The Acute Effects of Androstenedione Supplementation in Healthy Young Males

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Abstract/Résumé

We examined the effects of androstenedione supplementation on the hormonal profile of 10 males and its interaction with resistance exercise. Baseline testosterone, luteinizing hormone, estradiol, and androstenedione concentrations were established by venous sampling at 3 hr intervals over 24 hr. Subjects ingested 200 mg of androstenedione daily for 2 days, with second and third day blood samples. Two weeks later, they ingested androstenedione or a placebo for 2 days, in a double-blind, cross-over design. On day 2, they performed heavy resistance exercise with blood sampled before, after, and 90 min post. The supplement elevated plasma androstenedione 2-3-fold and luteinizing hormone ~70% but did not alter testosterone concentration. Exercise elevated testosterone, with no difference between conditions. Exercise in the supplemented condition significantly elevated plasma estradiol by ~83% for 90 min. Androstenedione supplementation, thus, is unlikely to provide male athletes with any anabolic benefit and, with heavy resistance exercise, elevates estrogen.

Nous avons analysé les effets d’un apport complémentaire d’androstènedione sur le profil hormonal de jeunes hommes en bonne santé avant et après une séance d’exercices de musculation. Toutes les 3 heures d’une période de 24 heures, des échantillons de sang veineux ont été prélevés chez 10 volontaires pour établir le niveau de base de testostérone totale et

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The potent anabolic effect that testosterone can exert on skeletal muscle is well known (Bhasin et al., 1996; Brodsky et al., 1996). Because of this, bodybuilders and strength and power athletes view any manipulation that might elevate endogenous testosterone as a potential ergogenic aid. The initial step in the synthesis of testosterone in the testis of the adult male is the cleavage of the cholesterol side chain to form pregnenolone. This step is catalyzed by the enzyme 20,22-desmolase and is controlled by luteinizing hormone, or LH (Berne and Levy, 1988; Rommerts, 1990). As summarized in Figure 1, pregnenolone is then converted to a variety of biologically inactive C-19 steroids, which are precursors of either testosterone or estradiol. In males, the most common pathway is the stepwise degradation of pregnenolone to 17α-hydroxypregnenolone to dehydroepiandrosterone to androstenediol to testosterone. Testosterone, however, can also be synthesized by a parallel pathway that is initiated by the conversion of pregnenolone to progesterone and the subsequent formation of 17-hydroxyprogesterone and androstenedione, which can be converted to either estrone (and then estradiol) or testosterone. This final step is catalyzed by an isozyme of 17 β-hydroxysteroid dehydrogenase (Andersson et al., 1995; Penning, 1997). In addition, interaction can occur between these 2 pathways by the oxidation of the A ring of 17α-hydroxyprogrenolone and its conversion to 17α-hydroxyprogesterone, or of that of dehydroepiandrosterone and its conversion to androstenedione (Rommerts, 1990; Van Haren et al., 1989). Some of these inactive intermediates, such as androstenedione, leak out of the testis and are found in relatively high concentrations in the circulation.

Synthetic androstenedione is marketed by a number of sport nutritional supplement companies as a “prohormone,” which does not require a prescription and, when taken orally, will “boost testosterone” and subsequently enhance muscle size and strength. Whether or not significant amounts of testosterone can be derived from the peripheral conversion of androstenedione is somewhat questionable (Horton and Tait, 1966). The purposes of the present study were to determine the acute effects of androstenedione supplementation in healthy young males and
its possible interactive effect with heavy resistance exercise. This latter purpose is based on reports that plasma testosterone can be acutely elevated following a bout of heavy resistance exercise (Hakkinen and Pakarinen, 1993; Kraemer et al., 1990; Kraemer et al., 1992) and the suggestion by some authors that this response may promote muscle anabolism (Gotshalk et al., 1997). Since the completion of the present study, an investigation of the effects of 8 weeks of androstenedione supplementation, combined with resistance training, has been published by King et al. (1999). Using a 2-group randomized trial design, these authors concluded that supplementation did not increase serum testosterone concentration or enhance muscle size and strength and may have an adverse effect on serum lipid profiles.

