

Diet and Sex Hormones in Girls: Findings From a Randomized Controlled Clinical Trial

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Background: Results of several studies have suggested that diet during adolescence may influence the risk of breast cancer in adulthood. We evaluated whether an intervention to lower fat intake among adolescent girls altered their serum concentrations of sex hormones that, in adults, are related to breast cancer development. **Methods:** We conducted an ancillary hormone study among 286 of the 301 girls who participated between 1988 and 1997 in the Dietary Intervention Study in Children, in which healthy, prepubertal, 8- to 10-year-olds with elevated low-density lipoprotein cholesterol were randomly assigned to usual care or to a behavioral intervention that promoted a low-fat diet. Median time on the intervention was 7 years. Blood samples collected before randomization and at the year 1, year 3, year 5, and last visits were assayed to determine the girls' serum levels of sex hormones. All *P* values are two-sided. **Results:** At the year 5 visit, girls in the intervention group had 29.8% (95% confidence interval [CI] = 5.4% to 47.9%; *P* = .02) lower estradiol, 30.2% (95% CI = 7.0% to 47.7%; *P* = .02) lower non-sex hormone binding globulin-bound estradiol, 20.7% (95% CI = 4.7% to 34.0%; *P* = .02) lower estrone, and 28.7% (95% CI = 5.1% to 46.5%; *P* = .02) lower estrone sulfate levels during the follicular phase of the menstrual cycle and 27.2% (95% CI = 5.7% to 53.1%; *P* = .01) higher testosterone levels during the luteal phase of the menstrual cycle than did girls in the usual care group. At the last visit, the luteal phase progesterone level was 52.9% (95% CI = 20.0% to 72.3%) lower for girls in the intervention group than for girls in the usual care group (*P* = .007). **Conclusion:** Modest reductions in fat intake during puberty are associated with changes in sex hormone concentrations that are consistent with alterations in the function of the hypothalamic-pituitary-ovarian axis. Whether these changes influence breast cancer risk is currently unknown. [J Natl Cancer Inst 2003;95:132-41]

The relationship between dietary fat and breast cancer risk has been studied extensively since the 1940s, when Tannenbaum (1) observed that rats fed a high-fat diet had a higher incidence

of mammary tumors than rats fed a low-fat diet. Comparisons of the breast cancer mortality rates among different countries according to the per capita fat consumption support the hypothesis that a high-fat diet may increase the incidence and mortality from breast cancer (2). However, results of case-control studies that have examined the relationship between adult diet and breast cancer risk are inconclusive, and cohort studies generally do not support such an association (3).

Adolescence is a time of rapid growth and maturation of the breasts, and a woman's diet as an adolescent could potentially affect her risk of developing breast cancer more than her diet as an adult (4-6). Early age at menarche is an established risk factor for breast cancer (7), and diet is associated with the timing of menses onset (8-10). Furthermore, adult height is influenced by childhood diet and is positively associated with breast cancer risk (7). Although results of case-control studies generally do not support an association between childhood diet and breast cancer risk (11-13), those studies relied on recall of diet in the

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distant past, which may have caused some individuals to be misclassified, thus biasing the results.

Breast cancer is a hormone-dependent cancer, and postmenopausal women with elevated serum estrogen and androgen concentrations are more likely to develop breast cancer than postmenopausal women with low levels of these hormones (14–18). A pooled analysis of prospective studies (19) found that postmenopausal women whose serum estradiol, estrone, estrone sulfate, testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS) levels were in the highest quintile were approximately twice as likely to develop breast cancer as postmenopausal women whose serum levels of those hormones were in the lowest quintile. By contrast, postmenopausal women with higher levels of sex hormone binding globulin (SHBG), a carrier protein that regulates the amount of bioavailable estradiol and testosterone in the blood, were less likely to develop breast cancer than postmenopausal women with low levels. Estrogens and progesterone are essential for normal mammary gland growth and differentiation (20). Lower levels of sex hormones during adolescence could potentially protect against breast cancer by altering breast morphology, by decreasing the rate of cell turnover and proliferation, which would decrease the occurrence of chance mutations, or by decreasing exposure of the breast to carcinogenic estrogen metabolites (21–25).

We conducted a study ancillary to the Dietary Intervention Study in Children (DISC) to examine the effects of a dietary intervention to lower fat intake on serum levels of sex hormones in girls and boys. Here we report our results for girls; the results for boys will be reported separately.

