Dexamethasone in resting and exercising men.
II. Effects on adrenocortical hormones

G. Lac, P. Marquet, A. P. Chassain, and F. X. Galen

Dexamethasone in resting and exercising men. II. Effects on adrenocortical hormones. J. Appl. Physiol. 87(1): 183–188, 1999.—This study presents the reactions of adrenocorticotropin (ACTH) suppression to a dexamethasone (Dex) treatment, which is expected to lower steroid levels via the ACTH blockade, and to exercise, especially in athletes whose testosterone sulfate (DHAS), androgens, dehydroepiandrosterone (DHA), dehydroepiandrosterone sulfate (DHAS), and testosterone are expected to increase steroid production via ACTH stimulation. Consistent with the decrease in ACTH, all steroids except testosterone reacted negatively to Dex, independently of the dose (0.5 and 1.5 mg administered twice daily for 4.5 days). After exercise, plasma ACTH rose to 600% of basal value, resulting in a significant increase in aldosterone and adrenal androgens, but cortisol and DHAS were unaffected. This apparently surprising result can be explained by differences in peripheral metabolism: a theoretical calculation predicted that after 15 min the increase in hormone concentration may only reach 12% for cortisol and 2% for DHAS. For cortisol and adrenal androgens, assays were carried out using plasma and saliva. The consistent results obtained from the two matrices allow us to consider salivary assays as a useful tool for steroid abuse detection.

MATERIALS AND METHODS

For a more detailed description see our first study (18). Briefly, 24 informed, consenting healthy men, aged 24.0 ± 3.7 yr, volunteered for the study. They were organized in three Latin square repetitions (6 subjects, 3 treatments) to determine the possible effects arising from subjects, groups of subjects, time periods (P1–P3 following order of treatment), and treatments on the variables studied. Treatments were administered in double-blind, random order at 3-wk intervals. They consisted of nine capsules (375 mg per capsule, administered twice daily for 4.5 days) containing a placebo, 0.5 mg of Dex per capsule (low dose), or 1.5 mg of Dex per capsule (high dose).

On the morning of the 5th day, the subjects came to the laboratory. They carried out a 12- to 18-min incremental test on a cycle ergometer to determine their aerobic capacity (V\text{O}_{2\text{max}}) in similar and defined conditions with use of an Oxycon 4 Mijnhardt system. Just before and at the end of the test, 100 ml of blood were drawn from a catheter (introduced into the forearm vein 1 h before the test). Simultaneously, they gave a saliva sample (unstimulated) in a plastic disposable tube (2-4 ml). Blood was immediately centrifuged, and plasma and saliva samples were divided into aliquots and stored at −25°C until assayed.

Steroids (androstenedione, DHA, DHAS, cortisol, and testosterone) were assayed following a routine method developed in the laboratory and previously described by Lac et al. (14). The validity criteria are as follows: sensitivity of 15 pg, accuracy of 8.6 pg, and interassay reproducibility of 11.3%. ACTH and aldosterone were assayed with EISA-ACTH-125I and SB-ALDO-H-M-3H kits, respectively; owing to small
saliva sample volume, some hormones were assayed only in plasma (testosterone and aldosterone), as was ACTH, which cannot be determined in saliva. For a given hormone, all the assays for plasma or saliva were performed in the same run to avoid inter assay variations.

Statistical analysis. Using ANOVA (18), we found that the Latin square schedule, the fitness status, and the time period (order of treatment) had no effect on steroid hormones at rest or after exercise. ANOVAs were applied to treatments and to exercise effects, and when a significant effect was found, 2 × 2 comparisons were carried out using the paired t-test (significance threshold set at 0.05). Regression analyses were done to test the correlation (Pearson’s r) between saliva and plasma concentrations.

RESULTS

Plasma and saliva hormone concentrations with placebo and with low- and high-dose Dex at rest are presented in Table 1 and those at the end of the exercise bouts are presented in Table 2, together with the results of statistical comparisons. All these effects are illustrated in Fig. 1, expressed as percent changes from resting levels (with placebo), which were taken as references. This presentation allows a direct comparison of the results obtained in the two biological fluids.

