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ORIGINAL CONTRIBUTION

T. Remer
K. Pietrzik
F. Manz

The short-term effect of dietary pectin on plasma levels and renal excretion of dehydroepiandrosterone sulfate

Der Kurzzeiteffekt von diätetischem Pektin auf den Plasmaspiegel und die renale Ausscheidung von Dehydroepiandrosteronsulfat

Summary Studies specifically investigating the effects of single dietary components on plasma levels of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are rare. Especially no data is available with regard to specific dietary fibers. Therefore, the impact of pectin (a representative fiber that affects the enterohepatic recirculation of bile acids) was studied in a randomized crossover trial consisting of three diet periods characterized by the same food supply and daily doses of 0 g, 15 g or 30 g pectin. Blood and 24-h-urine samples were collected at the end of each 4-day diet period from 6 healthy male volunteers. Plasma levels of DHEA, cortisol and the major binding protein of DHEAS albumin remained unchanged with the varying pectin supplements. Also, no changes were observed for several urinary analytes including urinary DHEAS. However, effects of pectin intake (30, 15 versus 0 g/d) were seen for plasma DHEAS (9.3 ± 2.8, 9.2 ± 2.6, 8.0 ± 3.1 μmol/L, $p < 0.01$) and total plasma cholesterol (4.4 ± 0.7, 4.5 ± 0.7, 4.7 ± 0.8 mmol/L, $p = 0.1$). Obviously, the altered intake of fiber in the form of pectin affects plasma concentrations of DHEAS and cholesterol in an opposite direction. The reason for this is not known but a dietetically induced modulation of the binding properties of plasma albumin for DHEAS appears possible. Our findings suggest that the target tissue-available, not protein-bound fraction of circulating DHEAS (as reflected by the renal DHEAS output) is not necessarily altered when total plasma concentrations of DHEAS vary.

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Dr. T. Remer (✉) · F. Manz
Forschungsinstitut für Kinderernährung
Heinstück 11
44225 Dortmund

K. Pietrzik
Institut für Ernährungswissenschaft der Universität Bonn
Abteilung Pathophysiologie der Ernährung des Menschen
Endenicher Allee 11–13
53115 Bonn

Zusammenfassung Es gibt kaum Untersuchungen zu den Auswirkungen einzelner Nahrungskomponenten auf die Plasmaspiegel von Dehydroepiandrosteron (DHEA) und Dehydroepiandrosteron-Sulfat (DHEAS). Insbesondere fehlen Studien zum Einfluß von speziellen Ballaststoffen. Der Effekt von Pektin (ein typischer Ballaststoff mit Wirkung auf die enterohepatische Rezirkulation von Gallensäuren) wurde in 3 aufeinanderfolgenden, jeweils 4tägigen Diätphasen an 6 männlichen Erwachsenen im Rahmen einer randomisierten cross-over-Studie überprüft. Am Ende jeder Diätphase (konstante Nahrungszusammensetzung, Pektinzusatz: 0, 15 bzw. 30 g/die) wurden 24h-Urine gesammelt und venöses Blut entnommen. Die Pektinzulagen führten weder bei DHEA und Cortisol noch beim wichtigsten Plasmabindungsprotein von DHEAS, dem Albumin, zu Veränderungen der Plasmakonzentrationen. Auch blieb die Ausscheidung verschiedener Urin-Analyte (einschließlich des DHEAS) konstant. Allerdings zeigten sich bei 30 bzw. 15 versus 0 g Pektin/die Effekte in bezug auf Plasma-DHEAS (9,3 ± 2,8; 9,2 ± 2,6; 8,0 ± 3,1 μmol/L, $p < 0,01$) und Plasma-Cholesterin (4,4 ± 0,7; 4,5 ± 0,7; 4,7 ± 0,8 mmol/L, $p = 0,1$). Die Auswirkungen einer veränderten Pektinzufuhr auf zirkulierendes DHEAS und Cholesterin sind also offensichtlich entgegengerichtet. Die Ursache hier-

für ist nicht bekannt, allerdings wird eine diätetisch induzierte Änderung der Bindungseigenschaften von Albumin für DHEAS vermutet. Die Befunde deuten an, daß der biologisch aktive, nicht proteingebundene Anteil des zirkulierenden DHEAS (der durch die renale DHEAS-Ausscheidung reflektiert wird) nicht notwendigerweise verändert sein muß, wenn das Gesamt-DHEAS im Plasma variiert.

