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Nutrition–endocrine interactions: Induction of reciprocal changes in the Δ^4-5α-reduction of testosterone and the cytochrome P-450-dependent oxidation of estradiol by dietary macronutrients in man

(dietary protein-to-carbohydrate ratio/Δ^4-steroid-5α-reductase/estrogen 2-hydroxylase/catechol estrogens/cytochrome P-450 induction)

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ABSTRACT **The *in vivo* biotransformations of drugs known to be metabolized by enzymes localized in the endoplasmic reticulum of liver can be greatly altered by diet in humans, as we have shown previously. Steroid hormones also are metabolized extensively by hepatic microsomal enzymes; therefore, we examined the possibility that testosterone and estradiol biotransformations, as assessed with radiolabeled tracer methods, could be influenced by dietary macronutrients. Normal males were fed a high-protein diet for 2 weeks, followed by a high-carbohydrate diet for an additional 2 weeks. The Δ^4-5α-reduction of testosterone was considerably diminished, while the cytochrome P-450-dependent hydroxylation of estradiol at the C2 position was substantially enhanced during ingestion of the high-protein diet as compared with the high-carbohydrate diet. These results indicate that dietary macronutrients can significantly alter major metabolic pathways for testosterone and estradiol in man. The mechanism by which reciprocal changes in the Δ^4-5α-reduction of testosterone and the cytochrome P-450-mediated oxidation of estradiol are produced by diet is not known. Similar changes in steroid Δ^4-5α-reduction and cytochrome P-450-dependent chemical oxidations have been observed in circumstances in which the mixed-function oxidase system in liver is induced by agents such as phenobarbital, hexachlorobenzene, dioxin, and polyhalogenated biphenyls. Thus, the alterations in steroid hormone metabolism produced by dietary macronutrients in man mimic those that can be produced by drugs and environmental chemicals.**

The oxidative metabolism of drugs can be greatly influenced by specific components of the human diet, as studies from our laboratories have shown (1–8). These studies demonstrated that feeding humans cruciferous vegetables (5), charcoal-broiled beef (6–8), or a high-protein/low-carbohydrate diet (1–4) stimulates the metabolism of certain drugs by the microsomal cytochrome P-450-dependent mixed-function oxidase system.

Natural steroid hormones, like many drugs and other foreign chemicals, also undergo biotransformations mediated by microsomal enzymes (9–12); therefore, it was of interest to determine whether the dietary content of protein and carbohydrate could alter the metabolism of such hormones in humans. The major sex steroids, testosterone and estradiol, were chosen for study because cytochrome P-450-dependent oxidation is the principal metabolic fate of the latter compound (12, 13), whereas reduction by a microsomal Δ^4-5α-reductase represents a primary biotransformation for the androgen (14). Thus by changing, in a carefully controlled fashion, the dietary intakes of protein and carbohydrate in the same individuals, the role of specific nutritional factors in determining the metabolic fate of these steroid hormone substrates of microsomal enzymes could be determined. The metabolism of antipyrine also was studied because the effects of protein and carbohydrate in the diet on the oxidative disposition of this cytochrome P-450 substrate have been well defined (1, 2, 4).

We report here that the isocaloric substitution of protein for carbohydrate in the diet decreases the Δ^4-5α-reduction of testosterone while concurrently increasing the oxidative metabolism of estradiol and also of antipyrine in man. The changes in the 5α-reductive metabolism of testosterone and in the cytochrome P-450-dependent steroid and drug oxidations produced by diet resemble those previously shown to result from phenobarbital administration in humans (15) or from certain environmental chemical exposures in animals (15–33). Thus, the protein/carbohydrate ratio of the human diet significantly alters the patterns of metabolism of natural steroid hormones, and these alterations can mimic those produced by exogenous chemicals.

METHODS

Eight normal male volunteers (ages 20–35 yr, weights 54–95 kg) were studied. All were nonsmokers, ingested no drugs other than occasional aspirin, and were normal by history, physical examination, and common clinical laboratory tests. They ingested no alcohol during or for at least 2 weeks prior to the study. The metabolism of testosterone, estradiol, and antipyrine was studied during two periods of feeding of controlled, calculated diets. An initial assessment of the usual caloric intake of each subject was made prior to the study so that the amounts of all foods could be adjusted proportionally to provide an optimal caloric intake for each subject. No significant weight loss or gain occurred for any subject during the study.