Testosterone was the only hormone not affected by the treatment at rest (Table 1, Fig. 1G) or at the end of the exercise bout (Table 2, Fig. 1G). In both situations, Dex induced a major decrease in plasma levels of ACTH (−67% at rest and −78% after exercise with low-dose Dex and −85% at rest and −94% after exercise with high-dose Dex; Tables 1 and 2, Fig. 1A). Except for ACTH, high-dose Dex did not induce a significantly greater reduction in the hormone levels than low-dose Dex. For cortisol this reduction was −86 to −91% in both cases and in both matrices. It was smaller (−45 to −60%) for the other steroids (Tables 1 and 2, Fig. 1, B–F).

The statistical significance of the effects of exercise (exercise vs. rest) is noted in Table 3 for the three treatment modalities (placebo and low- and high-dose Dex) independently of the matrices used, since the results were identical in blood and saliva. As shown in Fig. 1, the greatest effects of the short and intense exercise bouts performed by the subjects appeared in ACTH plasma levels, which were increased by +553% above rest level with placebo and +350 and +146% with low- and high-dose Dex, respectively (Fig. 1A). Aldosterone presented the same significant increase (about +100%) after exercise with placebo and low- and high-dose Dex (Fig. 1B), whereas for androstenedione and DHA a rise of ~50% occurred with placebo but no further significant change was observed with low- and high-dose Dex (Fig. 1C and D). Testosterone, cortisol, and DHAS levels did not change with exercise regardless of treatment (Fig. 1, E–G).

As illustrated in Fig. 1, C–F, and Tables 1–3, results from saliva were the same as those from plasma for cortisol, androstenedione, DHA, and DHAS. To confirm the validity of these saliva assays, Table 4 shows the Pearson coefficient of correlation between plasma and saliva concentrations from the 72 pairs of results. All were highly significant.

<table>
<thead>
<tr>
<th>hormone</th>
<th>plasma</th>
<th>saliva</th>
<th>correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>DHA</td>
<td>0.04</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>DHAS</td>
<td>0.05</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.07</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.08</td>
<td>0.07</td>
<td>0.01</td>
</tr>
</tbody>
</table>

DISCUSSION

The procedure used in this study highlighted the effects on adrenal hormones of a double, opposite modulation of ACTH: its suppression by Dex and its stimulation by exercise. Possible variations of hormone levels due to plasma shift (8, 28) or to a lowering of
Fig. 1. Dexamethasone and exercise effects on plasma levels of ACTH (A), aldosterone (B), and testosterone (G) and plasma and saliva levels of dehydroepiandrosterone (DHA; C), aldosterone (D), cortisol (E), and DHA sulfate (DHAS; F). Concentrations are expressed as percentage of resting level with placebo (P) to allow a direct comparison of results obtained in plasma and saliva. Lo, low-dose dexamethasone; Hi, high-dose dexamethasone.
hepatic clearance linked to exercise (7) can be excluded, since no variation was noted in the plasma concentration of testosterone, a testicular steroid not dependent on ACTH control. Moreover, because testosterone levels remained stable, variations of luteinizing hormone (LH) and, consequently, any eventual effects of this pituitary stimulation on adrenal androgens can also be excluded. Therefore, variations in levels of adrenal androgens found in the present study can be attributed only to regulation of the hypothalamic-pituitary-adrenal axis by ACTH.

The administration of low- and high-dose Dex during a short time period to healthy subjects induced a dose-dependent decrease in ACTH plasma level, which in turn induced a decrease in cortisol, aldosterone, androstenedione, DHA, and DHAS levels, but no significant dose effect, inasmuch as the maximum effect was reached with low-dose Dex.