Key words Dietary fiber – dehydroepiandrosterone sulfate – dehydroepiandrosterone – 3α-androstanediol glucuronide – peptide – steroid metabolism

Schlüsselwörter Diätetischer Ballaststoff – Dehydroepiandrosteronsulfat – Dehydroepiandrosteron – 3α-Androstendiol glucuronide – steroidmetabolismus

Introduction

Dehydroepiandrosterone-sulfate (DHEAS) is the most abundantly circulating steroid hormone in the blood of both men and women. It is derived primarily from secretion by the adrenal glands. Contrary to adrenal glucocorticoids DHEAS and its unconjugated parent steroid, dehydroepiandrosterone (DHEA), demonstrate a striking and unexplained decline with aging. Peak serum DHEAS and DHEA levels occur around 20–25 years of age, decrease progressively thereafter, and diminish by more than 80 % by age > 70 years (29).

Principally DHEA and DHEAS are mild androgens. However, as metabolically active prehormones (14–16, 27) both serve as precursor steroids for the synthesis of biologically potent estrogens as well as androgens. The conversion to either sex steroid depends on sex, genetics, age, metabolic state, and target tissues (14–16, 23, 27, 40). Especially the conversion to biologically active metabolites in certain target tissues appears to be one of the important functions of DHEA and DHEAS. For example, the skin, which is the largest organ of the body, possesses all enzymes required for transformation of DHEA and DHEAS into active sex steroids. The skin, therefore, can be considered as a major site of androgen formation (23). Other experimentally confirmed properties of adrenal androgens (DHEA and DHEAS) are their immunomodulatory functions and antiglucocorticoid effects (3, 6).

Several epidemiological studies have identified a reduced serum DHEAS concentration as risk factor for atherosclerosis (for literature, see Nestler et al. (26)). However, cardioprotective effects were not consistently observed by all investigators (7).

Although clear evidence has been provided that the production rate of DHEA/-S is strongly affected by the nutritional status (obese vs. nonobese) and by the intake of energy (fasting vs. dietary restriction vs. adequate energy intake) (11, 12, 17) only inconsistent findings are available with regard to possible effects of diet composition on circulating adrenal androgens (1, 2, 4, 18–20). Especially well-controlled diet studies, in which the impact of only one dietary component on adrenal androgens is specifically investigated, are lacking. Therefore, the present study was performed.

We have decided to study the impact of pectin (as a representative fiber that affects the enterohepatic recirculation of bile acids) because experimental evidence suggests that the drop in plasma levels and urinary excretion of other (non-bile acid) steroids, namely estrogens, observed with high fiber diets is attributable to an impaired enterohepatic recirculation (4, 13). DHEA and DHEAS circulate in the bile as well (35).

Dietary periods of 4 days duration were chosen to identify real short-term (no acute) effects and to assure full dietary compliance. Acute responses of serum DHEAS to "dietary manipulations" can occur even after 1–2 h as has been demonstrated by means of an oral glucose load (21).

Material and methods

Subjects

Six male volunteers, aged 20–39 years, were recruited for this study. All subjects described themselves as healthy and none were using oral medications or had a past medical history of renal, endocrine or cardiovascular disease. A daily intake of total dietary fiber < 35 g/d, as determined from a 3-day weighed diet record, was necessary for inclusion in the study. Subjects were of normal body weight, 73.8 ± 5.5 kg. The study protocol was approved by the institutional review board of the research institute of child nutrition. Written informed consent was received from each participant.

