Plasma Adrenal, Gonadal, and Conjugated Steroids Following Long-Term Exercise-Induced Negative Energy Balance in Identical Twins

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There are few reports of the change in sex hormone levels accompanying a weight change in men, although an excessive decline in testosterone (TESTO) has been described as an associate of stress-induced weight loss. Plasma levels of cortisol, TESTO, dihydrotestosterone (DHT), dehydroepiandrosterone sulfate (DHEA-S), androsterone glucuronide (ADT-G), and androstane-3α, 17β-diol glucuronide (3αDIOL-G) were measured in seven pairs of sedentary male monozygotic twins (age, 21.0 ± 0.8 years; body mass index [BMI], 26.2 ± 5.5 kg/m²) before and after 93 days of standardized submaximal (50% to 55% maximum oxygen consumption) cycle-ergometer exercise. A total energy deficit of 244 ± 9.7 MJ induced significant changes ($P < .0001$) in body weight ([BW] −5.0 ± 2.2 kg) and body fatness measures. Plasma TESTO and DHEA-S increased and 3αDIOL-G decreased. The increase in TESTO was a significant inverse correlate of loss in all measures of body fat, particularly central adiposity ($r = -.58$ to $-.86$, $P < .001$, fat loss–adjusted). Lower postexercise levels of 3αDIOL-G correlated positively with decreased body composition measures ($r = .65$ to $.68$, $P < .01$). The increase in plasma TESTO accompanying the loss of abdominal visceral fat (AVF) was greater in men with lower fasting insulin levels ($P < .0001$). The baseline within–twin-pair resemblance in TESTO and 3αDIOL-G (intraclass correlation coefficients [ICC] = .83 and .78, respectively, $P < .01$) was lost with intervention. Cortisol, DHEA-S, and ADT-G developed within–twin-pair similarity (ICC adjusted for fat loss: cortisol, .72; ADT-G, .62, $P < .05$; DHEA-S, .85, $P < .002$). We conclude that a steroid profile characterized by high TESTO and low androgen metabolite levels accompanied the changes in body composition and body fat distribution generated by the exercise-induced negative energy balance. Furthermore, these changes were characterized by a significant resemblance within identical-twin pairs.

THE EFFECT OF EXERCISE on circulating adrenal and gonadal steroid levels has been the subject of many studies reported over several decades,[1-3] the interest presumably arising from the potent anabolic response to plasma androgens in muscle and bone. While reports on the circulating steroids in response to exercise have been varied and contradictory, the factors that influence the response and create divergent effects are emerging.[4,5] The exercise mode, intensity, and duration have been described as major influences determining whether there will be an increase or a decrease of circulating testosterone (TESTO) and adrenal steroid levels. Weight change has not been described as a critical factor influencing circulating androgens in healthy young men, although Zumoff and Strain[6] have demonstrated a significant increase in TESTO in response to a large weight loss in markedly obese subjects. In instances where weight loss has been induced by stress developed through excessive training or excessive exertion, acutely depressed TESTO levels have been reported.[7-9]

Reports based on cross-sectional studies of adrenal steroid levels in twin and family studies concluded that variations in plasma hormone levels were partly determined by genetic factors.[10,11] Variations in plasma hormone levels have also been attributed to the influence of body composition, with TESTO levels, adrenal steroid precursors, and peripheral tissue metabolites being dependent on the total amount of body fat or lean tissue and fat topography.[12-14] Familial determinants of steroid patterns were maintained when energy balance was altered with overfeeding,[15] but whether they are maintained when body composition is altered by exercise-induced negative energy balance has not been determined.

The objective of the current study was to describe the changes in adrenal, gonadal, and conjugated steroids associated with the changes in body composition accompanying exercise-induced negative energy balance in identical twins. Another purpose was to test the hypothesis that individual differences before and after exercise-induced weight loss are characterized by intrapair resemblance.

