A Moderate Increase in Daily Protein Intake Causing an Enhanced Endogenous Insulin Secretion Does Not Alter Circulating Levels or Urinary Excretion of Dehydroepiandrosterone Sulfate

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To study the effect of a moderate increase in insulin secretion produced by an increased daily protein intake on dehydroepiandrosterone sulfate (DHEAS), a balanced randomized crossover trial consisting of three strictly controlled dietary regimens was performed in six healthy male volunteers. The basic diet (B) contained 50 g protein/d; diets P and M (also basic diets) were enriched with either 32 g protein/d (P) or 10 mmol L-methionine/d (M). Methionine was given (as a specific nonprotein source of endogenously derived sulfate) to control for possible confounding effects on DHEAS due to an increased sulfate supply. At the end of each 4-day diet period, blood and 24-hour urine samples were collected. Fasting plasma levels of testosterone, cortisol, insulin-like growth factor-I (IGF-I), and insulin, as well as urinary output of total (hot acid-cleaved) testosterone conjugates and 3α-androstanediol glucuronide, did not show significant changes in response to dietary manipulations. Endogenous sulfate availability (as reflected by renal sulfate output per 24 hours) approximately doubled with diets P and M. However, plasma levels (6.3 ± 1.5, 6.8 ± 1.8, and 6.9 ± 2.1 μmol/L for B, P, and M, respectively) and urinary excretion (8.8 ± 9.8, 9.4 ± 11.2, 8.0 ± 8.3 μmol/d) of DHEAS remained unaffected. Considering the clear increments ($P < .01$) in urinary C-peptide excretion with diet P (20.4 ± 10.3 nmol/d) versus diets B and M (12.6 ± 5.1 and 13.2 ± 3.6 nmol/d), respectively, our results suggest that a moderately strong diet-induced increase in daily insulin secretion does not alter urinary and plasma levels of DHEAS.

THE ADRENAL GLAND secretes a variety of steroids in addition to cortisol and aldosterone. The quantitatively most important steroid released from this gland is dehydroepiandrosterone sulfate (DHEAS). As metabolically active prehormones, DHEAS and its unconjugated parent steroid, DHEA, serve as precursor steroids for the synthesis of biologically potent sex steroids. The conversion to active androgens and partly to estrogens depends on sex, genetics, age, metabolic state, and target tissues.[1-4]

Apart from its direct androgenic (or estrogenic) action in target tissues, DHEAS also appears to have immunomodulatory functions,[5] antiglucocorticoid effects,[6] and positive effects on circulating insulin-like growth factor-I (IGF-I) and insulin sensitivity.[3] Insulin itself, when chronically but not excessively elevated, may stimulate DHEA and/or DHEAS production.[7,8] On the other hand, in studying the direct (acute) impact of insulin on adrenal androgens, Nestler et al[9] noted a rapid decrease in circulating DHEAS (and DHEA) after an insulin infusion (hyperinsulinemic-euglycemic clamp technique) in normal men. Urinary DHEAS output was increased during the latter study.

The inverse association of circulating DHEAS with circulating insulin was confirmed in a number of investigations.[10-12] An acute suppression of DHEAS blood levels has even been observed after an oral glucose load.[13] However, no studies have investigated whether alterations in daily nutrient intake that moderately affect endogenous insulin production also influence DHEAS levels. We now report on the effect of an increased daily protein intake on DHEAS. Protein is a known stimulus of pancreatic β-cell insulin release.

SUBJECTS AND METHODS

Subjects

Six healthy male volunteers aged 23 to 51 years (body weight, 73.8 ± 5.8 kg) were studied. None were using oral medications or had a history of renal, endocrine, or cardiovascular disease. The study was approved by the institutional review board of the Research Institute of Child Nutrition Dortmund, and written informed consent was obtained from each subject.

Study Design

The study design was a balanced randomized crossover trial consisting of three strictly controlled isoenergetic (9,140 kJ/d) dietary regimens, each of which lasted 4 days. The basic diet (B) contained 50 g protein/d, 101 g fat/d, and 282 g carbohydrate/d. For diet P (total protein content, 82 g), 14.4 g of fat was isoenergetically replaced with 32 g egg white protein. Regimen M was diet B enriched with 10.0 mmol L-methionine/d (Acimethin; Gry-Pharma, Kirchzarten, Germany). L-Methionine was given with diet regimen M (as a specific nonprotein source of endogenously derived sulfate) to control for possible confounding effects on DHEAS due to an increased sulfate availability.

