

Phytoestrogens Alter Adrenocortical Function: Genistein and Daidzein Suppress Glucocorticoid and Stimulate Androgen Production by Cultured Adrenal Cortical Cells*

SAM MESIANO[†], STEVEN L. KATZ[‡], JANET Y. LEE, AND ROBERT B. JAFFE

Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, California 94143-0556

ABSTRACT

Phytoestrogens influence a variety of biological processes. As 17β -estradiol alters adrenocortical cell function, we examined whether the dietary phytoestrogens, genistein and daidzein, have related effects. In cultured human fetal and postnatal adrenal cortical cells, genistein and daidzein (both 0.4–40 $\mu\text{mol/L}$) decreased ACTH-stimulated cortisol production to basal levels (ED_{50} , 1–4 $\mu\text{mol/L}$). In the adult adrenocortical cell line, H295, genistein, daidzein, and 17β -estradiol (10 $\mu\text{mol/L}$) decreased cAMP-stimulated cortisol synthesis in a similar fashion. Neither genistein nor daidzein altered basal or ACTH-stimulated dehydroepiandrosterone sulfate (DHEA-S) production in fetal adrenocortical cells, whereas in postnatal adrenocortical cells, DHEA and DHEA-S were markedly increased (ED_{50} , 1–4 $\mu\text{mol/L}$). In

H295 cells, basal and cAMP-stimulated DHEA production were similarly increased by the phytoestrogens and 17β -estradiol. Genistein and daidzein did not affect the expression of steroid-metabolizing enzymes. However, genistein and daidzein specifically inhibited the activity of 21-hydroxylase (P450c21); the activities of other steroidogenic enzymes were not affected. Thus, phytoestrogens may decrease cortisol synthesis by suppressing the activity of P450c21 and, as a consequence, increase DHEA/DHEA-S synthesis by shunting metabolites away from the glucocorticoid synthetic pathway. Therefore, consumption of foods containing phytoestrogens may alter adrenocortical function by decreasing cortisol and increasing androgen production. (*J Clin Endocrinol Metab* 84: 2443–2448, 1999)

PHYTOESTROGENS are plant (predominantly legumes and grasses) substances, that have structural and functional similarity to 17β -estradiol (1). Plant-derived isoflavonoids, coumestans and ligands compete with estradiol with varying affinities for binding to estrogen receptors, induce transcription of estrogen-responsive genes (2), and, depending on the outcome measured, either mimic or antagonize the actions of steroidal estrogens (3). Humans are exposed to phytoestrogens through their diet, a major source being soy and soy-derived foods, which contain high levels of the isoflavone class of nonsteroidal estrogenic compounds, genistein and daidzein (4–8). The impact of dietary phytoestrogens on normal biological processes was first recognized in sheep, when it was found that ewes grazing on clover for prolonged periods became sterile (9). Subsequently, dietary phytoestrogens have been found to influence virtually every component of the mammalian repro-

ductive system, including the morphology and function of the reproductive organs and sexual behavior (10–13). Among their widespread clinical effects (14), dietary phytoestrogens are purported to reduce cancer risk (15), be antioxidants and free radical scavengers (16), reduce serum cholesterol (17), induce cellular differentiation (18), and inhibit angiogenesis (19).

To date, no studies of phytoestrogen actions on adrenal function have been reported. However, we and others previously found that steroid production by human fetal adrenal cortical cells is modulated by estrogen (20–22). In cultured human fetal adrenal cortical cells, 17β -estradiol at relatively high concentrations (1–10 $\mu\text{mol/L}$), augmented corticotropin (ACTH)-stimulated androgen [mainly dehydroepiandrosterone sulfate (DHEA-S)] production, whereas glucocorticoid (cortisol) synthesis was significantly inhibited (22). Although these observations showed that *in vitro*, adrenal cortical cell function can be modulated by estrogen, their physiological significance is unclear, as endogenous estrogen concentrations do not normally reach micromole per L levels in nonpregnant adults. However, it is possible that exogenous estrogen-related compounds, such as dietary phytoestrogens, could reach circulating levels high enough to exert estrogenic actions on the adrenal cortex. This is particularly the case in infants and adults who consume large amounts of soy-derived foods. For example, circulating concentrations of total phytoestrogens as a result of the typical Japanese diet are in the 1–5 $\mu\text{mol/L}$ range (23, 24), and in infants fed exclusively soy-based formula, plasma genistein and daidzein concentrations are approximately 4 $\mu\text{mol/L}$,

Received November 11, 1998. Revision received March 11, 1999. Accepted March 31, 1999.

Address all correspondence and requests for reprints to: Robert B. Jaffe, M.D., Reproductive Endocrinology Center, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, California 94143-0556. E-mail: robert.jaffe@quickmail.ucsf.edu.

