2,134
HIV RNA copy no. $\times 10^3/mL$ (log no./mL)	1998 $\pm$ 3451	200	50–9877
Reproductive status	(6.30 $\pm$ 6.54)	(5.30)	(4.70–6.99)
Hysterectomized	3		
Amenorrhea	6		
Regular cycles	22		
Uncertain	6		

free ultrafiltrate of normal human serum (131 mmol Na, 4.3 mmol K, 1.9 mmol Ca, 1.0 mmol Mg, 98 mmol Cl, 1.3 mmol PO<sub>4</sub>, 1.3 nmol SO<sub>4</sub>, 5.4 nmol lactate, 3.3 nmol glutamate, and 8 mmol urea). In preliminary experiments, we demonstrated that equilibrium was reached in dialysis in 8 h; no significant differences in the dialyzed fraction of testosterone were detected after 8, 16, and 24 h of dialysis. Therefore, in subsequent experiments, dialysis was performed overnight for 16 h at 37 C. Four hundred microliters of the dialysate from the outer compartment were assayed in duplicate for testosterone.

To examine the effect of serum protein concentration on the performance of dialysis, we diluted serum samples 1:2 (serum to total volume), 1:5, and 1:10 with the assay buffer. Although the 1:2 dilution of serum did not affect the fractional amount of testosterone dialyzed, higher dilutions of serum increased the percentage dialyzed in a nonlinear manner, indicating that marked changes in serum protein concentrations affect the performance of dialysis. Therefore, only undiluted serum was used for dialysis in subsequent experiments.

### Testosterone RIA

Testosterone concentrations in the dialysate were measured by an iodinated RIA (26), using <sup>125</sup>I-labeled testosterone purchased from ICN Pharmaceutical Co. (Irvine, CA).

To enhance assay sensitivity, we used a larger volume of serum, a smaller amount of the antitestosterone antibody (final concentration, 1:4,000,000), and a longer incubation time (16 h at 4 C). The samples were preincubated with primary antibody for 2 h; at this point, tracer was added, and the incubation was continued for an additional 16 h at 4 C. The 20%, 50%, and 80% bound/free ratio (B/B<sub>0</sub>) points on the displacement curve corresponded to 4.3, 0.27, and 0.03 nmol/L, respectively (Fig. 1). The sensitivity, defined as the hormone concentration corresponding to the 90% B/B<sub>0</sub> point, was 0.008 nmol/L (0.22 ng/dL). By using 400  $\mu$ L of the sample against 100  $\mu$ L of the standards, the functional sensitivity of the assay was further increased to 0.002 nmol/L (0.06 ng/dL).

To define the intraassay coefficient of variation, 10 replicates of 4 serum pools in low, medium, and high ranges were assayed. The respective intraassay coefficients of variation were  $\pm 5.6\%$  for the low pool,  $\pm 4.6\%$  for medium pool, and  $\pm 2.6\%$  for high pool. Free and total testosterone for the same subject were measured in the same assay to avoid interassay variation.

### Measurement of total testosterone concentrations

Serum total testosterone concentrations were measured in 20  $\mu$ L serum using an iodinated testosterone assay, as described above (26).

### Measurement of serum FSH, LH, progesterone, and SHBG

Serum LH and FSH levels were measured by a sensitive, two-site-directed, immunofluorometric assay, (Delfia-Wallac, Gaithersburg,