To study separately the specific impact of protein on renal ammonium and net acid excretion capacity,[14] daily doses of 6.67 mmol trisodium citrate-dihydrate (for foodstuffs; Merck, Darmstadt, Germany) were additionally administrated (for urine pH adjustment) during diet periods P and M. The corresponding results (on acid-base metabolism, eg, nearly identical 24-hour urine pH and renal net acid excretion levels were found during all diet periods) have been published elsewhere.[14] That report also contains further details on the dietary regimens (meals, beverages, etc.).

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Sampling and Analytical Procedures

Twenty-four-hour urine was collected on day 3 and day 4 of each diet period and immediately stored below −20°C. Fasting blood samples were obtained by venipuncture between 8:00 and 9:00 AM on day 5 (shortly after the subjects had completed the second 24-hour urine collection).

Commercial solid-phase ^{125}I radioimmunoassays (coated-tube methodology) were used for measurement of DHEAS, cortisol, testosterone (all three kits from Diagnostic Products, Los Angeles, CA), and 3α-androstanediol glucuronide (AdiolG [5α-androstan-3α,17β-diol glucuronide]; Diagnostic Systems Laboratories, Webster, TX). C-peptide (a good measure of endogenous insulin production)[15] was analyzed by a radioimmunoassay using the PEG-accelerated double-antibody method to separate bound ^{125}I-labeled C-peptide from the unbound fraction (Diagnostic Products). IGF-I was quantified with an immunoradiometric ^{125}I assay (Diagnostic Systems Laboratories). All ^{125}I assays were purchased from DPC Biermann (Bad Nauheim, Germany). Insulin was analyzed by a highly sensitive (insulin detection limit ~1 pmol/L) and specific (no cross-reactivity to human proinsulin) enzyme immunoassay (Insulin MTPL EIA; DRG Instruments, Marburg, Germany).

Assays of plasma hormones, as well as urinary hot acid-cleaved testosterone conjugates and urinary C-peptide, were performed according to the respective manufacturers' instructions. Urinary DHEAS was quantified directly (like plasma DHEAS), without kit modification or specific sample preparation.[16] Quantification of urinary AdiolG was performed with modifications described elsewhere.[17] Urinary nitrogen and sulfate were analyzed using a modified Kjehldahl method and a Dionex 2000 i/SP Ion-Chromatograph (Dionex, Idstein, Germany), respectively.

Statistical Analysis

Changes in the dependent variables during diet periods were compared by one-way ANOVA with repeated measures. All data are expressed as the mean ± SD. F values are provided wherever ANOVA-derived *P* values are less than .05.

RESULTS

The isoenergetic replacement of fat with egg white protein resulted in clear increases in urinary nitrogen and C-peptide excretion (diet P *v* diets B and M; Fig 1). The slight elevation in circulating C-peptide and insulin seen with diet P failed to reach statistical significance (Table 1).

Endogenous sulfate availability (as reflected by renal sulfate output per 24 hours) doubled or nearly doubled with diets P or M (Table 2), but plasma levels and urinary excretion of DHEAS remained largely unaffected (Tables 1 and 2).

Plasma concentrations of testosterone, cortisol, and IGF-I (Table 1), as well as urinary output of total testosterone conjugates and AdiolG (Table 2), did not show significant changes.

DISCUSSION

Both insulin and nutritional status have proved to be determinants of circulating IGF-I. The increase in daily protein intake of 32 g/d that caused a clearly elevated overall insulin production during the day (as evidenced by 24-hour urinary C-peptide excretion) did not significantly affect fasting plasma levels of insulin, C-peptide, and IGF-I.

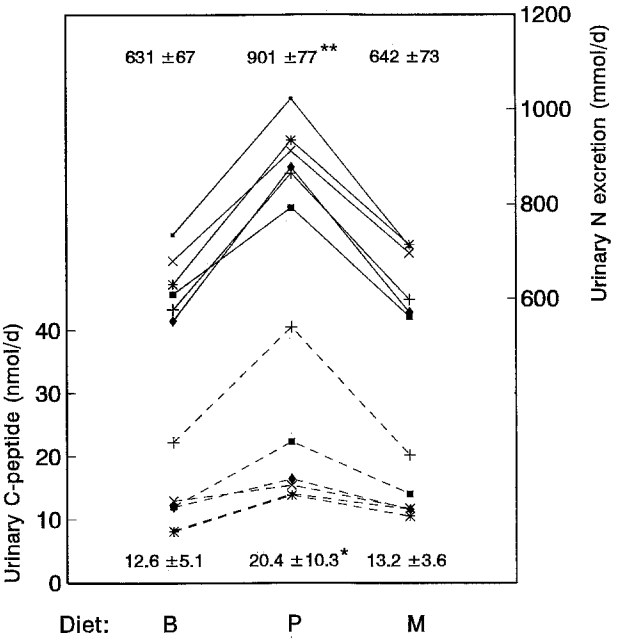

Fig 1. Individual and mean ± SD values for urinary C-peptide (- - - -) and nitrogen (—) excretion with diets B, P, and M. *P < .01 (F = 8.1); **P < .001 (F = 126.1).

