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Effect of Amino Acid Supplementation on the Urinary Steroid Profile
Concentrations

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Effect of amino acid supplementation on the urinary steroid profile concentrations

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ABSTRACT

We have investigated the possible effects of the administration of branched chain amino acids (BCAAs) on the urinary concentration of androgens and estrogens. The urines of 20 male healthy volunteers (age 25-40) moderately trained (1-2.5 hrs x 3 times/week) and taking food supplements were analyzed prior to, during and after the period of supplementation with (a) minerals (b) vitamins and (c) BCAA. Circadian variability was studied for all subjects participating to the study: the analysis of all samples (at least 2 times/months for each subject) randomly collected at 10 am for 4 months. Supplementation with minerals and vitamins did not provoke any alteration of the steroid profile, while BCAAs caused a statistically significant reduction of the absolute urinary concentration values of androgenic steroids.

INTRODUCTION

Food supplements containing vitamins, minerals and the branched chain amino acids (BCAA) valine, leucine and isoleucine, are widely used as a treatment of nutritional problems, primarily among them the loss of nutritional constituents. These products can also be used by sportsmen, not only at elite level, mainly to sustain the effort during heavy training sessions and/or performed in extreme environmental conditions (1). All these products are not forbidden by the current antidoping regulation.

The detection of doping by endogenous steroid hormones and/or metabolites/precursors is currently performed by following all those urinary samples showing abnormal values of relative urinary concentrations of two or more endogenous markers (for instance, any sample showing a concentration ratio testosterone/epitestosterone higher than

the allowed limit deserves further investigation). The study of the stability of those values is therefore relevant not only to sport physiology, but also to doping control tests. Most of the literature data concerning the concentration values of androgen and estrogen steroids before, during and after supplementation with branched chain amino acids (BCAA) is limited to the study of blood levels, referring to elite athletes, and mainly concerned with transient alterations of blood concentration values (2-4).

The aim of this study was to verify whether the administration of different nutritional supplement to moderately trained male healthy volunteers could induce (i) statistically significant changes in the urinary steroid profile, and/or (ii) the biosynthesis of pseudo-endogenous anabolic steroids and/or their metabolites.

EXPERIMENTAL SECTION

Nutritional supplements

The following products, preliminary tested to confirm the absence of any anabolic agent (either endogenous or synthetic) and/or metabolites (5-6), have been used:

- vitamins-based product (vit. C 150 mg; vit. D 400 U.I.; alpha tocopherol 2.05 mg; vit. A 3.333 U.I.; vit. B1 20 mg; vit. B2 5 mg; vit. B6 10 mg; nicotinamide 50 mg; calcium pantothenate 11.6 mg; biotine 1.8 mg; cyanocobalamine 5 µg, CuSO₄ 3.9 mg; ZnSO₄ 2.3 mg; MoSO₄ 0.5 mg): 1 tablet/day;
- minerals (Cl 239 mg; Na 225 mg; K 200 mg; Mg 90 mg; vit. C 60 mg): 2 sachets/day;
- branched chain amino acids (L-Leucine 1000 mg ; L- Isoleucine 500 mg; L-Valine 500 mg): 2 tablet/day.

Urine pretreatment and GC-MS instrumental conditions

All urine sample (in duplicate) were passed on C18 cartridges, the MeOH eluate was hydrolyzed by β-glucuronidase, pre-concentrated under N₂ stream, derivatized to form TMS-derivatives, and assayed by GC-MS (7-8).

Administration study

The relative concentrations of testosterone, epitestosterone, androsterone, etiocholanolone, 11β-OH-androsterone, 11 β-OH-etiocholanolone, estrone, estradiol, estriol were performed on all samples before, during and after the period of administration of nutritional supplements. In the pre-administration period, urine samples were collected at 10 am, at least 2 times/months for 4 months, to 20 male healthy volunteers (age 25-40, normal

BMI) not administered with nutritional supplements. In the administration period, urine samples were collected daily at 10 am to 10 male healthy volunteers; in the post-administration period, urine samples were collected daily at 10 am for at least one week to the same male healthy volunteers previously involved in the administration phase.