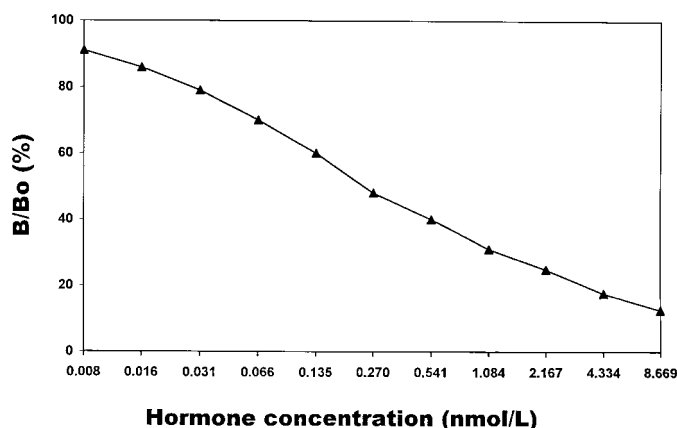


FIG. 1. Log/logit transformation of the testosterone displacement curve. The amount of bound hormone in each tube, expressed as a percentage of the amount bound in the absence of any added cold hormone, is plotted against the log of the hormone concentration in each tube. The ED<sub>20</sub>, ED<sub>50</sub>, and ED<sub>80</sub> points corresponded to 4.3, 0.27, and 0.03 nmol/L, respectively. The sensitivity of the assay, defined as the hormone concentration corresponding to the 90% B/B<sub>0</sub> point was 0.002 nmol/L.

MD), as described previously (26). The sensitivity of this assay is 0.04 U/L for LH and 0.06 U/L for FSH. The cross-reactivity with TSH, hCG, and free  $\alpha$ -subunit of pituitary glycoprotein hormones is less than 1%. Serum SHBG levels were measured by an immunofluorometric assay (26). Serum progesterone levels were measured by an immunoassay (28).

#### Statistical analysis

Correlation tests were estimated as McPearson's product-moment correlation coefficients. Also, because the hormone and CD4<sup>+</sup> and CD8<sup>+</sup> data did not meet the assumptions of normality, the Mann-Whitney U test was used for two-group comparisons, and a nonparametric one-way ANOVA was used when several groups were compared. Results are presented as the mean  $\pm$  SD or as the median and range.  $P < 0.05$  was considered significant. Because of considerable heterogeneity and lack of normal distribution, plasma HIV copy numbers were log transformed before analysis.

### Results

#### Serum total and free testosterone levels in healthy women

In healthy women with regular menstrual cycles, serum total testosterone levels varied during different phases of the menstrual cycle. During the follicular phase, serum testosterone levels increased from  $1.04 \pm 0.76$  nmol/L ( $30 \pm 22$  ng/dL) 12 days before ovulation to a peak of  $1.52 \pm 1.03$  nmol/L ( $44 \pm 31$  ng/dL) 3 days before the LH peak (Fig. 2). Total testosterone levels gradually decreased during the luteal phase to  $1.1$  nmol/L ( $31$  ng/dL) 9 days after ovulation. Mean testosterone levels during the luteal and follicular phases were not significantly different ( $1.18 \pm 1.21$  vs.  $1.21 \pm 1.21$  nmol/L;  $P = \text{NS}$ ).

Serum free testosterone tended to increase during the follicular phase from  $11.1 \pm 5.6$  pmol/L ( $3.2 \pm 1.6$  pg/mL) 12 days before ovulation to a peak of  $14.6 \pm 6.9$  pmol/L ( $4.2 \pm 2.0$  pg/mL) 3 days before the LH peak. During the luteal phase, serum free testosterone levels remained unchanged. Mean free testosterone levels during the luteal and follicular phases were not significantly different ( $12.8 \pm 6.6$  vs.  $12.8 \pm 6.9$  pmol/L;  $P = \text{NS}$ ).

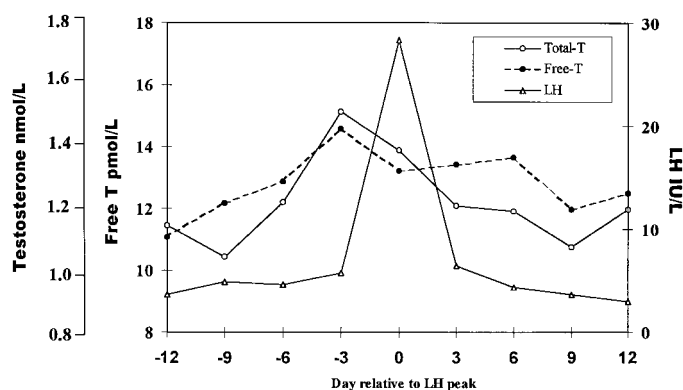


FIG. 2. Mean serum total and free testosterone and LH levels during the normal menstrual cycle in 34 healthy women. Data are the mean  $\pm$  SD.