SUBJECTS AND METHODS

Subjects

Eleven pairs of sedentary, young male identical twins (aged 21.0 ± 0.8 years) provided written consent to participate in a study of exercise-induced negative energy balance approved by the Laval University Medical Ethics Committee and the Office for the Protection from Research Risks of the National Institutes of Health (Bethesda, MD). This report is based on findings from the intervention undertaken by the seven pairs who completed the full study protocol. The specific study aims, design, and methodology have been described in detail.[16]

In summary, the 14 men, free of illness and without a history of disease, were required to exercise to achieve a negative energy balance of 4.2 MJ/d (1,000 kcal/d) over 93 days. A mean daily energy intake of 14.1 ± 0.6 MJ (3,370 ± 130 kcal) with a range of 10.8 to 17.8 MJ (2,584 to 4,246 kcal) was maintained, equivalent to the energy cost of weight maintenance. Subjects were housed off-campus from Laval University for 117 days, under 24-hour supervision such that the diet

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and physical activity were closely monitored to ensure essentially perfect compliance. The first 17 ± 2 days constituted a baseline period involving the establishment of the energy cost of weight maintenance while subjects ate freely from prepared diets and maintained a sedentary life-style. Exercise testing and anthropometric, metabolic, biochemical, and hematological measurements were performed preintervention and repeated during the postintervention period. During the intervention, the exercise sessions lasted for 57.0 ± 0.8 minutes and were performed twice daily, one session midmorning and one midafternoon. The intensity of the exercise sessions was individually controlled to be 50% to 55% of maximum oxygen consumption, monitored using the heart rate at all times during each session and verified by cycle ergometry every 25 days throughout the intervention. The mean energy cost of the exercise was measured as 38.9 kJ (9.3 kcal) per minute above the resting metabolic rate, achieving an estimated total energy deficit above the cost of weight maintenance of 244 ± 9.8 MJ (58,000 ± 2,000 kcal) over 93 days. There was a postintervention testing period of 7 days.

Body Composition

Observations before and after intervention, as well as daily measurements during the intervention, involved the following: body weight (BW) measured at the same time daily with the subject wearing light exercise shorts; body density determined before and after the exercise intervention by hydrostatic weighing,[17] using the helium-dilution technique to measure pulmonary residual volume[18] and the Siri equation to estimate percent body fat (%BF)[19], fat mass (FM) and fat-free mass (FFM) obtained from %BF and BW in kilograms; and circumference measurements of the waist and hip and skinfold thickness measurements in millimeters at 10 sites, five trunk (subscapular, suprailiac, abdominal, midaxillary, and chest) and five extremity (biceps, triceps, front midthigh, suprapatellar, and medial calf), made according to standardized procedures.[20] Computed tomography (CT) was performed before and after intervention with a Siemens Somatom DRH scanner (Siemens, Erlangen, Germany) according to the method described by Sjöstrom et al[21] at L4-L5 to obtain the abdominal visceral fat (AVF), abdominal subcutaneous fat, total abdominal fat, and, at the midthigh, total femoral fat (TFF) areas (centimeters squared).

Biochemical Analyses

Blood samples were obtained after an overnight fast between 7:30 and 8:00 AM for determination of plasma steroid and insulin levels. Plasma insulin levels were determined by radioimmunoassay as described by Oppert et al.[22] The insulin assay reliability was analyzed in a batch at completion of the study (coefficient of variation [CV], 10.8%). Steroid levels were measured by radioimmunoassay after separation of conjugated and unconjugated steroids by C_{18} column chromatography as described previously by Bélanger et al.[23] Sulfate derivatives were submitted to hydrolysis. Glucuronide conjugates were also submitted to hydrolysis with β-glucuronidase. Steroids from each fraction were further separated by elution on LH-20 columns. Steroid levels were measured by radioimmunoassay as previously described.[24] Six steroids have been identified as having high assay reproducibility estimated in regard to analytical error. The CVs were between 6.6% and 11.9% (except for androstane-3α, 17β-diol glucuronide [3αDIOL-G] and androsterone glucuronide [ADT-G], which had a CV of 19.4% and 21.5%, respectively) and the intraclass correlation coefficient (ICC) was greater than .96 (except for 3αDIOL-G and ADT-G, which had an ICC of .80 and .81, respectively). The day-to-day variability for three of the six steroids showed a CV between 8.8% and 13.5% (3αDIOL-G and ADT-G had a CV of 22.3% and 23.4%, respectively), with an ICC greater than .94 (except for 3αDIOL-G and ADT-G, with an ICC of .73 and .77, and cortisol). Cortisol has a day-to-day variation that produced an ICC of .55 (CV 26.0%), but its analytical error was low (CV 6.6%, ICC = .98) and thus it has been included in this report. Baseline plasma levels of these steroids for each subject were assessed to be within the normal range for adult men.[25]