The volunteers were fed a high-protein diet for 2 weeks and then a high-carbohydrate diet for a second 2-week period; there was an interval of 3 weeks between the study periods during which they consumed their usual home diets. The high-protein diet consisted of 44% of total calories as protein, 35% as carbohydrate, and 21% as fat; the high-carbohydrate diet consisted of 10% of total calories as protein, 70% as carbohydrate, and 20% as fat. These diets were identical to those used in our previous clinical studies on the influences of dietary protein and carbohydrate on the metabolism of antipyrine and theophylline (1, 2).

The subjects were required to consume all food provided and were not permitted to eat any other foods during each of the

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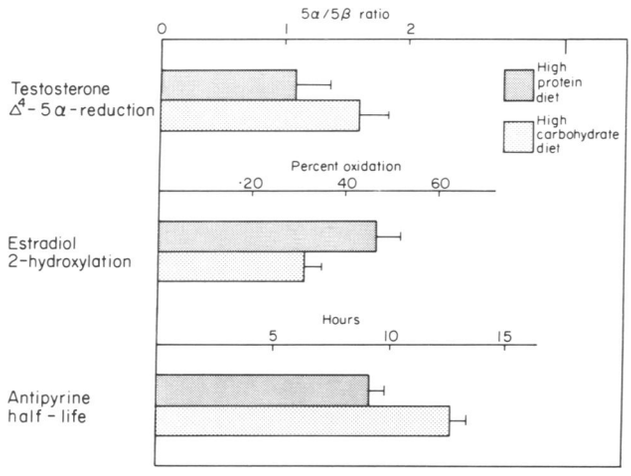

FIG. 1. Effect of changes in the amounts of dietary protein and carbohydrate on testosterone Δ^4-5α-reduction, estradiol 2-hydroxylation, and antipyrine half lives in normal males. Testosterone and antipyrine metabolism were studied in eight subjects and estrogen oxidation in four of the eight subjects. The changes in the mean values shown were significant by the paired *t* test.

2-week dietary periods. All lunches and suppers were supervised at The Rockefeller University Hospital, and an evening snack and breakfast were packed to be eaten at home. Antipyrine metabolism was studied on day 10, testosterone Δ^4-5α-reduction on days 11 and 12, and estradiol 2-hydroxylation on days 13 and 14 of both dietary periods.

The Δ^4-5α-reductive metabolism of testosterone was measured in all eight subjects by intravenous injection of a single 2.0-μCi (1 Ci = 37 GBq) dose of [4-^{14}C]testosterone (50 μCi/mmol), followed by isolation and measurement of its radiolabeled 5α and 5β metabolites excreted in urine as described (34, 35). This technique has been applied extensively to the study of steroid Δ^4-5α-reductase activity in man and permits quantitation of this metabolic pathway for steroid substrates of appropriate structure. Antipyrine metabolism was measured in the same eight subjects after administration by mouth of a single dose (1 mg/kg) of the drug in the fasting state and quantitation of the drug in saliva by radioimmunoassay as described (36). The metabolism of estradiol by oxidation at the 2-position was assessed in four of the eight subjects by injection of a single dose (6.0 μCi) of [2-^{3}H]estradiol; serial plasma samples were obtained to measure the amount of [^{3}H]H_2O distributed in total body water after release of ^{3}H from the C2 position on the steroid nucleus. This method for assessing site-specific oxidations of the steroid nucleus of estrogens has been described in detail (37). Total body water was measured independently by using ^2H_2O (37). With the experimental design used in this study, the metabolic transformations examined are known to occur predominantly in the liver. Statistical analysis was by the paired *t* test.