* Presented in part at the 1997 Annual Meeting of The Endocrine Society, Minneapolis, MI, June 13, 1997. This work was supported in part by NIH Grants HD-08478 and P3011979.

[†] Present address: Mothers and Babies Research Centre, John Hunter Hospital, Newcastle, New South Wales 2310, Australia.

[‡] Present address: California North Bay Fertility Associates, 1111 Sonoma Avenue, Santa Rosa, California 95405.

10,000–20,000 times higher than plasma 17β -estradiol concentrations (25). More importantly, on a per weight basis, levels of genistein and daidzein in those infants were an order of magnitude higher than typical plasma concentrations in adults consuming soy foods, suggesting that young children may be particularly susceptible to phytoestrogenic actions. Thus, we hypothesized that the human adrenal cortex is susceptible to functional modulation by dietary phytoestrogens. Therefore, we examined the effects of two phytoestrogens, genistein and daidzein, on basal and ACTH-stimulated steroid production by human fetal and postnatal adrenocortical cell cultures and in the human adult adrenal cortical cell line, H295R.

Materials and Methods

Materials

Human fetal adrenal glands were obtained from second trimester fetuses (16–23 weeks of gestation as assessed by foot length) after elective therapeutic termination of pregnancy by dilatation and evacuation. A pair of human postnatal (~1.5 yr of age) adrenals were obtained from an organ donor through the kidney transplant service at the University of California-San Francisco (UCSF). The study protocol was approved by the committee on human research, UCSF. The H295R cell line was provided by Dr. W. Rainey, University of Texas Southwestern Medical Center (Dallas, TX). Genistein and daidzein were obtained from Calbiochem (San Diego, CA). Human ACTH [ACTH-(1–24)] was obtained from Organon (West Orange, NJ). Progesterone, 17α -hydroxyprogesterone, 11-deoxycortisol, 17β -estradiol, and cAMP were obtained from Sigma Chemical Co. (St. Louis, MO). The full-length complementary DNAs (cDNAs) encoding human cytochrome P450 cholesterol side-chain cleavage (P450_{scc}), P450 17α -hydroxylase/17,20 lyase (P450_{c17}), and P450 21-hydroxylase (P450_{c21}) were provided by Dr. W. L. Miller, UCSF (26–28); human type II 3β -hydroxysteroid dehydrogenase/isomerase (3β HSD) was provided Dr. F. Labrie, University of Laval (Quebec, Canada) (29); human P450 11 β -hydroxylase (P450_{c11}) was provided by Dr. P. White, University of Texas Southwestern Medical School (30), and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was obtained from American Type Culture Collection (Manassas, VA).

Cell cultures

Primary cultures of human fetal and postnatal adrenocortical cells were prepared as previously described (31). Briefly, the adrenal capsule was carefully peeled away from the gland, and the remaining cortical cells were dispersed by enzymatic digestion and plated onto 6-cm diameter plastic culture dishes (Falcon Plastics, Los Angeles, CA) at a density of 5×10^5 cells/dish or onto 48-well culture dishes (Nunc, Naperville, IL) at a density of 1×10^5 cells/well. In each case, the culture medium was DMEM H-16/Ham's F-12 (1:1) medium containing non-essential amino acids and supplemented with 10% FCS, 2 mmol/L glutamine, and 50 μ g/mL gentamicin (Cell Culture Facility, UCSF). After 48 h, test substances were added. H295R cells were plated onto 48-well dishes in DMEM H-16/Ham's F-12 (1:1) medium supplemented with 2 mmol/L glutamine, 50 μ g/mL gentamicin, 2% FCS, and 1% ITS Plus (insulin, transferrin, and selenium, each 6.25 μ g/mL; Collaborative Research, Bedford, MA) and grown to confluence. All plates were incubated in a humidified environment at 37 C in 95% air-5% CO₂.

Ribonucleic acid (RNA) analysis

Northern blot analyses were used to assay the abundance of specific messenger RNA (mRNA) transcripts. Total RNA was extracted and purified from cultured cells using the method of Chomczynski and Sacchi (32). Total RNA (5–10 μ g) was denatured in formaldehyde, subjected to electrophoresis through a 1.2% agarose gel, and transferred to nitrocellulose membranes (Nytran, Schleicher & Schuell, Inc., Keene, NH) to which it was cross-linked by exposure to UV light (Stratalinker, Stratagene, La Jolla, CA). [³²P]deoxy-CTP-labeled cDNA probes (1–2 $\times$

10⁹ dpm/ μ g) were synthesized by random primer extension of denatured full-length cDNAs. Prehybridization was performed in hybridization buffer (Quickhyb buffer, Stratagene) at 68 C for 15 min. Denatured radiolabeled probe was then added to the membranes and incubated at 68 C for 1 h. To remove nonspecifically bound probe, membranes were washed in $2 \times$ SSC (1 $\times$ SSC is 0.15 mol/L NaCl and 0.015 mol/L sodium citrate)-0.1% SDS at room temperature for 15 min and then in 0.1 $\times$ SSC-0.1% SDS at 60 C for 30 min. Membranes were then subjected to autoradiography at –70 C. The relative abundance of mRNA transcripts was estimated by computer-assisted densitometry (BioImage, Ann Arbor, MI). All data were normalized to the abundance of transcripts encoding GAPDH, which was constitutively expressed. Probes were removed by washing the membranes in distilled water at 100 C. Complete removal of probe was confirmed by autoradiography before reprobing.