#### Total and free testosterone levels in HIV-infected women

Total testosterone levels were significantly lower in HIV-infected women than in healthy women (Table 2). Among the 37 HIV-infected women, all but 2 (95%) had total testosterone levels less than the median ( $<0.98$  nmol/L), 24 (65%) had levels below the 25th percentile ( $<0.65$  nmol/L), and 21 (57%) had levels below the 10th percentile ( $<0.51$  nmol/L) for healthy women.

Free testosterone levels constituted 0.5–1.2% of the total testosterone levels in the HIV-infected women compared to 0.6–2.0% in the controls ( $P = \text{NS}$ ), consistent with previously reported data on the dialyzable fraction.

Serum free testosterone levels were significantly lower in HIV-infected women than in healthy women [ $4.5 \pm 2.4$  pmol/L ( $1.3 \pm 0.7$  pg/mL) vs.  $12.8 \pm 5.5$  pmol/L ( $3.7 \pm 1.6$  pg/mL);  $P < 0.0001$ , healthy vs. HIV-infected women, respectively]. Only 1 of 37 HIV-infected women had free testosterone levels that exceeded the median value for healthy women ( $>12.5$  pmol/L); 33 (89%) had levels below the 25th percentile (8.7 pmol/L), and 22 (59%) had levels below the 10th percentile (5.6 pmol/L) for healthy controls (Fig. 3).

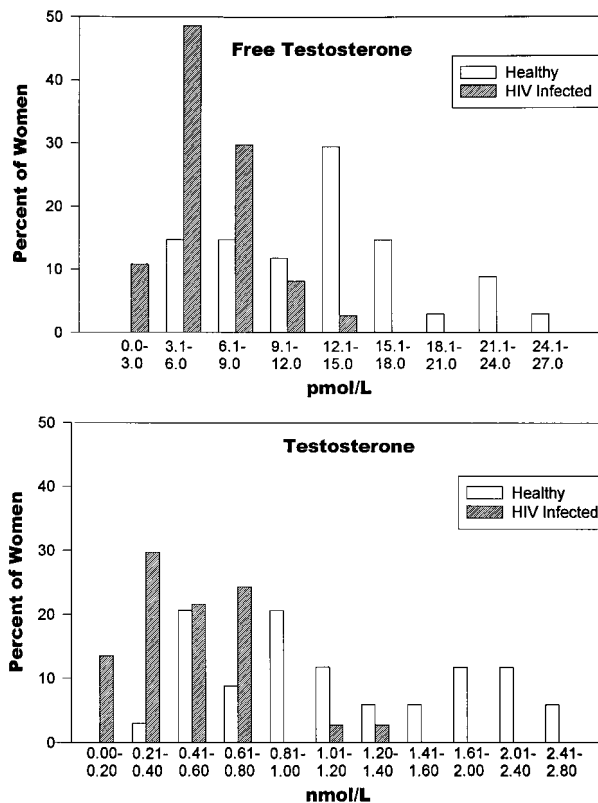
Of 31 women who were not menopausal and provided menstrual histories, 22 (71%) had normal menstrual cycles. We examined whether low testosterone levels were unique to women who had ceased to menstruate. The subgroup of HIV-infected women with regular menstrual cycles also had significantly lower total and free testosterone levels than the control group [total testosterone,  $0.5 \pm 0.4$  nmol/L ( $13.7 \pm 10.2$  ng/dL) vs.  $1.2 \pm 0.7$  nmol/L ( $34.7 \pm 19.8$  ng/dL);  $P < 0.0001$ , healthy vs. HIV-infected women, respectively; Fig. 4, A and B].

Eight (26%) HIV-infected women had experienced significant weight loss. Serum total and free testosterone levels were not significantly different in HIV-infected women who had lost weight and those who had not [total testosterone,  $0.53 \pm 0.36$  vs.  $0.50 \pm 0.31$  nmol/L ( $P = \text{NS}$ ); free testosterone,  $5.46 \pm 3.63$  vs.  $5.60 \pm 2.41$  pmol/L ( $P = \text{NS}$ , women with weight loss vs. women without weight loss); Fig. 5, A and B].