RESULTS

The results of the [4-^{14}C]testosterone studies in the eight subjects are shown in Fig. 1 and Table 1. Average values for recovery of the administered isotope dose in urine, as neutral steroid metabolites (i.e., after hydrolysis of glucuronides and sulfates combined), in the glucuronide fraction, or in the "polar" fraction (34, 35) did not differ significantly during the two dietary periods. There was a small but statistically significant difference ($P < 0.01$) in the amount of radioactivity recovered in the sulfate fraction during the two dietary periods, with a greater amount of radiolabel recovered as sulfates during the high-protein diet (Table 1). The relative proportion of radiolabeled testosterone metabolized to the 5α-reduced metabolite androsterone and the 5β-reduced metabolite etiocholanolone was significantly influenced by diet (Fig. 1). Thus, the mean 5α/5β ratio of these isolated and individually quantitated metabolites showed an approximately 50% increase (from 1.1 to 1.6) when the subjects were changed from a high-protein to a high-carbohydrate diet ($P < 0.025$), and this change was consistent in direction for all subjects. The average radioactivity excreted as androsterone increased when the subjects were changed from the high-protein diet to the high-carbohydrate diet (Table 1), whereas there was no statistically significant change in the average radioactivity excreted as etiocholanolone, indicating that the diet changes primarily influenced the 5α-reductive metabolism of the testosterone tracer and not its 5β-reduction.

The mean percentage oxidation of [2-^{3}H]estradiol in the four subjects studied was substantially greater during consumption of the high-protein diet than during the high-carbohydrate diet (Fig. 1). Thus, 45.7 ± 5.8% (mean ± SEM) of the ^{3}H in the tracer dose was released during the high-protein diet, and 31.2 ± 3.5% was released during the high-carbohydrate diet ($P < 0.02$). The direction of change in the percentage of oxidation at the C2 position of estradiol was the same in all subjects when the diet was altered from high-carbohydrate to high-protein content, although the degree of change varied with each individual.

As shown in Fig. 1, the average plasma half-life of antipyrine for the eight subjects was significantly lower during the high-protein diet than during the high-carbohydrate dietary period ($P < 0.001$). There were no significant changes in the apparent volume of distribution for the drug, whereas the average calculated metabolic clearance rate was significantly ($P < 0.005$) greater during the high-protein, as compared with the high-carbohydrate, diet period (data not shown).

DISCUSSION

The results of this study demonstrate that macronutrient components of the diet can concurrently alter both the reductive metabolism and the oxidative biotransformation of natural steroid hormones in man. Testosterone metabolism by the major hepatic pathway of metabolism in humans, microsomal Δ^4-5α-reduction, was significantly diminished by ingestion of a high-

Table 1. Metabolism of [4-^{14}C]testosterone in eight subjects studied during the consumption of a high-protein diet and a high-carbohydrate diet

Diet	% of dose				% of glucuronide and sulfate fractions	
	Total ^{14}C in urine	Glucuronide fraction	Sulfate fraction	Polar metabolites	Androsterone (5α)	Etiocholanolone (5β)
High protein	75.2 ± 5.4	62.1 ± 4.3	11.1 ± 1.6*	3.7 ± 0.3	32.9 ± 2.5†	30.5 ± 1.7
High carbohydrate	70.3 ± 6.7	59.1 ± 6.1	8.2 ± 1.1*	3.5 ± 0.2	39.2 ± 3.0†	27.9 ± 3.0

Values shown are means ± SEM for the urinary excretion of testosterone metabolites.
*Statistically significant difference ($P < 0.01$) by paired *t* test.
†Statistically significant difference ($P < 0.02$) by paired *t* test.

Table 2. Effects of diet and of various chemicals on hepatic Δ^4-5α-steroid reductase activity and on the cytochrome P-450-dependent mixed-function oxidation of drugs in humans or the content of cytochrome P-450 and P-448 in rat liver microsomes

Chemical agent or dietary regimen	Δ^4-5α-Steroid reduction				Cytochrome P-450 content or mixed-function oxidation*			
	Effect	Substrate	Species	Ref.	Effect	Substrate or cytochrome content	Species	Ref.
Increased dietary protein/carbohydrate ratio in the diet	↓	Testosterone	Human	(This study)	↑	Antipyrine, theophylline	Human	(This study; 1–3)
Phenobarbital	↓	Testosterone	Human	(15)	↑	Antipyrine (and others)	Human	(15)
	↓	11-Hydroxy-Δ^4-androstene-3,17-dione	Human	(15)				
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	↓	Δ^4-Androstene-3,17-dione	Rat	(16)	↑	Cytochrome P-450	Rat	(16, 17)
	↓	Δ^4-Pregnene-3,20-dione	Rat	(16)				
Polychlorinated biphenyl (3,4,3′,4′-tetrachlorobiphenyl)	↓	Testosterone	Rat	(18)	↑	Cytochrome P-450	Rat	(18–20)
Polychlorinated dibenzofurans	↓	Testosterone	Rat	(21)	↑	Cytochrome P-450	Rat	(22)
Polybrominated biphenyl (Firemaster BP-6)	↓	Testosterone	Rat	(23)	↑	Cytochrome P-450	Rat	(24–26)
Hexachlorobenzene	↓	Testosterone	Rat	(27)	↑	Cytochrome P-450	Rat	(28, 29)
16α-Cyanopregnenolone (Δ^4-Pregnene-3β-ol-16α-carbonitrile-20-one)	↓	Δ^4-Pregnene-3,20-dione	Rat	(30, 31)	↑	Cytochrome P-450	Rat	(31–33)