Study design and sample collection

In a randomized, crossover trial, which was divided into three diet periods (each lasting 4 days), subjects were given daily either 0 g (control period), 15 g or 30 g of a pectin supplement (Herbstreith & Fox, Neuenbürg, Germany; methoxyl content: 10.1 %, galacturonic acid content: 81 %). A 10-day washout was followed by a crossover to another diet period. Apart from the varying pectin supplements food intake was kept constant during the three diet periods (see, *study diets*). Total daily amounts of pectin were given in three equally divided doses of

5 g or 10 g each. Pectin was added to fruit juices or desserts. These juices or desserts – either with pectin supplements or without (control period) – were ingested with the second breakfast, with lunch, and as an individual afternoon meal (no pectin was added to the early breakfast and the dinner).

Twenty-four-hour urine was collected on day 3 and day 4 of each diet period and was immediately stored below -20 °C. Venous blood samples (10-mL each) were obtained on the morning of day 4 and day 5, ca. 40–50 min after the subjects had ingested their standardized early breakfast. EDTA was used as an anticoagulant. After providing blood on day 5, subjects were free to consume their normal diets. One participant refused to provide blood samples. Aliquots of plasma were stored below -20 °C until analyzed. The total urine volume of each 24-h collection was recorded after the samples were thawed. Aliquots of the thoroughly mixed 24-h urine samples were stored below -20 °C until analysis.

Study diets

From all participants a 3-day weighed food protocol was obtained during the pre-study period. The recorded data were used to design individualized study diets based on satiety and food preferences. These study diets (relatively high in protein but low in pectin rich foods) were composed in close consultation with an experienced dietitian. Subjects were given their own study diets so that they consumed the same foods on days 1 through 4 (and in the morning of day 5) of each diet period. Average daily intake of energy, protein and total fiber (without pectin supplements) was 11 516 ± 787.5 kJ, 96.4 ± 6.4 g and 26.4 ± 5.6 g, respectively (data were calculated using the nutrient data base of the research institute of child nutrition primarily based on Souci, Fachmann and Kraut (36)). The only beverage allowed ad libitum was a mineral water with low mineral content (Volvic, Puy-De-Dome, France). Coffee and energy-containing refreshment drinks of constant volume and composition (consumed at definite times) were additionally permitted.

Probably due to a potential gain in energy from fermentation of the added pectin (pectin is fermented in the large intestine) the study diets remained not totally isoenergetic during the three diet periods. On the other hand, there are also reports indicating that net availability of energy may be slightly decreased with added pectin (8, 9).

Analytical procedures

Commercial solid-phase ^{125}I-radioimmunoassays (coated-tube methodology) were used for the measurements of DHEAS, DHEA, cortisol (these three kits: Diagnostic Products, Los Angeles, CA, USA), and 3α-androstanediol glucuronide (AdiolG [5α-androstan-3α, 17β-diol glucuronide]) (Diagnostic Systems Laboratories, Webster, TX, USA). Urinary C-peptide was analyzed by a radioimmunoassay using the PEG-accelerated double-antibody method to separate the bound ^{125}I-labeled C-peptide from the unbound fraction (Diagnostic Products, Los Angeles, CA, USA). All radioimmunoassays were purchased from DPC Biermann GmbH, Bad Nauheim, Germany.