RIAs

Cortisol, DHEA, and DHEA-S were measured in conditioned medium using specific RIAs. Unconjugated cortisol was assayed using a kit purchased from Diagnostic Products (Los Angeles, CA). DHEA and DHEA-S were assayed using specific kits obtained from ICN Biomedicals, Inc. (Costa Mesa, CA), with charcoal separation of free from bound steroid. All assays were validated for use on conditioned medium from fetal adrenal cortical cell cultures, and each had an inter- and intraassay coefficient of variation of less than 10%.

Statistical analyses

All steroid data were normalized for cell number [measured using a particle counter (Coulter Electronics, Hialeah, FL)] and time of exposure to ACTH and are presented as the mean $\pm$ SEM. All experiments were performed in triplicate and repeated at least three times. Statistical analyses were conducted by ANOVA followed by *post-hoc* tests (Dunnett's, Student-Newman-Keuls, or Bonferroni), and differences were considered statistically significant when $P < 0.05$.

Results

Steroid synthesis

In all cell types studied, genistein and daidzein decreased agonist (ACTH or cAMP)-stimulated cortisol production in a dose-dependent fashion with an ED₅₀ of 1–4 μ mol/L (Fig. 1, A and B, *upper panels*). At the maximum phytoestrogen dose (40 μ mol/L), cortisol production was decreased to levels approaching the lower limit of assay detection in all cell types. In postnatal and H295R cells, genistein and daidzein also decreased basal cortisol production in a similar dose-dependent fashion. In fetal adrenal cortical cells, basal cortisol production was close to the lower limit of assay detection; therefore, any further decrease induced by phytoestrogens was not apparent.

As expected, both basal and ACTH-stimulated DHEA-S productions were high (10- to 20-fold that in postnatal and H295R cells) in fetal adrenal cells. In these fetal cell cultures, genistein and daidzein did not alter DHEA-S production. In contrast, basal and ACTH-stimulated production of DHEA-S and DHEA by postnatal adrenal cortical cells were increased by genistein. In postnatal cells, the increases in DHEA and DHEA-S induced by genistein were dose dependent, whereas in H295 cells, levels of DHEA and DHEA-S were maximally increased at all genistein and daidzein concentrations. We also examined the effect of 17β -estradiol (10 μ mol/L) on steroid production by H295 cells and found that it also decreased basal and cAMP-stimulated cortisol production to minimal levels and increased basal and cAMP-stimulated DHEA production.