Reference standards

The standards were obtained by NARL-Australia (testosterone, epitestosterone), Sigma Aldrich (androsterone, estrone, estradiol, estriol) and Steraloids (etiocholanolone, 11 β -OH-androsterone, 11 β OH-etiocholanolone).

RESULTS AND DISCUSSION

Single concentration data for each urine sample (2 aliquots, each assayed twice) were normalized as a function of the individual reference concentration, the latter being, for each hormone, the mean of the concentrations recorded for each volunteer in the pre-administration period. Urinary baseline levels of the selected androgens (A) and estrogens (B) in volunteers not treated with food supplements are shown in figure 1; while the effects of nutritional supplement are shown in figures 2 (minerals), 3(vitamins) and 4 (BCAA).

The preliminary longitudinal study, carried out over a period of 4 months, of the urinary steroid (androgens and estrogens) profile in moderately trained male volunteers showed that, taken into account the circadian fluctuations, the relative hormonal concentrations are stable as a function of the time.

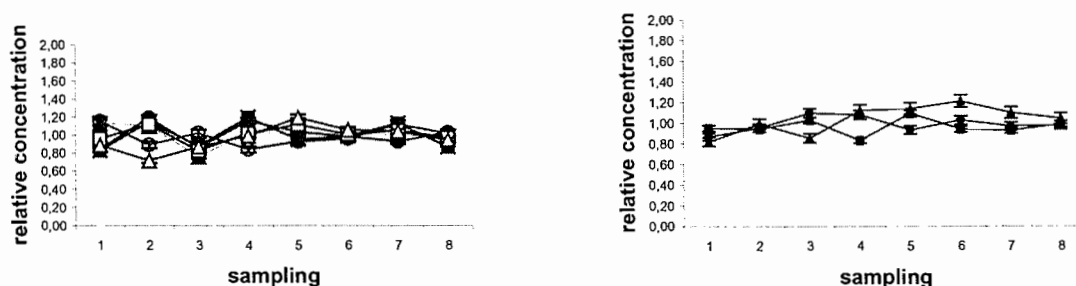


Figure 1. Stability of relative androgen (left) and estrogen (right) concentrations, evaluated in the pre-administration period. ■=testosterone; ●=epitestosterone; σ=androsterone; □=etiocholanolone; ○=11 β -OH androsterone; △=11 β -OH etiocholanolone; σ=estriol; ■=estrone; ●=estradiol

The overall stability of the urinary steroid profile allowed to evaluate the effect of different nutritional supplements. Particularly, the supplementation by either vitamins and/or mineral salts did not influence the urinary steroid profile. On the contrary, the supplementation by BCAA causes a statistically significant ($p < 0.05$, paired T-test) variation of the urinary steroid concentrations from normalized baseline values: reduced values of both androgens and estrogens concentrations were observed in all volunteers.

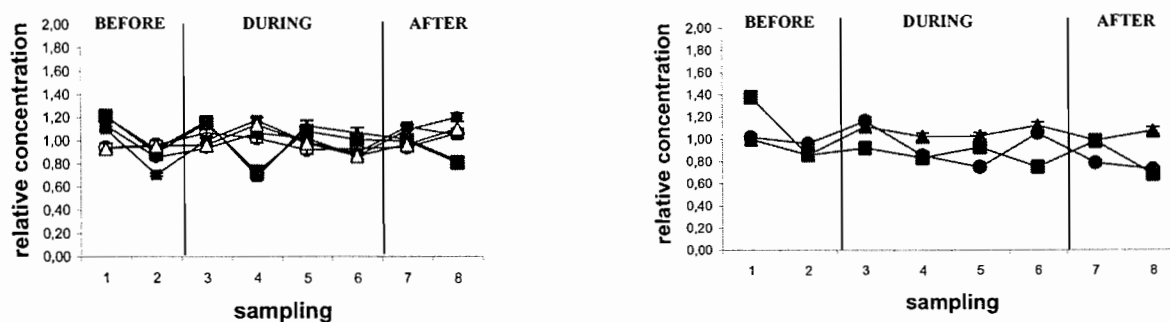


Figure 2. Relative concentration of androgens (left) and estrogens (right) before, during and after the administration of mineral salts. Symbols represent the same hormones as in fig. 1.