Statistical Analysis

The effects of negative energy balance on the body fat and steroid phenotypes and the interaction between genotype and phenotype changes following the exercise intervention were assessed with a two-way ANOVA for repeated measures on one factor—time.[26] The twins were considered nested within the pair, whereas the treatment effect was considered a fixed variable. The ICC for the changes produced by negative energy balance was computed from the between-pair and within-pair square means and used to quantify the similarity within pairs for plasma adrenal and sex steroids before and after intervention. Correlation analysis was undertaken to quantify the association between the changes in body fat with exercise-induced negative energy balance and (1) baseline plasma adrenal, gonadal, and conjugated steroid levels and (2) negative energy balance–induced changes in plasma adrenal, gonadal, and conjugated steroid levels, with 14 subjects considered as independent individuals. All analyses were performed before and after adjustment for changes in total FM. Statistical analyses were performed with the SAS statistical package (Version 6.12 for Windows; SAS Institute, Cary, NC).

RESULTS

Body Composition

The negative energy balance induced significant changes in all measures of BW and body composition except FFM ($P < .05$ to $P < .001$; Table 1). The mean body mass index (BMI) decreased to within the healthy weight range (24.6 ± 5.3 kg/m²). The changes in BW and measures of body composition in the Québec Negative Energy Balance Study were characterized by significant within–twin-pair resemblance as reported previously.[16]

Table 1. Effect of Exercise-Induced Negative Energy Balance on Body Composition in Identical Male Twins

Variable	Before Intervention	After Intervention	Change
BW (kg)	82.1 ± 19.9	77.1 ± 19.0	−5.0 ± 2.2‡
BMI (kg/m²)	26.2 ± 5.5	24.6 ± 5.3	−1.6 ± 0.6‡
Body composition			
FM (kg)	20.8 ± 13.0	15.9 ± 11.7	−4.9 ± 2.3†
%BF	23.6 ± 8.2	18.8 ± 8.5	−4 8 ± 2.1‡
FFM (kg)	61.2 ± 7.3	61.1 ± 7.7	−0.1 ± 1.0
Subcutaneous fat (skinfolds, mm)			
Total	203.0 ± 130.4	150.9 ± 111 7	−52.1 ± 26.3†
Trunk	118.9 ± 66.5	88.1 ± 66.3	−30.7 ± 11.6‡
Extremities	84.1 ± 64.8	62.8 ± 46.4	−21.4 ± 22.3*
CT-derived regional fat area (cm²)			
Abdominal			
Total	326.3 ± 166 9	231.3 ± 163.0	−95.0 ± 31.1‡
Subcutaneous	245.5 ± 154.3	179 3 ± 143.8	−66.2 ± 24.6†
Visceral	80.8 ± 19.0	52.1 ± 22.4	−28.8 ± 13.0†
Femoral	183.7 ± 52.1	138.9 ± 54.7	−44.8 ± 16.3

NOTE Values are the mean ± SD.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 2. Effect of Exercise-Induced Negative Energy Balance on Plasma Adrenal and Gonadal Steroid Levels and Within–Twin-Pair Resemblance in Response to the Intervention in Seven Pairs of Male Twins

Steroid (nmol/L)	Before Intervention*	After Intervention*	Change†	Intrapair Resemblance in Response	
				F Ratio	ICC
TESTO	12.3 ± 4.1	17.4 ± 5.1	5.1 ± 1.1‡	1.26	.12
DHT	2.4 ± 1.0	3.3 ± 1.8	0.9 ± 0.6	0.33	.00
Cortisol	148 ± 57	114 ± 32	−33.5 ± 15.5	1.38	.16
DHEA-S	3,407 ± 947	6,341 ± 2,722	2,933 ± 684‡	2.90	.49
ADT-G	39.9 ± 10.3	39.4 ± 6.2	−0.4 ± 2.4	1.06	.03
3αDIOL-G	7.5 ± 2.0	5.2 ± 1.5	−2.3 ± 0.5‡	0.82	.00

NOTE. Statistical significance was determined by a 2-way ANOVA with repeated measurements on 1 factor (time) and nested twins. The F ratio is the ratio of the variance between pairs to that within pairs. The ICC was used to assess similarity within pairs in response to the exercise-induced negative energy balance. An ICC close to 1.0 indicates perfect within-pair resemblance, whereas an ICC close to 0 implies that there is no within-pair resemblance in response to the intervention.

*Mean ± SD.

†Mean ± SE.

‡$P < .01$.