SUBJECTS AND METHODS

DISC Study Design

The DISC was a multicenter, randomized, controlled clinical trial sponsored by the National Heart, Lung, and Blood Institute to test the safety and efficacy of a dietary intervention to reduce serum low-density lipoprotein (LDL) cholesterol in children with elevated levels of LDL cholesterol. The design and results of the DISC have been described (26–28). Briefly, between 1988 and 1990, 663 children aged 8–10 years with elevated LDL cholesterol were entered into the DISC at one of six clinical centers: Children's Hospital, New Orleans, LA; Johns Hopkins University Hospital, Baltimore, MD; Kaiser Permanente Center for Health Research, Portland, OR; University of Medicine and Dentistry of New Jersey, Newark, NJ; Northwestern University Medical School, Chicago, IL; and University of Iowa Hospital and Clinics, Iowa City, IA. The children were randomly assigned to receive a dietary intervention to reduce fat intake or to usual care, according to randomization assignments provided by the coordinating center (Maryland Medical Research Institute, Baltimore, MD). In 1990 the National Cancer Institute initiated a study ancillary to the DISC, the Hormone Ancillary Study (HAS), to assess the effect of the reduced fat dietary intervention on serum levels of sex hormones during adolescence. The initial DISC protocol was designed for 3 years of intervention and was subsequently extended with planned intervention and follow-up until participants reached 18 years of age. However, the DISC was terminated in 1997, when the mean age of participants was 16.7 years, because the participants in the two treatment groups did not have statistically significantly different serum levels of

LDL cholesterol after their year 3 follow-up visits (28). Assent was obtained from DISC participants and written informed consent was obtained from their parents or guardians prior to randomization and again when the study was extended. The DISC protocol and HAS were approved by Institutional Review Boards at all participating centers, and a National Heart, Lung, and Blood Institute-appointed independent data and safety monitoring committee provided oversight.

Female Participants in the DISC and the HAS

The DISC recruited 301 girls through schools, health maintenance organizations, and pediatric practices. Girls were eligible if they were 7.8–10.1 years old, had a serum LDL cholesterol level in the 80th–98th percentiles (29), had no major illness and were not taking medications that could affect their blood lipid levels or growth, were at least in the 5th percentile for height and were in the 5th–90th percentiles for weight-for-height (according to growth data from the Bogalusa Heart Study; Weber LS: personal communication), were at Tanner stage 1 for breast and pubic hair development (30), and had normal cognitive and psychosocial development, as evaluated by progress in school and by use of the Achenbach Child Behavior Checklist (31). Girls were not eligible if they or their family members were already following a low-fat diet, if a parent had a history of early heart disease, if their family planned to move within 3 years, or if they were known to have behavioral problems. More than 90% of the girls in the DISC were Caucasian.

Girls in both the intervention and usual care groups remained under the care of their personal physicians during their participation in the DISC. LDL cholesterol eligibility criteria were established to include children who could be recommended for dietary intervention as their main treatment modality and to exclude children with severe hypercholesterolemia who might need medication. None of the girls in the intervention or usual care groups took cholesterol-lowering medications while they were enrolled in the DISC.

The numbers of girls who attended each DISC visit and who participated in the HAS at each visit are shown in Fig. 1. Of the 301 girls who were randomly assigned to one of the two study arms in the DISC, 286 participated in the HAS at one or more visits. Because the HAS was initiated after the commencement of randomization in the DISC, we had no baseline blood samples (which were collected prior to randomization) for 218 of the 286 girls and no year 1 blood samples for 97 of the 286 girls in our study. Girls were not eligible to participate in the HAS if they were pregnant or had used oral contraceptives within 3 months of blood collection, if they were postmenarcheal and had missing data on the date they started their next menses after blood collection, or if their next menses started more than 33 days after blood collection. Except where explicitly stated, results presented here pertain only to DISC girls who were included in the HAS. Their median duration on study at the last visit was 7.0 years (range = 6.4–9.1 years).

Dietary Intervention

The dietary goals of the DISC were to limit total fat intake to 28% of calories, with less than 8% of calories from saturated fat, 9% or fewer calories from polyunsaturated fat, and the remainder of fat-derived calories from monounsaturated fat. Cholesterol intake was limited to 75 mg/1000 calories and was not to exceed 150 mg/day. Dietary fiber intake was encouraged. Girls

