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DIET-HORMONE INTERACTIONS: PROTEIN/CARBOHYDRATE RATIO ALTERS RECIPROCALLY THE PLASMA LEVELS OF TESTOSTERONE AND CORTISOL AND THEIR RESPECTIVE BINDING GLOBULINS IN MAN

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Summary

The aim of this study was to determine if a change in protein/carbohydrate ratio influences plasma steroid hormone concentrations. There is little information about the effects of specific dietary components on steroid hormone metabolism in humans. Testosterone concentrations in seven normal men were consistently higher after ten days on a high carbohydrate diet (468 $\pm$ 34 ng/dl, mean $\pm$ S.E.) than during a high protein diet (371 $\pm$ 23 ng/dl, $p<0.05$) and were accompanied by parallel changes in sex hormone binding globulin (32.5 $\pm$ 2.8 nmol/l vs. 23.4 $\pm$ 1.6 nmol/l respectively, $p<0.01$). By contrast, cortisol concentrations were consistently lower during the high carbohydrate diet than during the high protein diet (7.74 $\pm$ 0.71 µg/dl vs. 10.6 $\pm$ 0.4 µg/dl respectively, $p<0.05$), and there were parallel changes in corticosteroid binding globulin concentrations (635 $\pm$ 60 nmol/l vs. 754 $\pm$ 31 nmol/l respectively, $p<0.05$). The diets were equal in total calories and fat. These consistent and reciprocal changes suggest that the ratio of protein to carbohydrate in the human diet is an important regulatory factor for steroid hormone plasma levels and for liver-derived hormone binding proteins.

The protein/carbohydrate dietary ratio in man can substantially influence oxidative drug metabolism, estradiol 2-hydroxylation, and testosterone Δ^4-5α-reduction, as previous studies from these laboratories have shown (1-6). In these studies it was demonstrated that the substitution of dietary protein for carbohydrate (or fat) can greatly increase the metabolic clearances of drugs such as antipyrine and theophylline (1-4) and the 2-hydroxylation of estradiol (6). Plasma clearances of these drugs and estrogen 2-hydroxylation are primarily dependent upon the activity of the hepatic mixed function oxidase system, for which a family of inducible hemoproteins in the endoplasmic reticulum, collectively termed cytochrome P-450, serve as terminal oxidases (7). In contrast, the Δ^4-5α-reduction of testosterone, which is not cytochrome P-450 dependent but is catalyzed by an enzyme system in the endoplasmic reticulum which also requires NADPH, was decreased by the same dietary regimen that increased antipyrine clearance and estrogen 2-hydroxylation (5). Substitutions of fat for carbohydrate, or unsaturated fat for saturated fat, when energy and protein intakes were kept constant did not alter drug oxidations in humans (3,4). Cruciferous

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vegetables and charcoal-broiled beef have also been shown to alter drug metabolism rates in normal subjects (4).

To our knowledge, there is no information on the effects of such specific dietary alterations on plasma concentrations of steroid hormones in humans. In this study we have examined the effects of a change in the dietary protein/carbohydrate ratio on plasma concentrations of major steroid hormones and their binding globulins in normal males.

Subjects and Methods

Seven men between 22 and 43 years of age, all of whom were normal by history, physical examination, and clinical laboratory tests, were nonsmokers, and were taking no drugs other than occasional aspirin, volunteered for this study. All were within 10 percent of ideal body weight (range 64-72 kg). No drugs or alcohol were ingested for at least 2 weeks before or during the study. Subjects maintained a consistent level of physical activity during the study. An initial assessment of the usual caloric intake of each subject was made before the study so that the amounts of all foods could be adjusted proportionally to provide an optimal caloric intake for each.

The subjects were studied during two periods of feeding of carefully controlled and calculated diets. The two diets employed in this study were designed to maximize changes in protein and carbohydrate, while keeping total dietary fat constant. Thus, our aim was not to replicate compositions of diets normally consumed (which for the United States is highly variable but is estimated to average about 12 percent of total calories as protein, 46 percent carbohydrate and 42 percent fat) (8). These diets were identical to those used in our previous studies on the effects of protein and carbohydrate on drug and steroid hormone metabolism (1,2,6). The first diet contained foods rich in protein, such as meat, fish, poultry, egg white, and a liquid dietary supplement (Sustacal, Mead Johnson and Company, Evansville, IN); in this diet 44% of total calories were protein, 35% were carbohydrate, and 21% were fat. The second diet contained carbohydrate-rich foods such as bread, vegetables, fruit, juices, pastry, and candy; in this diet 10% of total calories were protein, 70% were carbohydrate, and 20% were fat. To provide variety there were two alternating daily menus for each diet. All foods were individually weighed or their volumes measured. Charcoal-broiled foods and vegetables such as cabbage and brussels sprouts, known to influence cytochrome P-450-dependent chemical oxidations in humans (4), were excluded. Subjects were not permitted to consume food elsewhere during the study. All lunches and suppers were supervised; the diets included an evening snack and breakfast which were packed to be eaten at home. Body weights were measured daily.