Plasma Adrenal and Gonadal Steroids

Effects of exercise-induced negative energy balance. A significant increase was found for plasma levels of TESTO (41.5% ± 12.5%) and dehydroepiandrosterone sulfate ([DHEA-S] 86.1% ± 24.7%), while the level of the androgen metabolite 3αDIOL-G decreased (−30.7% ± 5.6%; $P < .01$). There was no change in cortisol, dihydrotestosterone (DHT), or the androgen metabolite ADT-G (Table 2). Figure 1 illustrates the percentage change in the steroids after intervention. Calculation of the correlation coefficients between steroid levels at baseline and their response to the intervention showed that baseline TESTO was negatively correlated with the change in its metabolites, ADT-G ($r = -.66$, $P < .01$) and 3αDIOL-G ($r = -.54$, $P < .05$). Baseline cortisol, DHT, ADT-G, and 3αDIOL-G were negatively correlated with their own degree of change ($r = -.85$, $P < .0001$, $r = -.60$, $P < .05$, $r = -.80$, $P < .001$, and $r = -.68$, $P < .01$, respectively). Baseline DHT correlated positively ($r = .61$, $P < .05$) and baseline 3αDIOL-G correlated negatively ($r = -.53$, $P < .05$) with the change in cortisol levels.

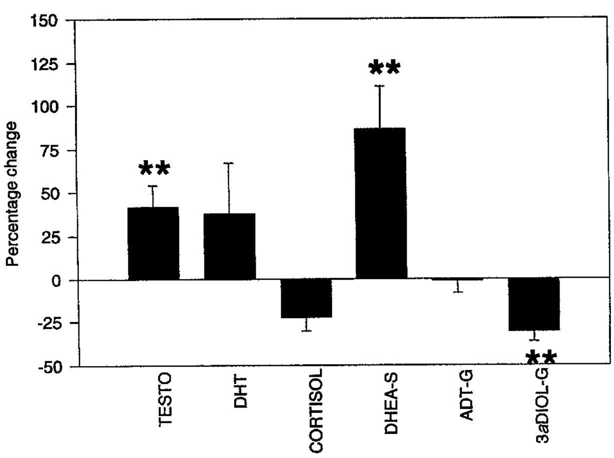

Fig 1. Percentage change (mean ± SEM) in plasma adrenal and gonadal steroid levels following exercise-induced negative energy balance. **$P < .01$.

Twin resemblance. Table 2 shows plasma steroid levels before and after intervention. The variance in response to the protocol of negative energy balance was just as large within twin pairs as between twin pairs. Table 3 shows that TESTO and 3αDIOL-G had significant within–twin-pair similarity before intervention (ICC = .83 and .78, respectively, $P < .01$), but with adjustment for the difference in FM at baseline, only TESTO retained this significance (ICC adjusted = .84, $P < .003$). After intervention, TESTO lost its within-pair resemblance and cortisol, DHEA-S, and ADT-G developed significant within-pair resemblance (Table 3) that was retained after adjustment for fat loss. Computation of within–twin-pair differences in the steroids demonstrated a reduction in the mean intrapair differences with intervention in DHEA-S and cortisol, but not TESTO (data not shown).

Association between steroid and fat phenotypes. The association between the body composition and steroid phenotypes was investigated by calculating the correlation coefficients with the men considered as independent individuals before and after intervention and before and after adjustment for loss of FM (Table 4). Of all the steroids, only baseline cortisol showed significant correlations with baseline body composition parameters (BW, $r = .62$, FM, $r = .60$; FFM, $r = .63$; all $P < .05$).

Table 3. Similarity Within Twin Pairs in Plasma Adrenal and Gonadal Steroids Before and After Exercise-Induced Negative Energy Balance in Identical Twins (n = 7 pairs)

Variable	Twin Resemblance Before Intervention			Twin Resemblance After Intervention		
	F Ratio	ICC	*P*	F Ratio	ICC	*P*
TESTO	10.82	.83	.01*	2.93	.49	.09
DHT	0.59	.26	.73	1.42	.17	.33
Cortisol	1.84	.30	.22	7.08	.75	.01†
DHEA-S	1.16	.07	.42	11.25	.84	.01†
ADT-G	2.63	.45	.12	7.06	.75	.01†
3αDIOL-G	8.01	.78	.01	1.84	.30	.22

NOTE. Results are from a 2-way ANOVA with repeated measurements on 1 factor (time) and nested twins.

*Significance remained after adjustment for difference in FM.

†Significance remained after adjustment for loss of FM.