Methods

SUBJECTS

Ten healthy, young males (mean age 24 ± 0.6 year, weight 79 ± 2.9 kg) volunteered to participate in the investigation. All had several years of previous resistance training experience and had never used anabolic steroids. They were advised of the purposes of the study and associated risks and gave their written consent. The project was approved by the Human Ethics Committee of McMaster University.

DESIGN

Baseline concentrations of plasma testosterone (total and free), luteinizing hormone, estradiol, and androstenedione were established by regular sampling of blood over 24 hr. Blood (5 ml) was drawn every 3 hr from an indwelling 22-gauge plastic catheter inserted into a forearm vein at 0830 hr and removed at 2030 hr of the same day, with an additional sample taken by venipuncture at 0830 hr the following morning. During that time, subjects were ambulatory and maintained their normal daily routine.
The following week, each subject was administered 200 mg of androstenedione per day for 2 days. The supplement was consumed in 100 mg capsules (Sports One, Klein Laboratories, Wallingford, CT) taken twice daily. On the second day, blood was sampled as described above and at the same time points. An additional sample was obtained by venipuncture at 1630 hr of the third day (24 hr after ingestion of the final capsule). Measurements were made on the same weekday, and subjects were instructed to replicate their activity patterns, as on the previous occasion, and to avoid any strenuous physical activity.

A minimum of 2 weeks later, subjects performed a typical resistance training session on two separate and randomly ordered occasions—a control condition, where they were administered placebo capsules for 2 days, and following 2 days of androstenedione supplementation as above. The two conditions were double-blinded and separated by a period of 2 weeks to allow for clearance of androstenedione. The exercise sessions were supervised by one of the investigators, and each consisted of 4 sets of 6 exercises (inverted leg press, lying hamstring curl, supine bench press, latissimus dorsi pulldown, shoulder press, and barbell biceps curl). Each set was performed at 80% of the predetermined 1RM and continued to failure with a 3-min recovery period between each set. Both placebo and androstenedione capsules were ingested at 0900 hr and 1500 hr for 2 days, with the exercise sessions beginning at 1600 hr and ending at 1730 hr on the second day. Blood (10 ml) was sampled by venipuncture immediately before (1600 hr) and after (1730 hr) exercise and at 90 min postexercise for hormonal analysis. Hematocrit was also determined at each time point in order to estimate plasma volume shifts, which are known to occur with such exercise (Ploutz-Snyder et al., 1995).

HORMONAL ASSAYS

Samples were collected in sodium-heparinized tubules and immediately centrifuged, and the plasma was frozen at −20°C until analysis. Plasma concentrations of total testosterone, free testosterone, androstenedione, luteinizing hormone, and estradiol were determined by radioimmunoassay by means of commercial assay kits (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA) for each hormone. In addition, the androstenedione supplement was assayed for purity. Assays were performed in duplicate, with each hormone measured in the same batch. The intraassay method error was less than 1.5%.

STATISTICS

All data were analyzed using a 2-way analysis of variance (Statistica, Statsoft Inc.). The level of significance was set at 0.05, and main effects were further analyzed by Tukey post-hoc test. Values are presented as means ± SE.

Results

BASELINE PROFILES

The supplement resulted in a 2–3 fold elevation \((P < 0.05)\) in plasma androstenedione concentration (Figure 2A). The effects of androstenedione supplementation on plasma total and free testosterone, over 36 hr of sampling, are illustrated in
Figures 2B, C and D. Figures 2B and C summarize mean data for the 10 subjects and, since grouped data for a pulsatile hormone can occasionally be misleading, we have also included the typical response for a single subject in Figure 2D. Although there was a trend toward a slight elevation in the early morning level of testosterone (by ~14%) in the supplemented condition, afternoon levels tended to be lower than in the control condition, with no statistically significant difference at any time point.

The effects of supplementation on LH are illustrated in Figure 2F. The supplement caused ~70% elevation in LH, which was statistically significant at most time points. Estradiol concentrations also appeared higher at all time points in the supplement condition (Figure 2E), but the difference was not statistically significant ($P > 0.05$).