Also, plasma concentrations of cortisol and testosterone were unaffected by the higher protein intake, although significant changes (reductions in testosterone and increases in cortisol) have previously been reported for both hormones following isocaloric dietary manipulations in healthy male volunteers.[18] However, in that study protein intake was increased along with a reduction in carbohydrate (including dietary fiber) intake, resulting in a decrease in sex hormone–binding globulin (SHBG) and an increase in corticosteroid-binding globulin. The fact that different vegetarian (vegan) diets with variable protein content induce increased SHBG[18-20] and/or testosterone[18,21] levels suggests combined food effects as a causal factor. This can explain the unresponsiveness of plasma testosterone in the present study.

The slightly but not significantly increased 24-hour urinary excretion rates for total testosterone conjugates and AdiolG with diet P (*v* diet B) correspond to the observation that omnivorous men (with higher protein intake) have a higher free androgen index (ratio of testosterone to SHBG) and higher AdiolG plasma levels than vegetarians.[19]

Nestler et al[11] have put forward the idea that insulin could act as a physiological regulator of DHEAS metabolism. In several clinical or experimental studies, drug-induced reductions in circulating insulin (accompanied by improvements in blood pressure and glucose tolerance) have been shown to be associated with striking increases in serum DHEAS (for literature, see Nestler et al[11]). However, it was not possible to clarify whether these increases were due to changes in production rates and/or metabolic clearance rates (MCRs) of DHEAS. Also, no definite conclusion could be drawn with regard to the question of whether the observed DHEAS changes resulted from

Table 1. Plasma Levels (mean ± SD) of DHEAS, Testosterone, Cortisol, IGF-I, Insulin, and C-Peptide in Response to Variations in Protein and/or Sulfur Intake

Dietary Regimen	DHEAS (μmol/L)	Testosterone (nmol/L)	Cortisol (nmol/L)	IGF-I (nmol/L)	Insulin (pmol/L)	C-Peptide (pmol/L)
B	6.3 ± 1.5	21.7 ± 2.1	494 ± 99	29.4 ± 7.0	36.0 ± 12.1	305 ± 63
P	6.8 ± 1.8	20.8 ± 2.2	500 ± 137	32.0 ± 8.0	52.5 ± 25.6	344 ± 92
M	6.9 ± 2.1	21.6 ± 4.8	538 ± 121	32.4 ± 8.6	46.9 ± 19.8	316 ± 73

reduced circulating insulin, improved insulin sensitivity, or direct drug action.[11]

Apart from these "chronic" studies, short-term experiments (hyperinsulinemic-euglycemic clamp and oral glucose tolerance test) have been performed to address the issue of hyperinsulinemia and suppression of circulating DHEAS.[9,13] The hyperinsulinemia-associated increases in urinary excretion of DHEAS (and DHEA glucuronide) of approximately 50% observed in one of these studies[9] suggest that acute insulin increases stimulate the metabolic clearance of DHEAS.

In principle, changes in 24-hour urinary DHEAS output are good indices of changes in overall DHEAS production rates,[16,17,22] but a stable metabolic situation (without acute changes in the respective prevailing DHEAS plasma level) is a prerequisite. However, if urinary DHEAS increases acutely and plasma DHEAS decreases simultaneously, the urinary change rather reflects an increased MCR. Another argument in favor of an acutely increased MCR (induced by hyperinsulinemia) might be that—at least in diabetics[23,24]—insulin infusions induce reductions in plasma albumin (albumin is the major transport protein for DHEAS in circulation). If this effect is also relevant in nondiabetics, the acute reductions in plasma DHEAS[9,13] could be explained in great part by an increased DHEAS delivery to the peripheral tissues (involved in metabolizing, storing, or eliminating steroids), including the kidney. Accordingly, the sudden increment in urinary DHEAS output by the kidney (observed by Nestler et al[9]) would reflect the acutely increased DHEAS supply to that organ, and thus can account only for a small proportion of the decrease in total plasma DHEAS. That has indeed been confirmed by Nestler et al.[9]