There were no significant differences in serum total ( $0.48 \pm 0.12$  nmol/L vs.  $0.44 \pm 0.24$  nmol/L) and free ( $1.7 \pm 0.8$  vs.  $1.4 \pm 0.5$  pmol/L;  $P = \text{NS}$ ) testosterone levels in HIV-infected

**TABLE 2.** Hormone levels in healthy (n = 34) and HIV-infected (n = 37) women

	Healthy women	HIV-infected women	P (healthy vs. HIV-infected women)
<b>Testosterone (nmol/L)</b>			
Mean ± SD	1.20 ± 0.69	0.51 ± 0.32	<0.0001
Median	0.98	0.45	
Range	0.4–2.7	0.1–2.0	
<b>Free testosterone (pmol/L)</b>			
Mean ± SD	12.80 ± 5.59	5.57 ± 2.70	<0.0001
Median	12.53	5.19	
Range	4.1–24.2	2.1–12.8	
<b>% Free T of total T</b>			
Mean ± SD	1.4 ± 1.1	1.5 ± 1.2	NS
Median	1.1	1.2	
Range	0.4–6.3	0.3–6.7	
<b>LH (IU/L)</b>			
Mean ± SD	7.2 ± 3.3	7.8 ± 8.3	NS
Median	6.7	4.6	
Range	3.0–18.7	0.4–33.4	
<b>FSH (IU/L)</b>			
Mean ± SD	4.7 ± 3.6	14.4 ± 19.8	<0.01
Median	4.2	6.3	
Range	1.5–21.4	1–85.2	
<b>SHBG (nmol/L)</b>			
Mean ± SD	66.1 ± 22.7	85.8 ± 48.1	NS
Median	71.0	83.7	
Range	17.8–114.0	19.0–207.4	

**FIG. 3.** Frequency distribution of total and free testosterone levels in healthy (n = 34) and HIV-infected women (n = 37)

women who were taking protease inhibitors and those who were not; in both groups of HIV-infected women, serum total and free testosterone levels were lower than levels in healthy controls.

Total and free testosterone levels were correlated ( $r = 0.62$ ;

$P < 0.0001$ ) with each other in the HIV-infected women, but there was no correlation between total or free testosterone and SHBG. Total testosterone levels were significantly inversely correlated to plasma HIV RNA copy number measured by RT-PCR ( $r = -0.49$ ;  $P < 0.05$ ). There was no significant correlation between serum total and free testosterone levels and body weight, body mass index, or weight change.

#### Serum LH, FSH, and SHBG levels

Serum FSH (Table 2) levels were significantly higher in HIV-infected women than in the control group (mean ± SD,  $14.4 \pm 19.8$  vs.  $4.7 \pm 3.6$  U/L; HIV-infected vs. healthy women;  $P < 0.01$ ). The subgroup of HIV-infected women with regular menstrual cycles (n = 22) also had significantly higher FSH levels than the controls (mean ± SD,  $11.7 \pm 20.0$  vs.  $4.7 \pm 3.2$  U/L;  $P < 0.05$ ; Fig. 4C). There were no significant differences in serum LH levels between the two groups (mean ± SD,  $7.8 \pm 8.3$  vs.  $7.2 \pm 3.3$  U/L;  $P = \text{NS}$ ; Table 2). Serum SHBG levels did not significantly differ between the HIV-infected women and the controls ( $85.8 \pm 48.1$  vs.  $66.1 \pm 22.7$  nmol/L; Table 2).

#### Discussion

In this study we measured serum total and free testosterone levels in healthy and HIV-infected women using assays that had sufficient sensitivity and precision for the measurement of the low levels of these hormones in women. We found that serum total and free testosterone levels were similar in the follicular and luteal phases, although there was a modest preovulatory rise in both in normal menstruating women. Using the normative database generated in healthy women, we demonstrated that total and free testosterone levels were lower, and FSH levels were higher in HIV-infected women. Low total testosterone levels correlated inversely with plasma HIV RNA, measured by RT-PCR. Serum