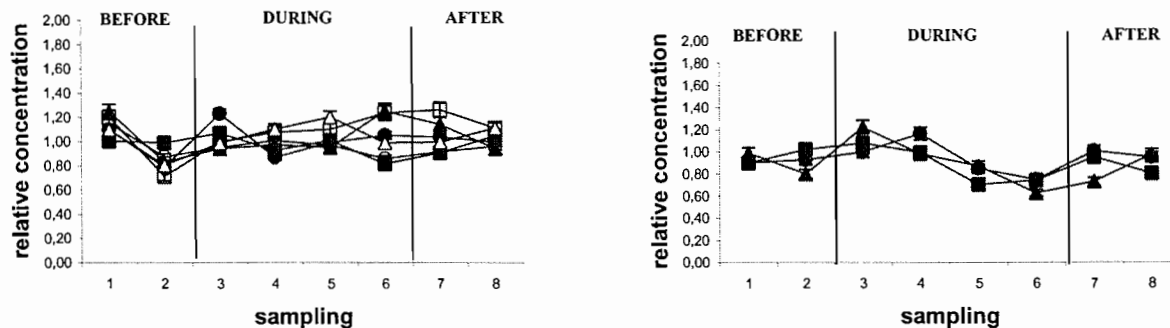


Figure 3. Relative concentration of androgens (left) and estrogens (right) before, during and after the administration of vitamins. Symbols represent the same hormones as in fig. 1.

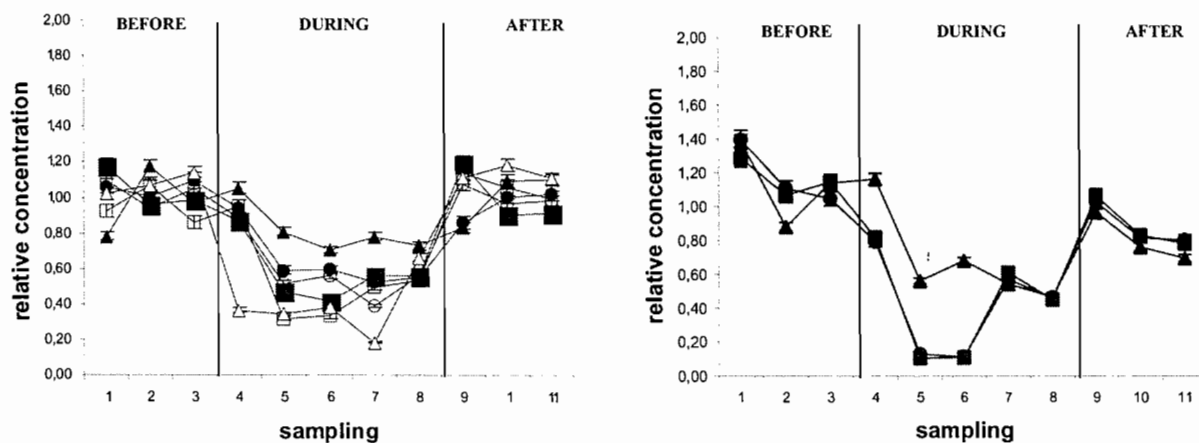


Figure 4. Relative concentration of androgens (left) and estrogens (right) before, during and after the administration of BCAA. Symbols represent the same hormones as in fig. 1

The interruption of supplementation caused the immediate return to the baseline values. No exogenous substance (e.g. synthetic androgenic anabolic steroids) nor metabolite was detected in any of the assayed samples.

Although very preliminary, and referred to a limited number of male subjects, our data seem to confirm that vitamins and mineral salts do not alter the urinary steroid profile, while the supplementation with BCAA induces a transient decrease of their concentration, the effect being fully reversible and limited to the duration of the supplementation period. The decrease of the relative concentration value observed after the administration of BCAA may be very pronounced (going down to 20% of the baseline value for 11β -OH androsterone, 11β -OH etiocholanolone, estrone and estradiol), suggesting that, as far as the concentration of these compounds is concerned, the concurrent administration of testosterone (and/or precursors) and BCAA could, in principle, show little difference with the baseline profile. Further light on the biochemical mechanism responsible for the observed effects may be shed by additional excretion studies, i.e. by monitoring the concentration of endogenous steroids under the concurrent administration of testosterone (and/or its precursors) and BCAA.

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