On the tenth day of each dietary period, blood samples were drawn every 2 hours from 8 AM until 8 PM, and an equal volume of plasma from each sample was added to a plasma pool for each subject. These plasma pools were kept refrigerated until the end of each 12 hour period, then divided into aliquots and frozen. Concentrations of steroid hormones, sex hormone binding globulin and corticosteroid binding globulin were measured using previously published methods (8-10).

Results

The plasma testosterone concentrations measured in 12-hour plasma pools for the seven subjects during each dietary period are shown in Figure 1. The mean testosterone level for the group of seven subjects was significantly greater during the high carbohydrate diet (468 $\pm$ 34 ng/dl, mean $\pm$ SE) than

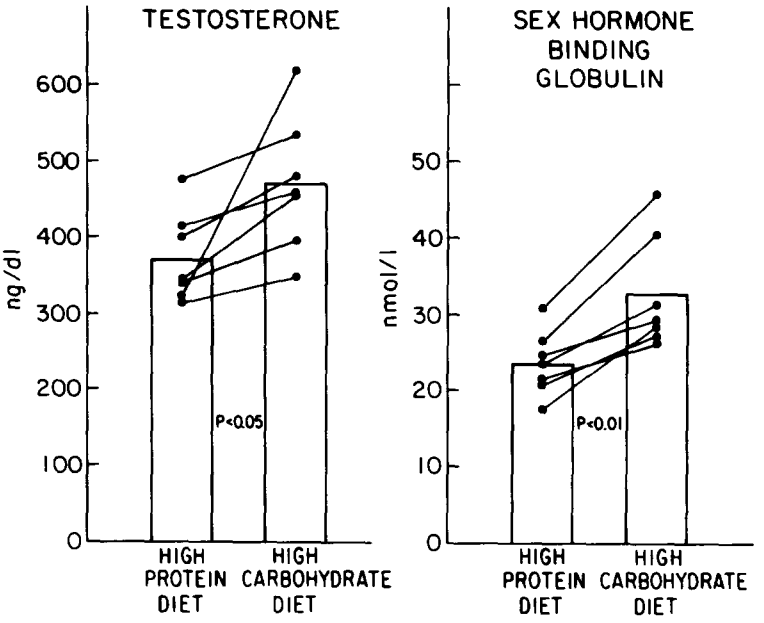

FIG. 1

Effects of an isocaloric change from a high protein diet to a high carbohydrate diet on plasma levels of testosterone and sex hormone binding globulin in seven normal males. Measurements were obtained after 10 days on each diet. Bars indicate mean values. See text for details.

during the high protein diet (371 ± 23 ng/dl, $p<0.05$, paired t test), with an average increase of 28 percent (range 10 to 93 percent). Plasma concentrations of dehydroepiandrosterone, Δ^4-androstenedione, and estradiol were not significantly different in this group of subjects during the two dietary periods (Table I).

TABLE I

Plasma levels of dehydroepiandrosterone, Δ^4-androstenedione and estradiol during the consumption of a high protein diet and a high carbohydrate diet

Diet	Dehydroepiandrosterone	Δ^4-Androstenedione	Estradiol
		(ng/dl)	
High protein	123 ± 17	83 ± 11	3.3 ± 0.5
High carbohydrate	157 ± 36	84 ± 9	3.0 ± 0.3

Values shown are means ± S.E. These were not significantly different during the two dietary periods (paired t test).

Sex hormone binding globulin concentrations were also higher in all subjects during the high carbohydrate dietary period, with increases ranging from 19 to 64 percent in the seven subjects (Fig.1). On average, values were 39 percent higher during the high carbohydrate diet than during the high protein diet (32.5 ± 2.8 nmol/l vs. 23.4 ± 1.6 nmol/l respectively, $p<0.01$).