*Cytochrome P-450 is used in the collective sense to refer to one or more isozymes of the hemeprotein.

protein diet as compared with a high-carbohydrate diet. No changes in the rate of Δ^4-5β-reduction of the hormone, a metabolic reaction localized to the cytosol, was produced by these diet changes. Concurrent with the decrease in the Δ^4-5α-reduction of testosterone, the high-protein diet elicited an increase in the cytochrome P-450-dependent oxidation of estradiol at the C2 position. Thus, selective and pronounced alterations in steroid hormone biotransformations in liver were produced by specific changes in dietary macronutrients in humans. The extent to which diet-induced alterations in steroid hormone metabolism may occur in endocrine target organs other than the liver is not known.

The proximate mechanism by which a high-protein/low-carbohydrate diet decreases the Δ^4-5α-reductive metabolism of testosterone while concurrently increasing the oxidation rate for estradiol (and antipyrine) in humans has not been defined. Both metabolic systems examined in this study (i.e., cytochrome P-450-dependent oxidation and Δ^4-5α-steroid reduction) are localized in endoplasmic reticulum membranes in the liver, but they appear to be regulated in a reciprocal fashion in certain circumstances. This reciprocal relationship extends beyond the dietary effects examined in this study because, as summarized in Table 2, a decrease in hepatic Δ^4-5α-steroid reductive activity also has been identified in animals or in humans at a time when enhanced activity or induction of the cytochrome P-450 system by drugs or environmental chemicals has been produced (15–33). The agents that have been shown to elicit these dual effects on cytochrome P-450-dependent oxidations and the Δ^4-5α-reductive metabolism of steroids include phenobarbital, hexachlorobenzene, dioxin, dibenzofurans, and polyhalogenated biphenyls. It is of interest in this respect that in anorexia nervosa, a disorder of unknown etiology but with complex psychiatric-metabolic aspects, a decrease in Δ^4-5α-steroid metabolism with an enhancement in 2-oxidation of estradiol has also been noted (38). Whether there is a nutritional basis for these metabolic changes in patients with anorexia nervosa is not known. Additionally, in subjects with the hereditary liver disease acute intermittent porphyria, a pronounced deficiency of Δ^4-5α-steroid reduction occurs only in those individuals in whom the disease has become clinically expressed (34, 35, 39)—i.e., in whom the heme pathway has become activated as reflected in the induction in liver of δ-aminolevulinate synthase, the rate-limiting enzyme of hepatic heme formation. Acute intermittent porphyria gene carriers in whom the gene defect has never become clinically manifest do not show this impairment of Δ^4-5α-steroid reduction (35). The findings of the present study and those summarized in Table 2 suggest that it may be fruitful to examine further the extent to which there may be an association between hepatic Δ^4-5α-reductive metabolism of steroids and activation of the heme biosynthetic pathway or cytochrome P-450-mediated chemical oxidations in certain disease states and in individuals treated with drugs or exposed to environmental chemicals.

The demonstration in this study that alterations in the composition of the human diet can produce consistent and specific changes in the patterns of steroid hormone biotransformation is of intrinsic endocrine interest and affirms that nutritional factors can play as significant a role in regulating the metabolic disposition of natural steroids as they do in regulating the biotransformation of exogenous chemicals such as drugs in man (1–8). In this context, the analogy between drugs and hormones extends further because many of the metabolites of natural steroid hormones, like those of numerous drugs, have been shown to exert potent biological actions independent of, and sometimes distinctive from, those of their parent compounds (40–52). Thus, nutrition-induced alterations of specific steroid biotransformations that are mediated by enzymes in the liver, and possibly in other tissues, have the potential for substantially modifying the biological impact of these hormones in humans.

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