The short, high-intensity exercise bouts performed by each subject induced a dramatic rise in plasma ACTH with placebo and a smaller, but significant increase with both Dex doses. Thus by acting through the hypothalamic-pituitary axis, exercise induced a release of ACTH, despite the Dex blockade, in the same way that ACTH and cortisol were reported to respond to corticotropin-releasing hormone after Dex blockade (17), although to a lesser extent. The best response to this rise in ACTH was in aldosterone, which increased significantly after exercise (Table 3, Fig. 1B) with both Dex doses, approximately to the same relative extent (+100%) as with placebo. DHA and androstenedione increased significantly with exercise only with placebo. Thus it seems that ACTH acts at an early step in the aldosterone biosynthesis pathway. ACTH blockade would thus reduce the pool of aldosterone precursor, inducing a lower absolute response but an equal relative response to its main regulator, the renin-angiotensin system.

Table 3. Statistical significance of effects of exercise on hormone plasma and saliva levels with placebo and low- and high-dose dexamethasone

<table>
<thead>
<tr>
<th>Exercise vs. Rest</th>
<th>P</th>
<th>Lo</th>
<th>Hi</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cortisol</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DHA</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DHAS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tbody>
</table>

NS, not significant.

Table 5. Plasma concentrations and characteristics of peripheral kinetics of steroid hormones

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Half-Life, min</th>
<th>MCR, µmol/24 h</th>
<th>PR, µmol/24 h</th>
</tr>
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<tr>
<td>Testosterone</td>
<td>21.67</td>
<td>30</td>
<td>1,100</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>4.89</td>
<td>20</td>
<td>1,600</td>
</tr>
<tr>
<td>DHA</td>
<td>14.23</td>
<td>20</td>
<td>1,600</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>0.0484</td>
<td>20</td>
<td>1,800</td>
</tr>
<tr>
<td>Cortisol</td>
<td>277.38</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>DHAS</td>
<td>6,661.1</td>
<td>600</td>
<td>10–15</td>
</tr>
</tbody>
</table>

MCR, metabolic clearance rate; PR, production rate.

Surprisingly, cortisol, which is the target of ACTH and responded strongly to Dex treatment, and DHAS, the nonconjugated parent compound (DHA) of which increased strongly during exercise, did not vary significantly from rest to the end of the exercise bout. These discrepancies among responses of different adrenal steroids to an incremental, maximal exercise lasting ~15 min can probably be explained by their respective peripheral kinetics: a hormone level (L) is the result of an equilibrium between its production rate (PR) and its catabolism rate, measured through its metabolic clearance rate (MCR). These three parameters are bound by the relation

\[ PR = L \times MCR \]

MCR is linked to the half-life of the product: the greater the MCR, the smaller the half-life. Table 5 presents the values used for the estimations below: the values attributed to half-life, MCR, and PR were obtained by averaging bibliographic data (2, 3, 6, 22, 23); the plasma hormone concentrations are those measured at rest with placebo in this study.

For cortisol, which is the most reactive hormone after stress, postexercise levels can be twice as high as rest levels, as generally reported in the literature (20, 30). To maintain such a level with a constant MCR, the PR must be doubled. Thus, given a doubling of PR for each hormone, it may be calculated\(^1\) that, after 15 min, there will appear an increase of 1) 40% for the two nonconjugated adrenal androgens androstenedione and DHA and for aldosterone (which presents approximately the same MCR), 2) 12% for cortisol, and 3) 2% for DHAS, the half-life of which is very long. These theoretical

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\(^1\) The theoretical percent increase of hormone levels after 15 min of exercise (average V\textsubscript{O2max} test duration) is calculated as follows. During a continuous infusion (which mimics the secretion of the gland into the bloodstream), the hormone level will reach a plateau. According to Tait (26), the kinetics of a hormone in this case are represented by the following equation: \[ Y = Y_0 \times e^{-\frac{t}{\text{t}_0/a}} \]