The measurements of urinary C-peptide and of all plasma steroids (conjugated as well as unconjugated) were carried out according to the respective kit instructions. For C-peptide quantification urine samples were diluted 1:40 (v/v) with a separately provided urinary diluent (PEDU, Diagnostic Products, Los Angeles, CA, USA). Urinary DHEAS was quantified directly (without kit modification or specific sample preparation). This direct radioimmunological determination of DHEAS in urine samples has previously been validated (34). Urinary AdiolG concentrations were determined after 1:5 (v/v) dilution of the specimens with the kit's zero standard. Average recoveries in parallelism (specimen dilution: 1:3, 1:5, 1:10, 1:20; v/v) and spiking experiments were found to lie between 79 and 120 %. Intra- and inter-assay coefficients of variation were 6.1 % and 7.6 %, respectively, at an urinary AdiolG concentration of 490 nmol/L. The quantification of urinary total 17-ketosteroid sulfates was done without previous hydrolysis after C_{18} reversed-phase extraction and LH-20 chromatography as described in detail elsewhere (31). Creatinine measurements were carried out with a Beckman-2 creatinine analyzer (Beckman Instruments, Inc., Fullerton, CA, USA), and albumin and total plasma cholesterol were quantified using photometric routine kits (albumin [BCP], Sigma, St. Louis, MO, USA; cholesterol, Boehringer Mannheim, Mannheim, Germany).

Data analysis

Changes in the dependent variables during diet periods were compared by one-way analysis of variance (ANOVA) with repeated measures. All data are expressed as mean ± SD. *F* statistic values are provided wherever ANOVA-derived p-values are given. When the ANOVA was significant comparisons between the means of paired observations were evaluated by linear contrasts.

Results

As shown in Table 1, increases in pectin ingestion failed to produce changes in any measured 24-h urine parameter. The constant renal output of DHEAS is also reflected in unchanged excretion levels of the group of 17-KSS which apart from DHEAS comprises further closely related C_{19}-17-keto-androgen metabolites (31). AdiolG, an indicator of peripheral (and hepatic) conver-

Table 1 Mean daily renal excretion[a] of DHEAS, total 17-ketosteroid sulfates (17-KSS), 3α-androstanediol glucuronide (AdiolG), C-peptide and creatinine after variations in pectin intake

Pectin supplement (g/d)	DHEAS (μmol/d)	17-KSS (μmol/d)	AdiolG (nmol/d)	C-peptide (nmol/d)	Creatinine (mmol/d)
0	9.4 ± 9.4	18.2 ± 9.2	690 ± 262	18.2 ± 3.7	16.4 ± 1.7
15	9.1 ± 10.1	17.8 ± 7.9	687 ± 326	17.7 ± 4.1	15.7 ± 2.2
30	9.6 ± 8.8	18.3 ± 10.8	697 ± 301	18.2 ± 4.3	16.4 ± 2.6

[a] Mean daily renal analyte excretion was calculated as $\bar{x} \pm SD$ (n = 6) from the individual average 24-h excretion data. The latter was obtained for each subject from the mean urinary output of the two 24-h specimens collected successively on days 3 and 4 of each diet period

Table 2 Plasma levels[a] of DHEAS, DHEA, cortisol, albumin and total cholesterol in response to variations in pectin intake

Pectin supplement (g/d)	DHEAS (μmol/L)	DHEA (nmol/L)	Cortisol (nmol/L)	Albumin (μmol/L)	Cholesterol (mmol/L)
0	8.0 ± 3.1[b]	16.9 ± 10.8	511 ± 129	660 ± 30	4.7 ± 0.8[c]
15	9.2 ± 2.6	16.0 ± 5.5	467 ± 111	658 ± 42	4.5 ± 0.7
30	9.3 ± 2.8	16.7 ± 8.6	461 ± 115	661 ± 44	4.4 ± 0.7

[a] Means ± SD of individual average analyte plasma levels. The latter were obtained for each subject (n = 5) from the mean of the two morning blood specimens collected successively on days 4 and 5 of each diet period
[b] $p < 0.01$ ($F = 15.96$); 0 versus 30 g pectin/d: $p < 0.01$
[c] $p = 0.1$ ($F = 3.12$)

sion of androgen precursors (including testosterone) to the active metabolite dihydrotestosterone, remained unchanged as did urinary creatinine (Table 1).