Table 4. Correlations Between Steroid Levels and Body Composition Phenotypes Before and After Exercise-Induced Negative Energy Balance

	Body Composition				
Steroid	BW	FM	FFM	AVF	TFF
Baseline					
TESTO	–	–	–		–
DHT	–	–	–	–	–
Cortisol	.62*	.60*	.63*	–	–
DHEA-S	–	–	–	–	–
ADT-G	–	–	–	–	–
3αDIOL-G	–	–	.54*	–	–
Postexercise					
TESTO	−.73†	−.77‡	−.62†	−.66†	–
DHT	–	–	–	–	–
Cortisol	–	–	–		–
DHEA-S	–	–	–	.63*	.74†
ADT-G	–	–	.59*		–
3αDIOL-G	.68†	.67†	.66†		

NOTE. Postexercise levels/measures are the levels/measures after intervention.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

Similarly, baseline 3αDIOL-G was positively correlated with FFM ($r = .54$, $P < .05$).

Cortisol did not retain its correlation with the measures of body composition following the negative energy balance protocol, but postexercise TESTO, DHEA-S, ADT-G, and 3αDIOL-G each developed a significant association with the measures of body composition after intervention. Postintervention plasma levels of TESTO were highly and inversely correlated with all measures of posttreatment body composition except TFF (Table 4). Postexercise plasma levels of 3αDIOL-G were correlated with postexercise BW, FM, and FFM (BW, $r = .68$; FM, $r = .67$; FFM, $r = .66$; all $P < .01$), but these correlations were not significant after adjustment for loss of FM. Postintervention plasma levels of DHEA-S were correlated with postexercise AVF ($r = .63$, $P < .05$) and TFF ($r = .74$, $P < .01$) but also lost significance when adjusted for FM.

Individual subjects were divided into groups of higher and lower loss of measures of body composition with intervention (BW, FM, FFM, AVF, and TFF). ANOVA was used to identify any significant differences in steroid levels before and after exercise between the low- and high-loss groups for these measures. Results of these analyses are shown in Fig 2 for BW, FM, and AVF. There was a negligible loss of FFM with intervention. Differences in baseline and postintervention steroid levels between TFF high- and low-loss groups were not evident.

Subjects who lost more BW (mean, 6.8 kg) with the intervention were those with significantly higher baseline TESTO levels (13.1 nmol/L) than the BW low-loss group (BW loss, 3.2 kg; baseline TESTO, 11.4 nmol/L; F ratio = 4.6, $P < .05$), and their postexercise TESTO levels showed a trend to be higher, but not significantly (Fig 2a). Subjects who lost more FM were those with higher baseline levels of TESTO (Fig 2b). A mean loss of 6.8 kg FM was achieved in men with a mean baseline TESTO level of 14.0 nmol/L, whereas men with a