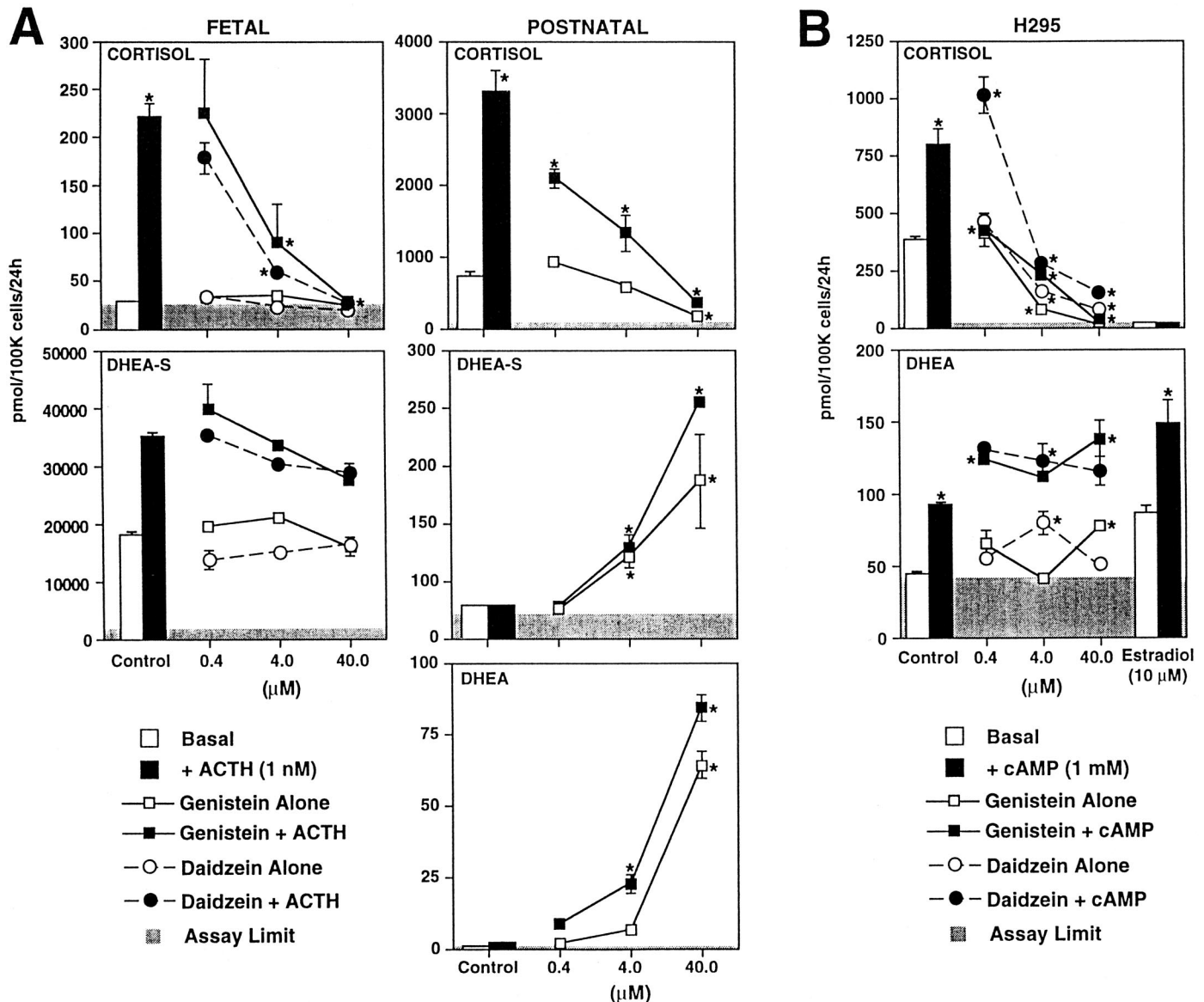


FIG. 1. Effects of genistein and daidzein on cortisol, DHEA-S, and DHEA production by primary cultures of human fetal and postnatal adrenal cortical cells (A) and the adult human adrenal cortical cell line, H295 (B). Cell cultures were exposed to genistein, daidzein, or 17 β -estradiol with and without ACTH (1 nmol/L) or cAMP (1 mmol/L) for 24 h. Media were then collected and assayed for cortisol, DHEA-S, and DHEA, and the remaining cells were harvested and counted in a particle counter. For postnatal adrenal cortical cells, only the effects of genistein were examined. All data are the mean $\pm$ SEM (n = 3). *, $P < 0.05$ compared to the appropriate (with or without ACTH or cAMP) control.

Steroidogenic enzyme expression

To examine the mechanism by which phytoestrogens modulate steroid production by adrenal cortical cells, we determined whether genistein and daidzein alter the expression of the steroid-metabolizing enzymes involved in DHEA and cortisol synthesis by human fetal adrenal cortical cells. The synthesis of cortisol from pregnenolone (Fig. 2A) is performed sequentially by four enzymes: 3 β HSD, P450c17, P450c21, and P450c11, whereas DHEA production requires only P450scc and P450c17. The effects of 24-h exposure of human fetal adrenal cortical cells to genistein and daidzein (both 40 μ M/L) in the presence and absence of ACTH on the expression of genes encoding these enzymes were examined by Northern blot analysis. Relative to the abundance

of mRNA encoding the constitutively expressed GAPDH, genistein and daidzein did not alter the basal or ACTH-stimulated abundance of mRNAs encoding P450scc, 3 β HSD, P450c17, P450c21, or P450c11. In the representative experiment shown in Fig. 2B, genistein and daidzein appeared to decrease 3 β HSD mRNA; however, this effect was not reproducible (n = 3).

Steroidogenic enzyme activity

We next examined whether the phytoestrogens modulate steroidogenic enzyme activity. To that end, fetal adrenal cortical cells were exposed for 24 h to genistein or daidzein with and without ACTH and in the presence of specific steroid precursors of cortisol synthesis. The rationale under-