C-peptide excretion representing an index of the "integrated" endogenous insulin secretion over a 24-h period did not show any variation in response to the altered pectin intake. Also, plasma levels of DHEA and cortisol as well as of albumin (which is the major binding protein of DHEAS in circulation) remained unchanged after 0, 15, and 30 g pectin/d, respectively. However, one-way repeated-measures ANOVA uncovered significant effects of pectin intake on plasma DHEAS (Table 2). Average DHEAS increments were 1.2 and 1.3 μmol/L, respectively. There was also a tendency towards lower total plasma cholesterol with the pectin enriched diets (Table 2).

Discussion

Studies comparing hormone levels between vegetarians (with generally high intake of dietary fiber) and omnivores yielded inconsistent results with regard to adrenal androgen levels. Adlercreutz et al. (1) found lower DHEAS plasma levels (-28 % on average, nonsignificant) in a group of postmenopausal vegetarians compared to omnivorous women. However, both groups differed significantly in energy intake, weight and BMI; and the average duration of menopause was 10.3 years for the vegetarians and only 7 years for the omnivores. In addition, the omnivorous women exhibited a higher mean sex-hormone-binding globuline (SHBG) level although ingestion of vegetarian diets usually results in SHBG increases, as discussed by the authors themselves.

On the other hand, higher DHEAS plasma concentrations have been observed in vegetarians versus nonvegetarian subjects (4). However, the difference was not statistically significant. Considering the high degree of intersubject variability that is characteristic of DHEAS (37) the number of subjects per group (n = 12) was probably too small in the latter study to obtain a clearer result. For an adequate evaluation of possible diet effects on DHEAS it appears appropriate to perform the investigations in the same subjects. Hill et al. (19) observed mean DHEA plasma levels of 352 ± 38 and 407 ± 70 ng/ml, respectively in healthy women switching from a Western diet to a vegetarian diet, but there was no statement whether the difference was significant. The authors only reported that the relative fall in DHEA was significantly stronger after adrenocortical suppression with dexamethasone when the vegetarian diet was fed. Clearly higher DHEA plasma levels were measured by the above authors (18) in premenopausal vegetarian women on their habitual diets before they changed to a Western diet. In a later study, Hill and coworkers (20) again found higher DHEA, and, in addition, higher testosterone plasma levels

in a group of males (elderly black South African men) on their customary vegetarian diet in comparison to a cafeteria-fed Western diet eaten for 3 weeks.

Similar results were obtained by Anderson et al. (2) in a group of healthy male volunteers. The authors observed that the isocaloric change from a high protein to a high carbohydrate (high dietary fiber) intake resulted in an average increase of 28 % for both plasma testosterone and plasma DHEA. However, the DHEA rise failed to reach statistical significance. DHEAS measurements were not performed. There are no studies which have specifically looked at the effect of a specific group of dietary complex carbohydrates on circulating and renally excreted adrenal androgens.

The present study demonstrates that a markedly raised intake of pectin can produce significant increases in circulating (but not in urinary) DHEAS. These increases averaged 16 % and thus the mean increment in plasma was nearly of the magnitude of the DHEAS rise (22 %) that has been observed after an endocrinological stimulation test (with hCG) in healthy males of comparable age (32).

The unresponsiveness of urinary DHEAS to pectin along with the elevated post-pectin DHEAS plasma levels strongly suggests that the mechanism(s) responsible for the cholesterol-lowering action of pectin is not effective for DHEAS. Dietary pectin was reported as early as 1961 to reduce blood cholesterol levels in humans (22) and this finding has since been confirmed by numerous human studies (30). In these studies, lasting between 2 and 9 weeks, total blood cholesterol decreases ranged from 3 to 31 %. Thus, the cholesterol lowering of 4 (15 g pectin) to 6 % (30 g pectin), observed in the present short-term study – although not statistically significant on the 5 % level – indicates that pectin exerts its metabolic effects even within a few days after switching from a low to a high pectin intake. The decrease would probably have been more pronounced in subjects with individually higher cholesterol levels (total plasma cholesterol concentrations were relatively low in the participants). In general, the strongest cholesterol-lowering effects of pectin are seen in subject groups with very high initial cholesterol levels (30).