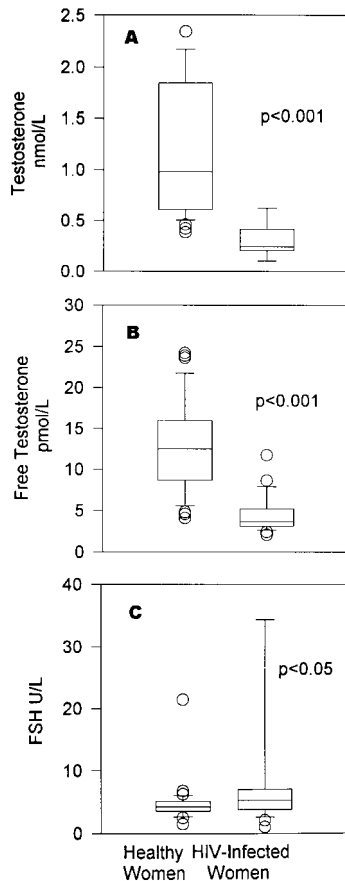


FIG. 4. Hormone levels in HIV-infected women with normal menstrual cycles ( $n = 22$ ) and healthy women ( $n = 34$ ). A, The box and whisker plots demonstrate the distribution of total testosterone levels (A), free testosterone levels (B), and FSH levels in healthy women ( $n = 34$ ) and HIV-infected women with normal menstrual cycles ( $n = 22$ ). In each plot, the *lower and upper boundaries* of the box represent the 25th and the 75th percentile values, respectively; the *line inside the box* represents the median; and the *bars below and above the box* represent the 10th and 90th percentile values, respectively.

total and free testosterone levels were similar in HIV-infected women who had experienced weight loss and those who had not. Furthermore, serum androgen levels were lower in HIV-infected women with normal menstrual cycles than in healthy women. These data suggest that serum total and free testosterone levels decline before these patients develop overt wasting or disruption of their menstrual cycles.

Circulating testosterone is bound predominantly to sex hormone-binding globulin and albumin (29–40). The free fraction, which constitutes 0.5–2% of total testosterone and is measured most accurately by equilibrium dialysis, is believed to be the biologically active fraction and, therefore, a better marker of serum androgen levels than total testosterone (29–38). Albumin-bound testosterone dissociates rapidly and is available for transport *in vivo* (36). Both the unbound fraction, measured by equilibrium dialysis (29–31), and the albumin-bound fraction, measured by ammonium sulfate precipitation (32–34), have been widely used as markers of free testosterone and have been shown to have a clinical correlation. Another method for the calculation of the free testosterone index uses algorithms (25, 40) based on the con-

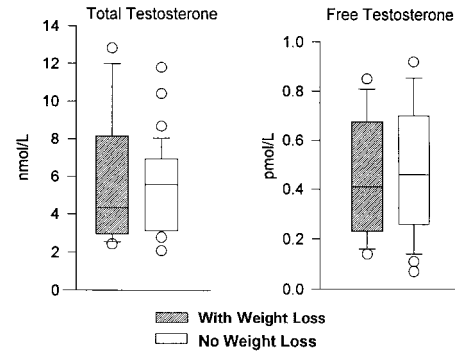


FIG. 5. Serum total and free testosterone levels in HIV-infected women with ( $n = 8$ ) and without ( $n = 29$ ) weight loss. The *lower and upper boundaries* of the box represent the 25th and the 75th percentile values, respectively; the *line inside the box* represents the median; and the *bars below and above the box* represent the 10th and the 90th percentile values.  $P = NS$ , HIV-infected women with weight loss vs. HIV-infected women without weight loss.

centrations of total testosterone and the binding proteins; however, one cannot assume that these algorithms for the computation of free testosterone concentrations from total testosterone and SHBG concentrations will be uniformly applicable in patients with different clinical disorders, especially those in whom binding protein concentrations are substantially altered. Nonisotopic methods for the estimation of free testosterone may lack sensitivity. A commercial kit for the measurement of free testosterone levels using a tracer analog method is available (23). Although the estimates of free testosterone by the tracer analog methods have been shown to correlate with the measurements of free testosterone by the equilibrium dialysis method in healthy men, the validity of the tracer analog method has been questioned (24, 25). There are reports of the use of labeled testosterone to estimate the percentage of tracer dialyzed and then calculate free testosterone from total testosterone levels and the percentage of tracer dialyzed. We used an equilibrium dialysis method in which the dialyzed fraction was directly measured by a sensitive immunoassay.