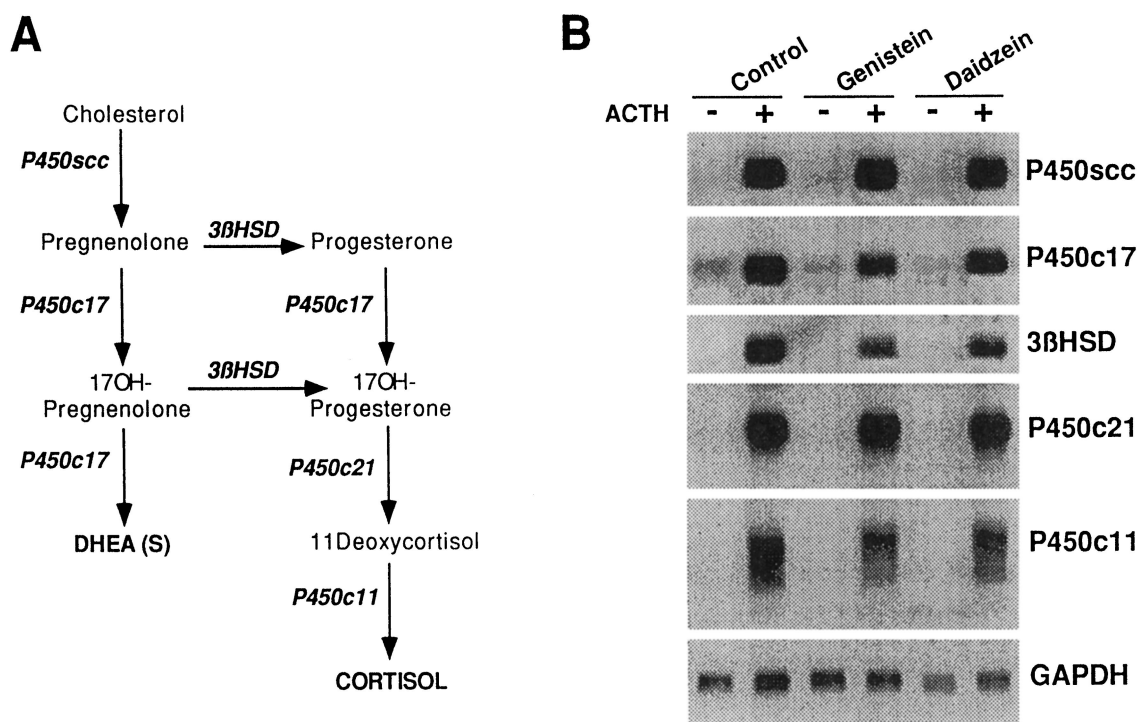


FIG. 2. A, Biosynthetic pathway for cortisol and DHEA from cholesterol. Steroidogenic enzymes are in *italics*. All enzymes are required for conversion of cholesterol to cortisol, whereas DHEA production requires only P450scc and P450c17. B, Northern blot analysis of total RNA extracted from primary cultures of midgestation human fetal adrenal cells exposed to genistein or daidzein (each 40 μ mol/L) with and without ACTH (1 nmol/L) for 24 h. Exposure times were 4 h for P450scc, 30 min for P450c17, 18 h for 3 β HSD, 5 h for P450c21, 6 h for P450c11, and 18 h for GAPDH. The results shown are representative of three separate experiments.

lying these experiments was that by providing different substrates we would bypass, and therefore identify, any step in the pathway (Fig. 2A) toward cortisol synthesis affected by the phytoestrogens. Both genistein and daidzein inhibited ACTH-stimulated cortisol production when progesterone (Fig. 3B) and 17 α -hydroxyprogesterone (Fig. 3C) were added to the medium. However, when 11-deoxycortisol was added to the cultures, neither genistein nor daidzein inhibited cortisol production (Fig. 3D). Similar results were obtained using H295R cells. Taken together, these data suggest that phytoestrogens suppress cortisol production by inhibiting P450c21.

Toxicity and effects on tyrosine kinase activity

Genistein and daidzein (each at 40 μ mol/L) were not toxic to human adrenal cortical cells, as assessed by cell number, and their effects were transient; exposure to genistein or daidzein for 24 h had no effect on the subsequent response to ACTH (data not shown). Thus, at the maximum concentrations studied, the phytoestrogens did not permanently alter the cell's steroidogenic phenotype. As genistein inhibits protein tyrosine kinase activity (33), we determined whether its effects were due to inhibition of tyrosine phosphorylation. By Western blot analysis, we found that genistein did not alter the spectrum of peptides containing phosphotyrosine residues in human fetal adrenal cortical cells (data not shown). That the actions of daidzein, which does not alter tyrosine kinase activity, were identical to those of genistein