The post-pectin DHEAS plasma responses seen in our diet experiment are in accord with the findings of Barbosa et al. (4) indicating a clear tendency towards higher DHEAS levels in vegetarian than in nonvegetarian women. On the other hand, we did not observe the changes in DHEA plasma levels that were reported by other investigators (2, 18, 20) following variations in the quantity of dietary fiber intake. Possibly, this could be due to the fact that in the other studies a marked change occurred also for dietary protein. A higher protein intake seems to affect 5α-reductase activity (2) which is responsible for the metabolism of testosterone (and consequently for the metabolism of the testosterone precursors androstenedione and DHEA) to the active metabolite dihydrotestosterone. The equal excretion rates of the major metabolite of dihydrotestosterone AdiolG and of total 17-keto-androgen-sulfates with all three pectin intake levels (Table 1) confirm that peripheral and/or hepatic androgen metabolism is not affected by variations in the ingestion of this soluble fiber. In addition the elevated DHEAS plasma levels with the pectin-added diets are not a result of some adrenocortical overactivity as can be deduced from cortisol measurements (Table 2).

The altogether relatively high plasma concentrations of cortisol and DHEA during all three diet periods are (at least partly) due to the physiological increases in the morning blood levels of these unconjugated steroids. Circulating DHEAS on the other hand, remain unaffected by the circadian rhythmicity of the adrenal glands' secretion of unconjugated steroids (29).

The mechanism by which an increase in the dietary fiber intake can produce a change in plasma concentrations of DHEAS is not known. In humans it has been shown that vegetarian diets increase the circulating levels of SHBG (2, 5). DHEAS and DHEA although largely bound to plasma albumin have also weak binding to SHBG (24). With regard to DHEA about 3 % circulates in association with SHBG (10). Even if a rise in pectin intake induced an increase in SHBG (SHBG measurements were not performed) the resulting increment of the fraction of SHBG-bound DHEAS would probably be too small to account for the observed post-pectin DHEAS increases.

The fact that total plasma albumin was constant at all pectin intake levels does by no means rule out an ameliorated binding of DHEAS to this protein. Binding studies have revealed that up to 20 binding sites (two initial and 18 nonspecific) seem to exist per albumin molecule (35). More than 99 % of the available steroid binding sites of albumin remain unoccupied in man (39). Albumin is involved in the transport of many very different substances such as hormones, fatty acids, drugs or calcium. One group of dietary compounds (free fatty acids) has been discussed to induce conformational changes of the albumin molecule with marked effects for the albumin binding of certain drugs (25).

Whether the binding properties of albumin for DHEAS are also subject to dietetic/metabolic manipulations remains open to further research. However, the existence of an altered protein binding of DHEAS (i.e., a slowed plasma clearance) appears rational. However, the bioactive nonprotein-bound fraction of circulating DHEAS, which is reflected by the renal DHEAS output, is not necessarily altered when the total plasma concentration of DHEAS is modulated by dietetic measures. Contrary to several clinical or endocrinological studies (21, 26, 28, 33, 38) the present dietetically induced variation in plasma DHEAS is not related to an altered exogenous or endogenous insulin stimulation (as confirmed by constant

daily C-peptide excretion rates). The unaltered C-peptide data are in accord with a number of findings showing that significant decreases in blood glucose (after raising pectin intake) are not necessarily associated with significant alterations in circulating insulin levels (a literature overview is given by Reiser (30)).

Taken together, during metabolic steady state ensured by almost constant energy and nutrient supply, a single dietetic component can obviously modify total plasma levels of DHEAS without affecting in parallel the pool of unbound DHEAS that is available for target tissues (or is excreted in urine). The possible existence of such metabolic constellations should be considered when interpreting findings on altered DHEAS plasma levels.

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