**EFFECTS OF EXERCISE**

The effects of resistance exercise on plasma hormonal concentrations are illustrated in Figures 3 and 4. Total and free testosterone concentrations were significantly elevated immediately following exercise but returned to baseline levels or lower by 90 min following exercise (Figure 3), with no difference as a result of supplementation. Hematocrit followed a similar profile, increasing significantly as a result of the exercise and returning to baseline within 90 min (Figure 3). Estradiol concentration increased significantly following exercise in the supplemented condition and remained elevated for at least 90 min (Figure 4). LH concentration was unaffected by exercise but showed a trend towards a decrease (by ~15%) immediately following exercise (Figure 4).

**Discussion**

Under both conditions, plasma testosterone concentrations were well within the normal range for healthy young men and displayed the typical pattern of peak values occurring in the morning and then declining throughout the day (Figure 2). Free testosterone also typically comprised less than 3% of total and tended to track total concentrations, indicating the general dynamic equilibrium between the bound and unbound fractions in both conditions. These results are in agreement with those of King et al. (1999), who examined testosterone concentration over 6 hr following administration of 100 mg of androstenedione or a placebo. Our finding that androstenedione supplementation had no significant effect on plasma testosterone concentrations over the 24 hr sampling period is also consistent with an earlier radioactive tracer study, which indicated that in males, less than 0.3% of circulating testosterone is derived from the peripheral conversion of androstenedione and that an acute intravenous infusion of labeled androstenedione elevated testosterone by less than 6% (Horton and Tait, 1966). In contrast, ~60% of the testosterone in females can be derived from androstenedione (Horton and Tait, 1966). Since androstenedione is, itself, biologically inactive (Rommerts, 1990), it is unlikely that oral supplementation of this hormone provides male athletes with any anabolic benefit.

Following supplementation, clearance of the excess androstenedione was complete by 24 hr after ingestion of the last capsule (Figure 2). We did not measure
Figure 2. The acute effects of androstenedione supplementation on the 36 h hormonal profile of 10 young men. The androstenedione condition is indicated by open circles and placebo by closed circles. The Δs indicate the times at which the 100 mg supplement was ingested. A summarizes mean (±SE) plasma androstenedione concentrations, B total testosterone, C free testosterone, E estradiol and F luteinizing hormone for the 10 subjects. D illustrates total plasma testosterone for a typical individual subject. * indicates significant difference (P < 0.05) between placebo and supplemented conditions.
Figure 3. The effects of a 90-min bout of resistance exercise on plasma total testosterone (upper panel) and free testosterone (middle panel) and hematocrit in the placebo (closed circles) and androstenedione supplemented condition (open circles). The timing of the ingestion of the last capsule is denoted by ▲. Blood was sampled immediately before, immediately after, and 90 min after exercise. Values and means (±SE), N = 10. * indicates significant (P < 0.05) difference from pre-exercise value.
The effects of a 90-min bout of resistance exercise on plasma estradiol (upper panel) and luteinizing hormone (lower panel) in the placebo (closed circles) and androstenedione supplemented (open circles) condition. Values are means (±SE), N = 10. * indicates significant (P < 0.05) difference between conditions.

Figure 4. The effects of a 90-min bout of resistance exercise on plasma estradiol (upper panel) and luteinizing hormone (lower panel) in the placebo (closed circles) and androstenedione supplemented (open circles) condition. Values are means (±SE), N = 10. * indicates significantly greater than placebo (P < .05).

urinary androstenedione or urinary 17-ketosteroid concentrations but expect that this was the main mechanism for clearance, with a small proportion of the metabolite being converted to estrone, estradiol, and perhaps testosterone. The increases in estradiol in the supplemented condition were not statistically significant and were of such a small magnitude (~20%) that they were probably of no biological significance.