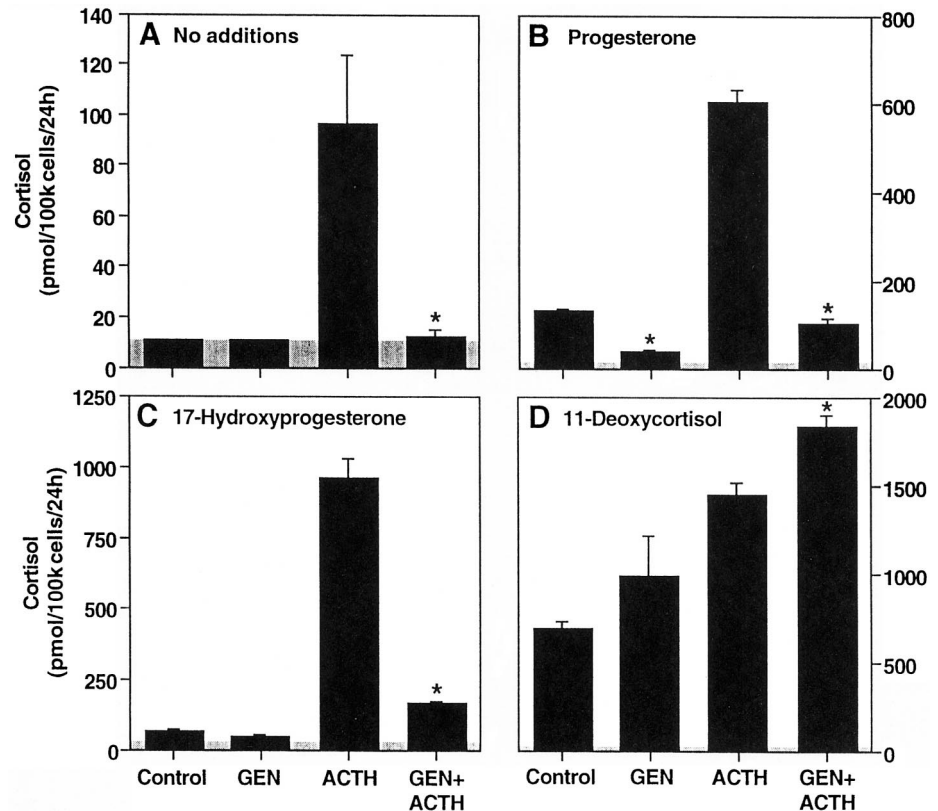
further indicates that the actions of genistein were not mediated by inhibition of tyrosine kinase activity.

Discussion

To our knowledge, this is the first study to suggest that dietary phytoestrogens influence human adrenal cortical function. We found that two phytoestrogens, genistein and daidzein, which are highly abundant in soy-based foods, increase androgen and decrease glucocorticoid production by cultured human adrenal cortical cells. Although these effects occurred at concentrations of genistein and daidzein that were within the reported range for infants and adults consuming a soy-rich diet, blood concentrations and tissue levels may differ markedly. Clearly, controlled *in vivo* studies are needed to firmly establish whether phytoestrogens modulate adrenal cortical steroid production.

Our data indicate that genistein and daidzein decreased cortisol synthesis by suppressing P450c21 enzymatic activity; expression of this gene was not affected. Although 3 β HSD activity was not assessed in the present studies, it is likely, based on previous findings, that genistein and daidzein inhibited cortisol synthesis by decreasing the activity of 3 β HSD. Studies in human adult adrenal microsomes (34) and in the H295R cell line (35) have demonstrated that estrogens inhibit cortisol synthesis by specifically inhibiting the activity of the 3 β HSD enzyme. The effects of phytoestrogens on H295R cells reported here were essentially identical to the effects of estradiol on H295R cells reported by Gell *et al.* (35).

FIG. 3. Effects of genistein (GEN; 40 $\mu\text{mol/L}$) on basal and ACTH (1 nmol/L)-stimulated cortisol production by primary cultures of human fetal adrenal cortical cells in the absence (A) or presence of precursors [progesterone (B), 17 α -hydroxyprogesterone (C), and 11-deoxycortisol (D); each 1 $\mu\text{mol/L}$] for cortisol biosynthesis. Cells were exposed to test substances for 24 h, after which the media were collected, and the cells were harvested and counted. Values are the mean $\pm$ SEM (n = 3). *, $P < 0.05$.



Interestingly, Gell *et al.* (35) also showed that the actions of estradiol on steroid production by H295R cells were not inhibited by the estrogen antagonist, ICI 182,780, suggesting that the estrogen effects were not mediated through estrogen receptors. Although Kuiper *et al.* (2) showed that phytoestrogens, including genistein and daidzein, compete with estradiol for binding to estrogen receptors (both the α - and β -subtypes) and regulate estrogen-responsive gene expression, these processes do not appear to be involved in the estrogen/phytoestrogen modulation of adrenal cortical cell steroidogenesis. We previously showed that estradiol inhibits cortisol synthesis by human fetal adrenal cortical cells without influencing the expression of P450scc, P450c17, and 3 β HSD (22). Similarly, in the present study, phytoestrogens altered steroid production without affecting the expression of steroidogenic enzymes. Taken together, these findings support the concept that estrogen and phytoestrogens modulate adrenal steroidogenic activity via nongenomic mechanisms, most likely by direct modulation of steroidogenic enzyme activity.