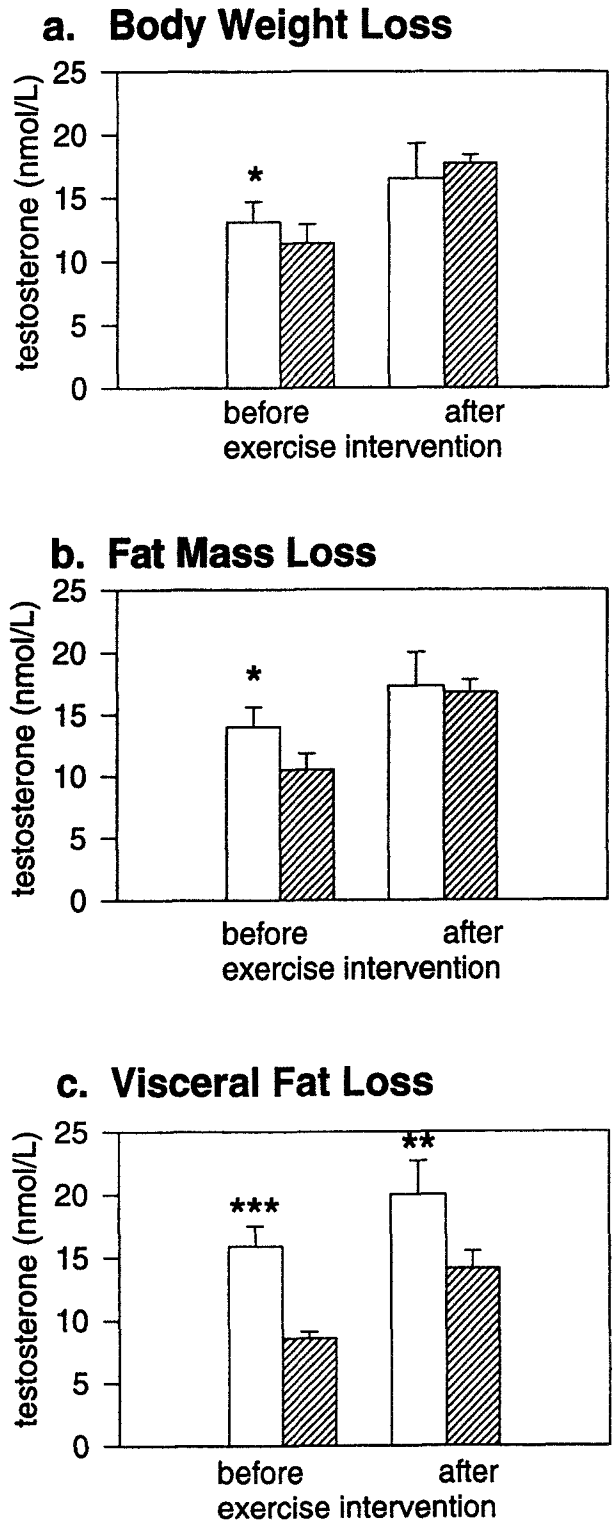

Fig 2. Mean TESTO levels before and after exercise-induced negative energy balance in subjects grouped according to (a) high and low loss of BW with intervention (mean high loss, 6.8 kg; mean low loss, 3.0 kg), (b) high and low loss of FM with intervention (mean high loss, 6.8 kg; mean low loss, 3.2 kg), and (c) high and low loss of AVF with intervention (mean high loss, 39.9 cm^2; mean low loss, 17.7 cm^2). (□) High loss; (■) low loss. *$P < .05$, **$P < .01$, ***$P < .001$.

mean loss of 3.0 kg FM had a baseline TESTO level of 10.5 nmol/L (F ratio = 7.3, $P < .05$). Postintervention TESTO levels did not differ between FM high- and low-loss groups. The strongest association was found between AVF high- and low-loss groups (Fig 2c). Men who lost more AVF (mean, 39.9 cm^2) were those with a higher mean baseline TESTO level of 15.9 nmol/L (F ratio = 75.8, $P < .0001$), and their postintervention TESTO levels were also significantly higher, 20.7 nmol/L, versus the AVF low-loss group (mean, 17.7 cm^2). The men in this category had a mean baseline TESTO level of 9.2 nmol/L, which increased to only 14.4 nmol/L after intervention (F ratio = 17.6, $P < .01$).

Association between insulin and steroids before and after intervention. The relationship between insulin and steroid levels before and after the intervention was examined. The mean ±SD postintervention fasting plasma insulin level of 65 ± 6 pmol/L was significantly lower than the preintervention level of 86 ± 10 pmol/L (mean ± SD) ($P < .01$).[22] Baseline fasting insulin showed no significant correlation with baseline DHT, DHEA-S, ADT-G, and 3αDIOL-G. However, it showed a strong negative correlation with baseline and postintervention TESTO levels ($r = -.66$, $P < .01$ and $r = -.86$, $P < .0001$, respectively). These levels of significance were retained after adjustment for FM at baseline and loss of FM postintervention. Similarly, postintervention fasting insulin was correlated negatively with posttreatment TESTO levels ($r = -.83$, $P < .0002$) and positively with postexercise 3αDIOL-G ($r = .61$, $P < .05$), but the latter correlations lost significance after adjustment for fat loss (Fig 3).

When subjects were divided into groups with high and low levels of fasting insulin before and after the intervention, subjects with lower baseline fasting plasma insulin (59.1 ± 13.9 pmol/L) showed significantly higher TESTO levels both at baseline and after intervention versus subjects with higher insulin levels (112.8 ± 33.5 pmol/L). This difference in TESTO levels was not evident with postintervention fasting plasma insulin levels. A two-way ANOVA using repeated measures on