In contrast to these findings and considerations, no information is available as to whether urinary and plasma DHEAS might also change when endogenous overall secretion of insulin—due to a defined dietary manipulation—is increased only moderately, but for a longer period (ie, several days). Recently, we observed that prolonged (not acutely induced) diet effects on circulating DHEAS can occur without clear variations in daily insulin production.[25] In the present study, the (physiological) hyperinsulinemic effect of protein failed to clearly alter DHEAS. The protein-induced change in daily insulin secretion is obviously reflected better by 24-hour urinary C-peptide output than by a single measurement of circulating insulin or C-peptide (although the latter blood components also showed moderate but nonsignificant elevations with diet P). No conclusions can be drawn from our data regarding possible acute diet effects on DHEAS (the occurrence of which cannot be excluded directly after ingestion of meals with different protein contents). Also, no information is available regarding the importance of an altered insulin sensitivity for circulating DHEAS.

Table 2. Mean 24-Hour Urinary Excretion of DHEAS, Total Testosterone Conjugates, AdiolG, and Sulfate With Diets B, P, and M

Dietary Regimen	DHEAS (μmol/d)	TC (nmol/d)	AdiolG (nmol/d)	Sulfate (mmol/d)
B	8.8 ± 9.8	405 ± 178	592 ± 213	14.9 ± 1.2*
P	9.4 ± 11.2	445 ± 177	673 ± 249	30.3 ± 1.5
M	8.0 ± 8.3	397 ± 185	670 ± 243	25.0 ± 1.8

NOTE. Mean 24-hour urinary analyte excretion was calculated as the mean ± SD (n = 6) from individual average daily excretion data. The latter was obtained for each subject from the mean urinary output of the 2 24-hour specimens collected successively on days 3 and 4 of each diet period.

Abbreviation: TC, total testosterone conjugates.

*$P < .001$ ($F = 493.8$): diet B *v* diets P and M.

The repeated-measures design used in the present study is a powerful tool to detect even moderate effects in individuals, provided the treatment under investigation is of physiological significance (as demonstrated by the protein-induced increase in urinary C-peptide) and the measurement variable is proved to be adequately responsive in corresponding treatment designs. The latter has been proven for plasma DHEAS in several experimental trials with comparable sample sizes (n = 5, 6, or 7).[9,13,17,25] Each of these studies detected statistically significant DHEAS changes of a magnitude of 15% to 22% (on average) with repeated-measures ANOVA. The statistical power for such a design to detect clearly smaller differences for plasma DHEAS is surely insufficient. Accordingly, the small trend for increased plasma DHEAS in response to diets P and M (Table 1) and increased urinary DHEAS in response to diet P (Table 2) suggested by the present results allows no further conclusions. Whether an increase in the sample sizes would have produced clearer results remains questionable; however, it cannot be excluded that a considerably higher protein intake might have induced significant DHEAS changes.

Altogether, the present results show—at least for healthy males—that DHEAS is not importantly influenced by moderate diet-induced alterations in daily insulin secretion. Neither a reduced plasma level nor an increased urinary output of DHEAS (both observed during hyperinsulinemic-euglycemic clamp[9]) was seen, indicating that the overall MCR of DHEAS is not substantially altered in response to a moderately elevated protein content of the diet.

Apart from these findings, the present study also provides evidence that clear elevations of sulfur intake, and thus endogenous sulfate production, do not necessarily

affect DHEAS levels. This DHEAS unresponsiveness occurred despite the fact that there exists a considerable metabolic interconversion of DHEA and its sulfate ester leading to clear DHEAS increments when, for example, the secretion of unsulfated androgens including DHEA is specifically stimulated.[17] Consequently, (under the conditions tested) the supply of inorganic sulfate appears not to be rate-limiting for the peripheral and/or hepatic hydroxysteroid sulfotransferase–catalyzed formation of DHEAS from unconjugated DHEA. In that respect, the action of steroid sulfotransferases is obviously different from the clear substrate (sulfate) dependency of phenol sulfotransferases, which convert xenobiotics (with a phenolic hydroxyl group) to their respective sulfate conjugates.[26] Our finding disproves the speculation of Gordon et al[27] that the rate of sulfate conjugation of steroids (especially sulfation to DHEAS) is in principle limited by the supply of inorganic sulfate.

In summary, the present study demonstrates that a chronic, moderately large diet-induced insulin increase (caused by variation of only one dietary component) does not alter urinary and plasma levels of DHEAS. This is in contrast to acute pharmacological effects of insulin on DHEAS.[9,11] It is concluded that (at least in healthy men) less severe physiological variations of insulin secretion do not influence DHEAS metabolism.

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