From this equation, the slope of the curve; \( a \) is calculated as follows: \[ a = \ln(2)/\text{t}_0 \] where \( Y_0 \) is the hormone concentration, \( Y \) the level at steady state, \( \text{t}_0 \) the exponential function, \( t \) the elapsed time (in this case, 15 min), and \( a \) the slope of the curve; \( t_0 \) is the half-life of the product. To produce a twofold increase in the level (\( Y_1 = 2 \times Y_0 \)), PR must be doubled. In this case, \( Y_1 \) will reach the new plateau (\( 2Y_0 \)) following the same equation, since it starts from \( Y_0 \) at steady state. An example of the calculation for cortisol with the values of Table 5 is as follows: \[ Y_0 = 100.5 \times 100.5e^{-0.0065 \times 15} = 12.7 \] Thus, after 15 min, an increase of 12.2 ng/ml (12%) in cortisol level will appear if its PR has doubled.
values agree with the data of this study, except for aldosterone, which responded more strongly for reasons mentioned above. Considering the fact that ACTH does not respond immediately at the onset of exercise (27, 31), these theoretical values are certainly overestimated, and it is not surprising that in this study the cortisol level remained unchanged during exercise, because the delay necessary to obtain a significant response for cortisol was not reached. This may explain the controversial results found in the literature: some authors described a regular rise during the $\dot{V}O_{2\text{max}}$ test (20); others reported a plateau, if not a fall, depending on individuals (30).

In fact, the cortisol increase reported for all kinds of tests of short duration (<15 min) could simply arise from an anticipation stress, such as can be observed in situations of psychological stress without exercise (4). This assessment (concerning the delay of response) is in accordance with results obtained after ACTH stimulation (27). Considering such a delay, only the adrenal androgens and aldosterone may present a rise strictly linked to exercise, a reason that may justify the determination for the analysis of the pituitary-adrenal axis response to these short intense exercises.

Contrary to the absence of a significant effect of this short exercise on cortisol and DHAS levels, Dex induced a strong effect on these two hormones, because concentrations were determined after 4.5 days of treatment. Even DHAS, with a half-life of 10–14 h, showed a decrease similar to that of its nonconjugated parent compound (DHA), the half-life of which is 20 min. Considering the very long half-life and the very large pool of DHAS, its level would return to normal very slowly after the end of Dex administration, or, in other words, Dex is likely to exert a long-lasting effect on this compound. Thus the determination of DHAS may be of interest for the screening of corticosteroid abuse. Unfortunately, we could not monitor the return to basal levels in this study. It was reported that (5), during a marathon run, DHAS began to rise at the 30th km only (thus after >1.5 h of running), whereas all other steroids exhibited a sharp rise beginning at the 10th km. Conversely, DHAS remained at a high level 2 h after exercise, contrary to other steroids, the levels of which had decreased at this time. These results agree well with the characteristics of the above-mentioned kinetic parameters of DHAS, although they were not discussed in this way (5).

The greatest effect of ACTH decrease with Dex, as expected, was noted for cortisol, the level of which fell by ~90%. This fall was ~50% for aldosterone, androstenedione, and DHA, meaning that if these three compounds did effectively react to the ACTH decrease, Dex did not abolish their secretion. In fact, their regulation does not depend on ACTH only. Another regulatory mechanism is well known for aldosterone (the renin-angiotensin-aldosterone system), although it is only suspected for the adrenal androgens (12, 19). After the present results, stimulation by LH may be discarded, since no variations occurred in testosterone level, a compound presenting quite the same peripheral kinetic parameters as androstenedione and DHA. Thus a non-ACTH, non-LH stimulation may be hypothesized for androstenedione and DHA (19).

Saliva has been revealed as a convenient and useful alternative medium to plasma for steroid screening, since the very same conclusions may be drawn from plasma and saliva levels of cortisol, androstenedione, DHA, and DHAS. Numerous studies have validated RIAs for steroids in this biological fluid (14, 21, 29). They are widely used in sport studies. Considering the advantages of saliva sampling, i.e., saliva sampling is noninvasive and nonstressful and in this case respects intimacy, and saliva is easy to collect, store, and assay, we suggest that this technique may be a useful tool in the detection of steroid drug abuse in sports.

Address for reprint requests and other correspondence: G. Lac, Dept. of Sports Sciences, UFR STAPS, University of Clermont-Ferrand, PO 104, 63172 Aubière Cedex, France (E-mail: glac@cicsun.univ-bpdermont.fr).

Received 4 June 1998; accepted in final form 25 February 1999.

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