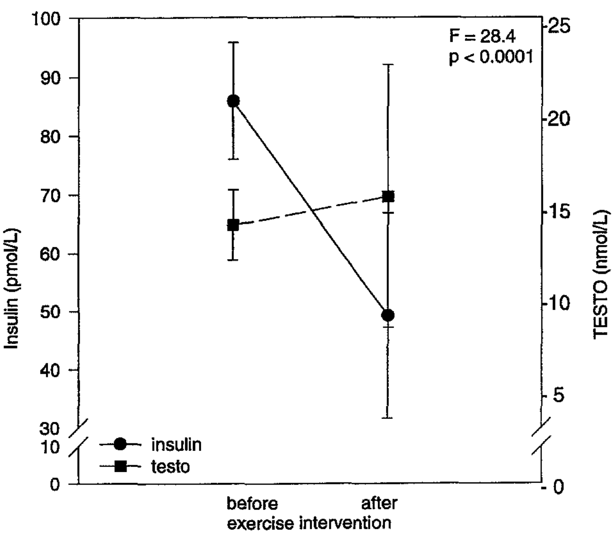

Fig 3. TESTO and insulin levels before and after exercise-induced negative energy balance.

time for both fasting insulin and TESTO levels before and after exercise intervention indicated a significant interaction (F ratio = 28.4, $P < .0001$). Figure 3 shows that postexercise TESTO levels increased the most in those with lower baseline fasting insulin levels and those with lower fasting insulin levels after the intervention protocol.

DISCUSSION

The Québec Negative Energy Balance Study has identified different patterns of within–twin-pair similarities in adrenal, gonadal, and conjugated steroids before and after an experimental exercise-induced energy deficit. In the mildly overweight young men, initial plasma levels of the major gonadal hormone TESTO and androgen metabolite 3αDIOL-G showed a highly significant within–twin-pair similarity (ICC for TESTO = .83 and ICC for 3αDIOL-G = .78). The within–twin-pair similarity for 3αDIOL-G was dependent on FM. Following the intervention and changes in body composition, the twin-pair resemblance for these steroids became nonsignificant, but the TESTO precursors cortisol and DHEA-S, as well as the metabolite ADT-G, gained a significant within–twin-pair resemblance. These findings confirm those of a previous Québec study with a larger sample of identical twin pairs before and after an overfeeding protocol.[26] In that study, a significant twin resemblance was observed for all baseline plasma levels of adrenal and gonadal steroids (except cortisol) in healthy lean young men.[15] With a larger sample of twin pairs, it is likely that we would also have detected here a significant twin-pair resemblance in the plasma levels of other steroids, in line with the familial effects previously described for plasma sex-steroid levels in related men[27] and for cortisol and DHEA-S in male monozygotic and dizygotic twins.[28]

Is the twin resemblance in plasma steroids retained with alterations in body composition accompanying the exercise-induced energy deficit? A strong intrapair resemblance was previously reported for all measures of body composition with either a positive or negative change in energy balance in identical twins.[29] In the present study, a significant identical twin–pair resemblance, and presumably a genetic effect, was evident for cortisol, DHEA-S, and ADT-G levels independently of the weight (and fat) loss accompanying the intervention. Cortisol has been positively associated with BW,[30] although the significance was lost with adjustment for FM.[31] DHEA has been reported to be negatively associated with FM.[32] It has been suggested that ADT-G, as an androgen metabolite, reflects androgen status.[33] In the current study, cortisol, DHEA-S, and ADT-G continued to be characterized by a significant within-pair resemblance when the data were adjusted for postexercise loss of fat. These results are generally in agreement with the genetic effect of 45% for total cortisol and 58% for DHEA-S reported by Meikle et al.[28] However, the small number of subjects in the present study and the use of only monozygotic twins make it difficult to compare the results of the present report with those of prior studies.[34]

There are many studies reporting decreased plasma levels of TESTO in men with overweight, obesity, or visceral obesity,[35-37] whereas we did not find any data showing an increase in TESTO with exercise-induced weight loss. Zumoff et al[38] have described a continuum of TESTO levels inversely related to BW in men across a range of BMI from very lean to morbidly obese. Leenen et al[39] reported a reduction in TESTO levels in women with a 12.4-kg weight loss, but men with a similar weight loss (13.5 kg) showed no change in TESTO. The loss of BW accompanying wasting in acquired immune deficiency syndrome was found to be characterized as much as by excessive loss of muscle mass as by fat loss.[40] Decrements in TESTO levels were correlated with loss of body potassium and muscle mass rather than loss of BW or body fat. It is noteworthy that the baseline BW of subjects in the current study (BMI, 26.2 ± 5.5 kg/m^2) was in the overweight range (BMI > 25.0) for healthy young men and the mean BW was within the healthy range (to a BMI of 24.6 kg/m^2) at the end of the exercise intervention. The loss of AVF and abdominal subcutaneous fat achieved by the exercise-induced weight loss[16] also reduced these fat measures to normal levels for healthy young men. The TESTO increment with decreasing abdominal fat in the current study is in accordance with the inverse relationship of TESTO with increasing abdominal fat in an overfeeding study[15] and the total and visceral fat described in overweight men in previous reports.[32,35]