To our knowledge, this is the first description of the changes in free testosterone levels measured using an equilibrium dialysis method in healthy women during different phases of the menstrual cycle. Most existing assays for the measurement of free testosterone levels lack sufficient sensitivity to measure the low levels in women. The total testosterone levels that we measured in healthy menstruating women are in agreement with those reported in the literature (41, 42).

Information regarding changes in gonadal function in general and serum testosterone levels in particular in women with HIV infection is limited. While this manuscript was in preparation, Grinspoon *et al.* (22) reported that free, but not total, testosterone levels were significantly lower in HIV-infected women with early and late wasting. Free testosterone levels correlated with muscle mass, leading these investigators to conclude that low testosterone levels contribute to wasting. However, the free testosterone levels reported in that paper were measured using a tracer analog method. The biological nature of the fraction measured by the tracer an-

alog method used in the previous publication remains unclear.

In healthy women, the ovaries and the adrenal glands contribute equally to the maintenance of circulating testosterone levels. We do not know whether lower testosterone levels in HIV-infected women are the result of impairment of ovarian or adrenal function, or both.

Previous studies have reported either no increase or only a modest increase in the frequency of menstrual irregularity in HIV-infected women (43–46). Our data are similar, in that 71% of HIV-infected women in whom a definite menstrual history was available, were menstruating regularly. Some researchers have suggested (43–46) that menstrual irregularities and hormonal changes in HIV-infected women are caused by the malnutrition associated with the disease. In our study, only eight women had lost weight; serum testosterone levels were lower even in HIV-infected women who had not lost any weight. Furthermore, there was no difference in serum testosterone levels between women who had lost weight and those who had not. Therefore, malnutrition alone does not adequately explain the decrease in serum testosterone levels. Dobs *et al.* (3) found that low bioavailable testosterone levels in HIV-infected men are observed early in the course of events that culminate in wasting. Therefore, although low testosterone levels correlate with weight loss, a decrease in serum testosterone levels is not necessarily the consequence of wasting. Our data suggest that either low testosterone levels may contribute to events that result in wasting, or similar pathogenic factors are responsible for both the wasting and low testosterone levels.

Serum FSH levels were higher in HIV-infected women compared to the mean levels in healthy women. Women who had regular menstrual cycles also had higher FSH levels, suggesting that a subset of HIV-infected women may have compensated or subclinical ovarian dysfunction. None of the patients was taking ketoconazole or other drugs known to inhibit steroidogenesis. The cause of subclinical ovarian dysfunction in HIV-infected women is not apparent, but the host immune response to the disease, the HIV virus itself, drugs, and malnutrition may contribute to the multifactorial etiology of gonadal dysfunction. Most of the HIV-infected women in our study had not lost weight; therefore, other factors must be invoked to explain gonadal dysfunction in these women. The plasma HIV RNA level is a good prognosticator of disease progression; therefore, an inverse relationship between testosterone levels and plasma HIV RNA suggests that low androgen levels in HIV-infected women may be associated with poor disease outcome.

Testosterone administration increases nitrogen retention, fat-free mass, muscle size, and strength in hypogonadal men, boys before puberty, and women (47–58). Supraphysiologic doses of testosterone further increase muscle mass and strength in eugonadal men (26, 54). Androgen deficiency in HIV-infected men correlates with adverse disease outcomes (13–14); those who have lost weight or progress to AIDS have lower testosterone levels than those without evidence of weight loss or disease progression. A number of studies have provided preliminary evidence that androgen administration to HIV-infected men promotes weight gain and accretion of lean body mass (16–21). However, the physiological

and therapeutic implications of androgen deficiency in HIV-infected women remain unclear. Testosterone administration increases markers of bone formation and fat-free mass in postmenopausal women (57, 58). Clinical trials to assess the effects of testosterone replacement on body composition, muscle function, and overall quality of life in HIV-infected women with weight loss are in progress.

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