As well as decreasing cortisol synthesis, genistein and daidzein modulated androgen production by increasing DHEA and DHEA-S synthesis. However, effects on androgen synthesis occurred only in postnatal adrenal cortical cells and in the H295 cell line; in fetal adrenal cortical cells, basal and ACTH-stimulated DHEA-S production were not altered by exposure to phytoestrogens. The mechanism by which the phytoestrogens increased androgen production is not clear. As with the decrease in cortisol production, it is possible that genistein and daidzein increased DHEA and DHEA-S production by directly modulating steroidogenic enzyme activ-

ity, *i.e.* by augmenting the activity of either P450scc or P450c17. However, the abundance of mRNAs encoding P450scc and P450c17 was not affected by genistein or daidzein. Furthermore, genistein and daidzein did not affect basal or ACTH-stimulated production of DHEA-S in fetal adrenal cortical cells, suggesting that the activities of these enzymes were not affected. It is more likely that androgen production was increased as a result of decreased cortisol synthesis, *i.e.* the block in the cortisol biosynthetic pathway caused an increase in the pool of precursors available for DHEA synthesis. Interestingly, basal DHEA and DHEA-S production was increased only in cells in which basal cortisol production was high. In postnatal adrenal cortical cells, genistein increased DHEA and DHEA-S production in a dose-responsive fashion, which mirrored the decrease in cortisol, suggesting that the two events were related.

The possible consequences of altered adrenal function by dietary phytoestrogens are intriguing. Suppression of cortisol production is not likely to have a major impact, as the hypothalamic-pituitary-adrenal axis is exquisitely sensitive to cortisol negative feedback and therefore would be expected to accommodate the increased ACTH secretion to maintain glucocorticoid homeostasis. We propose that the most significant physiological effect of dietary phytoestrogens is to increase adrenal androgen (DHEA and DHEA-S) secretion as a consequence of decreased cortisol production and increased ACTH secretion. Previous studies suggested that the timing of adrenarche (when adrenal androgen production resumes at 6–10 yr of age) is influenced by estrogens (36). Precocious adrenarche in girls was associated with increased estradiol levels. Those findings and our present data

raise the possibility that a phytoestrogen-rich diet may cause adrenarche to occur at a younger age and possibly promote masculinization, provided that the adrenal androgens are not converted to estrogens.

Our study brings together two somewhat controversial areas of biomedical research: 1) the effects of dietary phytoestrogens on human health, and 2) the role of the adrenal androgens DHEA and DHEA-S in human physiology. Despite relatively scant scientific evidence, the idea that DHEA promotes well-being has gained increasing popularity in recent years, and as a consequence, DHEA is readily available as an over the counter food supplement. Based upon our present findings, we postulate that a diet that includes a moderate amount of phytoestrogen-containing foods such as soy would be sufficient to raise endogenous DHEA and DHEA-S production to levels that may obviate the need for DHEA supplementation. Thus, it is possible that some of the actions of dietary phytoestrogens may be mediated via increased adrenal androgen production. It is of interest that DHEA is a precursor of androgenic and estrogenic endogenous sex steroids. Thus, by increasing DHEA production, dietary phytoestrogens may indirectly increase total estrogen and/or androgen levels. Therefore, it is possible that some of the estrogenic actions of dietary phytoestrogens may be mediated via their stimulation of adrenal androgen synthesis.

Acknowledgments

We thank Dr. Mary Dallman for helpful discussions, and Ms. Carmelita Aguirre for performing the RIAs.