Concentrations of cortisol and corticosteroid binding globulin (Fig.2) were also consistently changed when the diet was altered from a high protein to a high carbohydrate intake, but the direction of change was opposite to that observed for testosterone and sex hormone binding globulin. Thus, in the seven subjects cortisol was between 14 and 64 percent lower during the high carbohydrate dietary period than during the high protein dietary period (7.74 $\pm$ 0.71 μg/dl vs. 10.6 $\pm$ 0.4 μg/dl respectively, $p<0.001$). Corticosteroid binding globulin levels also decreased in all but one of the seven subjects and in six subjects were between 6 and 43 percent lower during the high carbohydrate diet than during the high protein diet. For the group of seven subjects the mean concentration of this protein was significantly lower during the high carbohydrate diet than during the high protein diet (635 $\pm$ 60 nmol/l vs. 754 $\pm$ 31 nmol/l respectively, $p<0.05$).

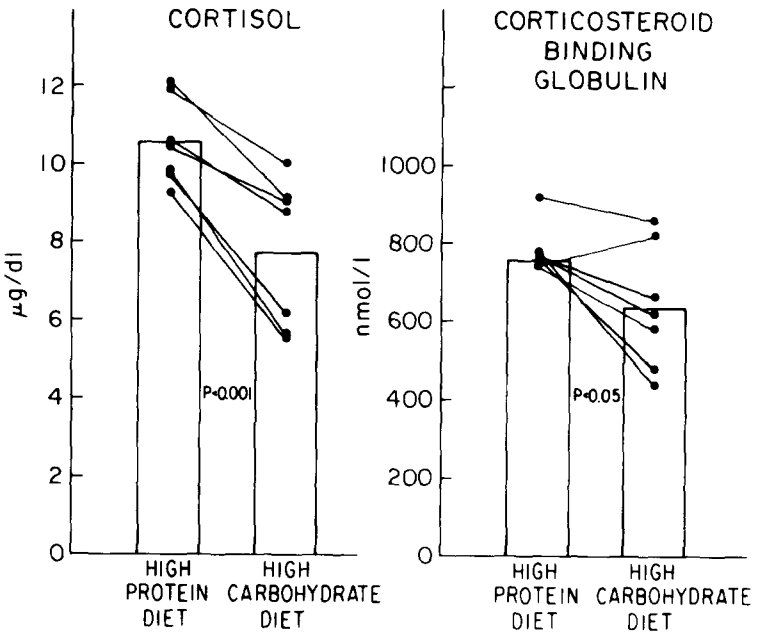

FIG. 2

Effects of a change from a high protein diet to a high carbohydrate diet on plasma levels of cortisol and corticosteroid binding globulin in seven normal men. Bars indicate mean values.

Discussion

The results of this study indicate that plasma concentrations of steroid hormones and their binding globulins are subject to dietary regulation; and that components of the diet such as protein and carbohydrate, even when the intakes of fat and energy remain unchanged, can produce consistent changes in concentrations of testosterone and its binding globulin which are reciprocal in direction to changes in cortisol and corticosteroid binding globulin. We found that the isocaloric change from a high protein to a high carbohydrate intake was associated with increases in plasma testosterone levels in all seven normal subjects studied. These increases averaged 28 percent, and were accompanied by increases in sex hormone binding globulin (all subjects) which averaged 39 percent (Fig.1). There were also consistent changes in concentrations of cortisol (all subjects) and corticosteroid binding

globulin (all but one subject) which were reciprocal to the changes in testosterone and its binding globulin (Fig.2).

Other nutritional factors, such as fasting (11), malnutrition (12,13), and obesity (14) are known to be associated with changes in steroid hormone concentrations or in metabolic pathways for these hormones, but these factors did not play a role in this study. Subjects in our study were normally nourished, and the test diets were tailored to the energy requirements of each individual such that there were no significant changes in body weight during the four-week study period. Other environmental factors that could influence hormone levels, such as smoking (15,16), stress, and exertion (17,18) were also excluded. Because the test diets employed in this study contained mostly solid foods, it is possible that components other than protein and carbohydrate contributed to the changes observed. However, we have shown previously that a change in the protein-carbohydrate ratio in the diet can substantially influence the Δ^4-5α-reduction of testosterone and the 2-hydroxylation of estradiol, as well as the oxidative metabolism of drugs such as antipyrine and theophylline (1-6). Although micronutrient deficiencies may alter drug metabolism (19), micronutrient changes in the absence of a deficiency have not, to our knowledge, been shown to have such effects. Thus, it is likely that the changes in plasma hormone and hormone-binding globulin concentrations which we have observed under well-controlled dietary and environmental conditions were produced by the major changes in dietary protein and carbohydrate content of the diet.