The significant elevation in basal plasma LH concentration that occurred with androstenedione supplementation was unexpected. LH synthesis and release is controlled by the hypothalamic hormone luteinizing hormone-releasing hormone, which is regulated, in turn, by negative feedback by the concentration of circulating testosterone. Since testosterone did not decrease in the supplemented condition, the increase in LH was surprising. Similarly, it was surprising that the increase in LH was not reflected by an increased secretion of testosterone. The half-life for circulating testosterone is as short as 12 min (Rommerts, 1990), whereas that of LH may be as long as 120 min (Santon and Bardin, 1973). We did not sample frequently enough to measure LH pulses, but it is possible that the increase in basal LH concentration may have been counteracted by a decrease in LH pulse frequency and-or amplitude or that any transient changes in testosterone went undetected with the timing of our 3 hr sampling protocol.
The bout of resistance exercise resulted in an increase in plasma testosterone and hematocrit, which was evident immediately following exercise but returned to baseline within 90 min (Figure 3). The elevated testosterone is consistent with a number of previous reports (Hakkinen and Pakarinen, 1993; Kraemer et al., 1990), as is the reduction in plasma volume (Collins et al., 1986; Ploutz-Snyder et al., 1995). Such shifts in fluid are thought to be caused by the movement of plasma from the vascular space to the interstitial space of the active muscles (Ploutz-Snyder et al., 1995) in response to the extreme elevations in mean arterial and intramuscular pressures, which occur with such exercise (MacDougall et al., 1992). Decreases in plasma volume are typically in the range of 15–20%, and normal volume is restored within 30 min (Collins et al., 1986).

More than 95% of the total circulating testosterone is bound to sex hormone-binding globulin or albumin. Since these plasma proteins are restricted in their ability to pass through the capillary endothelial wall, rapid shifts in plasma volume may result in a transient overconcentration of these carrier substances and their bound testosterone in the vascular space. It is possible, therefore, that much of the apparent increase in total plasma testosterone concentration immediately following resistance exercise could be secondary to the brief hemococoncentration that occurs, with normal levels being restored with the return to normal plasma volume (Figure 3). Our finding, that LH concentration did not increase as a result of exercise (Figure 4), further supports the view that the elevated testosterone concentration was not caused by an increase in its release or production rate. An alternative explanation is that its removal rate may have been reduced by decreased hepatic flow during the exercise session. In any event, the increase in testosterone following resistance exercise is relatively small (similar to normal morning concentrations) and lasts for less than 90 min. When considered in combination with the fact that the muscle protein synthesis stimulated by such exercise remains elevated for as long as 36 hr (MacDougall et al., 1995), we interpret this transient elevation in plasma testosterone as being of minimal anabolic importance.

Since estradiol concentration was unaffected by exercise in the control condition, our data indicate that the exercise interacted with the androstenedione supplement to exaggerate the conversion of androstenedione to estradiol, which was still ~83% above control levels 90 min after exercise (Figure 4). Whether or not such an elevation in estradiol is of sufficient magnitude and/or duration to have any adverse effects on healthy, young males who chronically ingest androstenedione and exercise daily is not known but probably warrants further investigation.

In summary, 200 mg of androstenedione supplementation per day (manufacturer’s recommended dosage) does not elevate plasma testosterone, as claimed. It does tend to elevate plasma estrogen, however, which is exaggerated by the interaction with resistance exercise. Thus, androstenedione supplementation appears to be of no significant anabolic benefit to the athlete. We recognize that these conclusions are based on findings following short-term supplementation and that some individuals might argue that, had the dosage been higher or the supplementation period longer, results may have differed. We think this unlikely, since the dosage that we used was sufficient to elevate plasma androstenedione 2–3 fold and suspect that higher dosages would result in a greater conversion to estrogen. In addition, we note that King et al. (1999) observed no significant increase
in testosterone following 8 weeks of supplementation at 300 mg per day. The supplement also causes an elevation of plasma luteinizing hormone, but the biological significance of this is unknown. An acute bout of heavy resistance exercise results in a transient elevation of plasma testosterone, which may be due to brief shifts in plasma volume rather than an increased secretion rate. The anabolic effects of this are probably minimal.

References


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