References

- Setchell KDR. 1985 Naturally occurring non-steroidal estrogens of dietary origin. In: McLachlan J, ed. Estrogens in the environment. New York: Elsevier; 69–85.
- Kuiper GG, Lemmen JG, Carlsson B, et al. 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology*. 139:4252–4263.
- Hughes C. 1996 Are the differences between estradiol and other estrogens merely semantical? *J Clin Endocrinol Metab*. 81:2405.
- Price KR, Fenwick GR. 1985 Naturally occurring oestrogens in foods—a review. *Food Addit Contam*. 2:73–106.
- Franke AA, Custer LJ. 1994 High-performance liquid chromatographic assay of isoflavonoids and coumestrol from human urine. *J Chromatogr*. 662:47–60.
- Franke AA, Custer LJ, Cerna CM, Narala K. 1995 Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Proc Soc Exp Biol Med*. 208:18–26.
- Stob M. 1983 Naturally occurring food toxicants: estrogens. In: Rechner MJ, ed. Handbook of naturally occurring food toxicants. Boca Raton: CRC Press; 81–100.
- Wang H, Murphy PA. 1994 Isoflavone content of commercial soybean foods. *J Agric Food Chem*. 42:1666–1673.
- Schinckel PG. 1948 Infertility in ewes grazing subterranean clover pastures. Observations on breeding following transfer to sound country. *Aust Vet J*. 24:289–294.
- Shutt DA. 1976 The effects of plant oestrogens on animal reproduction. *Endeavour*. 35:110–113.
- Kaldas RS, Hughes CLJ. 1989 Reproductive and general metabolic effects of phytoestrogens in mammals. *Reprod Toxicol*. 3:81–89.
- Hughes CLJ. 1988 Phytochemical mimicry of reproductive hormones and modulation of herbivore fertility by phytoestrogens. *Environ Health Perspect*. 78:171–175.
- Cassidy A, Bingham S, Setchell KDR. 1994 Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr*. 60:333–340.
- Knight DC, Eden JA. 1996 A review of the clinical effects of phytoestrogens. *Obstet Gynecol*. 87:897–904.
- Messina M, Persky V, Setchell KD, Barnes S. 1994 Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutr Cancer*. 21:113–131.
- Jha HC, von Recklinghausen G, Zilliken F. 1985 Inhibition of *in vitro* microsomal lipid peroxidation by isoflavonoids. *Biochem Pharmacol*. 34:1367–1369.
- Anderson JW, Johnstone BM, Cook-Newell ME. 1995 Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med*. 333:276–282.
- Constantinou A, Huberman E. 1995 Genistein as an inducer of tumor cell differentiation: possible mechanisms of action. *Proc Soc Exp Biol Med*. 208:109–115.
- Fotsis T, Pepper M, Adlercreutz H, et al. 1993 Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci USA*. 90:2690–2694.
- Voutilainen R, Kahri AI, Salmenpera M. 1979 The effects of progesterone, pregnenolone, estriol, ACTH and hCG on steroid secretion of cultured human fetal adrenals. *J Steroid Biochem*. 10:695–700.
- Fujieda K, Faiman C, Reyes FI, Winter JSD. 1982 The control of steroidogenesis by human fetal adrenal cells in tissue culture. IV. The effects of exposure to placental steroids. *J Clin Endocrinol Metab*. 54:89–94.
- Mesiano S, Jaffe RB. 1993 Interaction of insulin-like growth factor-II and estradiol directs steroidogenesis in the human fetal adrenal toward dehydroepiandrosterone sulfate production. *J Clin Endocrinol Metab*. 77:754–758.
- Morton MS, Wilcox G, Wahlqvist ML, Griffiths K. 1994 Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *J Endocrinol*. 142:251–259.
- Adlercreutz H, Markkanen H, Watanabe S. 1993 Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet*. 342:1209–1210.
- Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. 1997 Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet*. 350:23–27.
- Chung B, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL. 1986 Human cholesterol side-chain cleavage enzyme, P450_{sc}: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. *Proc Natl Acad Sci USA*. 83:8962–8966.
- Chung B, Picado-Leonard J, Haniu M, et al. 1987 Cytochrome P450c17 (steroid 17 α -hydroxylase/17,20 lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proc Natl Acad Sci USA*. 84:407–411.
- Bristow J, Gitelman SE, Tee MK, Staels B, Miller WL. 1993 Abundant adrenal-specific transcription of the human P450c21A pseudogene. *J Biol Chem*. 268:12919–12924.
- Rheume E, Lachance Y, Zhao HF, et al. 1991 Structure and expression of a new complementary DNA encoding the almost exclusive β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 -isomerase in human adrenals and gonads. *Mol Endocrinol*. 5:1147–1157.
- Chua SC, Szabo P, Vitek A, Grzeschik KH, John M, White P. 1987 Cloning of cDNA encoding steroid 11 beta-hydroxylase (P450c11). *Proc Natl Acad Sci USA*. 84:7193–7197.
- Mesiano S, Mellon SH, Gospodarowicz D, Di Blasio AM, Jaffe RB. 1991 Basic fibroblast growth factor expression is regulated by ACTH in the human fetal adrenal: a model for adrenal growth regulation. *Proc Natl Acad Sci USA*. 88:5428–5431.
- Chomczynski P, Sacchi N. 1987 Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 162:156–159.
- Akiyama T, Ishida J, Nakagawa S, et al. 1987 Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem*. 262:5592–5595.
- Byrne GC, Perry YS, Winter JS. 1986 Steroid inhibitory effects upon human adrenal β -hydroxysteroid dehydrogenase activity. *J Clin Endocrinol Metab*. 62:413–418.
- Gell JS, Oh J, Rainey WE, Carr BR. 1998 Effect of estradiol on DHEAS production in the human adrenocortical cell line, H295R. *J Soc Gynecol Invest*. 5:144–148.
- Warne BL, Carter JN, Faiman C, Reyes FL, Winter JS. 1978 Hormonal changes in girls with precocious adrenarche: a possible role for estradiol or prolactin. *J Pediatr*. 92:743–747.