Sutton et al[1] were among the first to record a rapid acute increase in TESTO levels in response to a short-term submaximal workload on a cycle ergometer, with the levels returning to basal in the postexercise period. A severe reduction of TESTO has been described with stressful excessive exercise training accompanied by a prolonged energy deficit.[7-9,12] Studies of a 62-day training program undertaken by US Army Ranger students in 1991 to 1992, which required extremely high levels of energy expenditure with food restriction under stressful, physically demanding environmental conditions, reported an average 11.3% loss of BW accompanied by an 86% decrease in TESTO.[9] Following a 95-day excursion involving a 2,300-km walk across Antarctica, excessive weight loss accompanied by malnutrition led to greater than 90% loss of TESTO.[8]

These stressful weight-loss conditions produced a response in TESTO reflecting the adverse nutritional status arising from the intervention. In the Québec Negative Energy Balance Study, nutritional status was maintained under the moderate controlled dietary and exercise conditions and the nonstressful nature of the intervention, as evidenced by the relatively low cortisol levels. There was no loss of FFM or change in hematological or biochemical parameters likely to create or reflect physically stressful conditions associated with the loss of weight.[16] The increase in TESTO levels accompanying the weight loss with conservation of FFM, produced with a moderate aerobic exercise program of 2 h/d, is not typical of a catabolic state. The fact that TESTO levels did not decrease is compatible with the reported TESTO relationship with FFM and not with FM.[41] However, although lean mass was entirely preserved in the current study, TESTO was consistently and inversely correlated with FM.

Zmuda et al,[42] who found that short-term exercise produces a transient elevation in TESTO in elderly men, proposed a decreased rate of TESTO clearance, similar to that described in dogs by Gagnon et al,[43] and/or hemoconcentration with exercise as the possible mechanisms underlying the higher levels in their observations. The retention of similar hemoglobin and hematocrit levels following intervention in the current study[16] implies that the postintervention TESTO levels were not likely an artifact arising from hemoconcentration. However, it is possible that a decrease in TESTO clearance could account for the increase in both DHEA-S and TESTO and the decrease in 3αDIOL-G following prolonged negative energy balance in the current study. The decrease in 3αDIOL-G, which was highly correlated with the decrease in FM ($r = .67$, $P < .01$) and total abdominal fat ($r = .65$, $P < .01$), is in accordance with the proposal that enzymatic conjugation of TESTO occurs in adipose tissue.[44,45]

Plasma levels of sex-hormone and cortisol binding globulins were not available in the present study. Further investigations should include such assays so that alterations in free hormone levels induced by negative energy balance may be understood. Also, there would be value in controlling for the effects of other confounder variables on plasma steroid levels, including the effects of circadian variations in hormone levels,[46] and the composition of the habitual diet.[47,48]

The current study has identified a significant interaction between TESTO and insulin. TESTO levels before and after intervention were higher in men with lower baseline fasting insulin levels. The relationship is closely allied with the changes in central fat. Significant increases in TESTO levels occurred after intervention in men with a greater loss of visceral fat. These findings are consistent with the observation of high insulin levels in visceral obesity,[36] and confirm the reported negative correlation of fasting insulin levels with TESTO in men with higher versus lower visceral fat.[49] These findings suggest that there may be metabolic benefits arising from weight loss achieved through exercise-induced negative energy balance: the greater the loss of FM and visceral fat and the protection of FFM, the greater the improvement in insulin (decrease) and TESTO (increase) levels.

In summary, this study identified changes in adrenal and gonadal steroids with exercise-induced negative energy balance in monozygous twins that were characterized by twin-pair resemblance and related to the amount of FM and abdominal fat loss. A high plasma TESTO level was associated with the leaner phenotype that accompanied the protocol of exercise-induced negative energy balance. We conclude from this study that the genotype is likely an important determinant of the plasma levels of adrenal, gonadal, and conjugated steroids during exercise-induced weight loss, and high plasma TESTO and low fasting insulin levels are associated with the preservation of FFM and major decreases in abdominal and visceral fat.

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