There have been only a few previous studies in which steroid hormone levels in the same subjects were examined during the consumption of two different diets. These studies were less well controlled and were not designed to identify the effects of specific components of the diet which might affect plasma steroid hormone levels, because both diets were not of known composition. Hill and coworkers (20-22) have studied the effects of changing groups of Black or Caucasian men and women from vegetarian diets to Western diets and found alterations in plasma and urinary hormone levels and in the length of the menstrual cycle. For example, in a group of 13 elderly black South African men, the change from their customary vegetarian diet to a cafeteria-fed Western diet for 3 weeks was associated with decreases in plasma testosterone, androstenedione, and dehydroepiandrosterone, no change in estradiol and an increase in estrone (20). Rosenthal et al. (23) found in a group of 21 men that a change from customary diets to a diet high in complex carbohydrates and low in fat (the Pritikin diet), and accompanied by an exercise program (and in some subjects by discontinuation of smoking, alcohol and medications) produced reductions in serum levels of estradiol but not testosterone. Comparisons of hormone levels in vegetarians and omnivores (24,25) also indicate that steroid hormone metabolism can be influenced by diet. The present study provides specific evidence that the ratio of two of the major macronutrients of the human diet should be recognized as having an important influence on plasma steroid hormone concentrations. Our results do not exclude the possibility that other dietary components, such as fat, may influence steroid hormone blood levels or metabolism in man. Although alterations in dietary fat have been found not to influence hepatic drug oxidations (3,4), effects of dietary fat and other specific dietary components on steroid hormone metabolism have not, to our knowledge, been reported. They are currently under investigation.

The mechanisms by which an alteration in the protein/carbohydrate ratio of the diet can produce changes in plasma concentrations of steroid hormones and their binding globulins are not known. In laboratory animals (19) and humans (1-6), dietary protein content is known to influence hepatic enzyme

systems that play a major role in the oxidative and reductive metabolism of steroids, drugs and other chemicals, and changes in activities of these hepatic enzymes might be reflected in altered plasma hormone concentrations. Also, the possibility that the changes in concentrations of certain steroid hormones reflect changes in concentrations of their binding globulins is suggested by the parallel changes in levels of testosterone and sex hormone binding globulin, and in cortisol and corticosteroid binding globulin. There were no significant changes in concentrations of two other androgens, dehydroepiandrosterone and Δ^4-androstenedione, which bind less avidly to sex hormone binding globulin. Changes in estradiol, which does bind avidly to this protein, were not observed in these male subjects, but might be detectable in women or in disease states in which estrogen levels are higher. Both sex hormone binding globulin and corticosteroid binding globulin are synthesized in the liver (26,27), and it is possible that the alterations in hormone binding globulins reported here represent additional examples of liver-derived proteins whose synthesis and/or degradation may be influenced by dietary factors. Plasma concentrations of these binding globulins are regulated in a differential fashion by factors such as thyroxine and estrogen (28,29), and it is perhaps not unexpected that these proteins underwent reciprocal changes during the same dietary alteration. Decreased testosterone and sex hormone binding globulin concentrations after glucocorticoid administration in patients with pulmonary disease (30,31), and decreased testosterone levels in Cushing's disease (32) and after ACTH administration (33) also suggests an inverse relationship between plasma concentrations of testosterone and glucocorticoids. Moreover, we have found that estrogen hydroxylation and testosterone 5α-reduction were affected by the protein/carbohydrate dietary ratio in a reciprocal fashion (5,6). Non-dietary environmental influences such as barbiturates (34), chlorinated hydrocarbon pollutants (see ref. 5 for review) and the genetic disease acute intermittent porphyria (35) can also substantially alter steroid metabolizing enzyme systems in a manner reminiscent of the reciprocal effects produced by a change in protein/carbohydrate ratio (5,6). Thus, there are likely to be differential influences of dietary factors such as the protein/carbohydrate ratio on a number of aspects of steroid hormone metabolism.

The relationships between diet-induced changes in important hepatic enzyme systems that metabolize hormones, the synthesis of liver-derived binding proteins, and the actions of steroid hormones and certain of their biologically active metabolites (36-38) are clearly complex, but have broad implications for understanding the influences of dietary composition on endocrine-mediated development, and on hormone actions in health and disease.

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