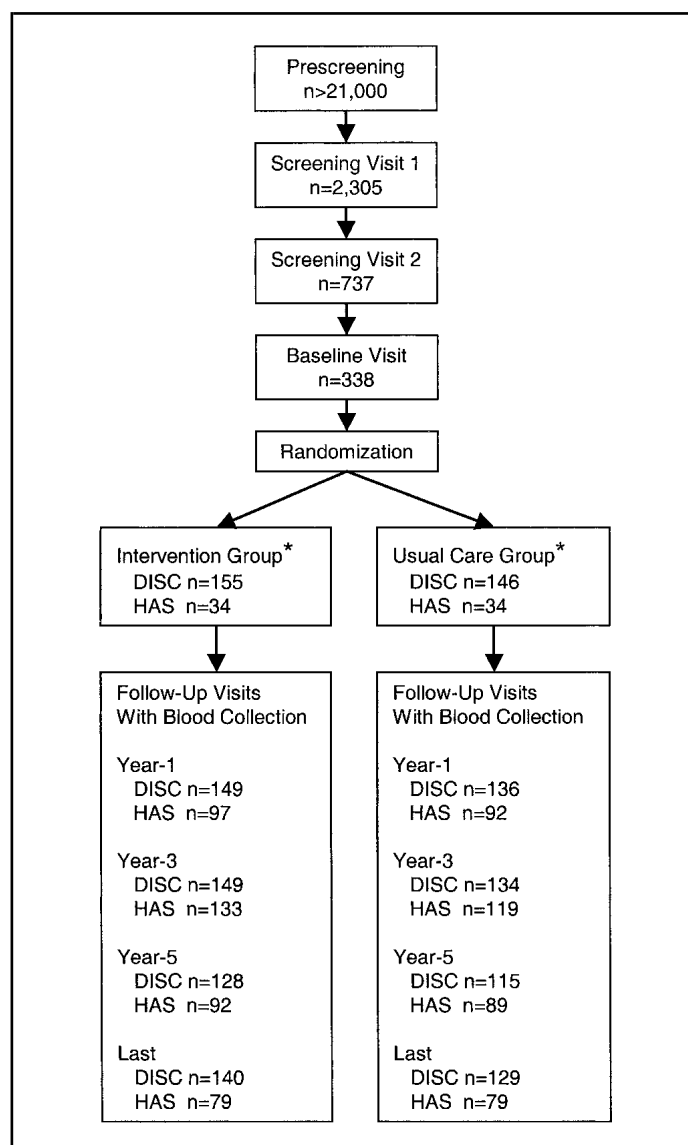


Fig. 1. Trial flow diagram for the Dietary Intervention Study in Children (DISC) and the Hormone Ancillary Study (HAS) showing the number of girls seen at screening visits, baseline visits, and follow-up visits at which blood was collected. * = Randomly assigned participants with baseline serum samples available.

in the intervention group and their families attended individual and group sessions led by nutritionists and behaviorists that were designed to teach them how to achieve the dietary goals of the DISC (32,33). Intervention visits initially occurred weekly, but their frequency was gradually decreased over the course of the trial so that four visits were planned each year during years 4 through 9. Girls in the usual care group and their families were given American Heart Association nutrition educational materials that are generally available to the public.

Data Collection

Data were collected at baseline (i.e., before randomization) and annually thereafter by trained staff who were blinded to the participants' treatment assignments. Information was obtained on demographics, medical history, use of medications, and smoking history. The height and weight of each participant was measured and was used to calculate body mass index (BMI);

weight [in kg] divided by height² [in m]). Each participant underwent a brief physical examination that included Tanner staging. Date of onset of first menses was ascertained annually until menarche. Girls were asked about use of oral contraceptives and pregnancy beginning with the year 3 visits.

Three nonconsecutive 24-hour dietary recalls were collected over a 2-week period at baseline, and at year 1, year 3, year 5, and last visits (34). Nutrient analyses were performed by the Nutrition Coordinating Center, University of Minnesota, using version 20 of their database. Data from the three recalls at each visit were averaged to estimate mean nutrient intake.

A single blood sample was collected by venipuncture in the morning after an overnight fast at baseline and at the year 1, year 3, year 5, and last visits. Serum was separated by centrifugation after the blood sample was kept at room temperature for at least 45 minutes to allow complete clotting. Serum was then aliquoted and stored in glass vials at -80°C until it was analyzed for hormone, lipid (27,28), and micronutrient levels (27,35). Blood collections were not timed to the menstrual cycles of the postmenarcheal girls. We determined the day of the menstrual cycle that corresponded to the date of blood collection from menstrual cycle calendars that were completed by the postmenarcheal girls for 6 weeks before and 6 weeks after their blood collections.

Hormone Measurements

Hormone assays were performed by Esoterix Endocrinology, Inc. (Calabasas Hills, CA). Steroid hormones were measured by radioimmunoassay, and SHBG was measured by an immunoradiometric assay (36,37). The concentration of non-SHBG-bound estradiol was calculated as the product of the total estradiol concentration and the percent non-SHBG-bound estradiol, which was measured by ammonium sulfate precipitation (37). Serum samples collected at the same clinic visit were grouped and assayed together in the same laboratory batches. Quality control samples were included in each batch, and laboratory personnel could not distinguish quality control samples from participant samples. These samples were aliquots from three serum quality control pools that were created by using charcoal-stripped serum to dilute serum from adults to achieve the range of steroid concentrations expected in the participants' samples (36). The within-visit coefficients of variation, as estimated from quality control samples, were 8%–29% for estradiol, 12%–31% for estrone, 12%–17% for estrone sulfate, 8%–17% for androstenedione, 9%–22% for testosterone, 5%–9% for DHEAS, 4%–10% for progesterone, and 15% for SHBG. The low concentrations of hormones in some quality control samples may have contributed to the higher coefficients of variation for some hormones. For example, the mean concentrations of estradiol in samples from the three quality control pools were 0.9 ng/dL, 2.8 ng/dL, and 11.3 ng/dL, and their corresponding within-visit coefficients of variation were 29%, 11%, and 8%.

Statistical Analysis

All analyses were performed by randomized treatment assignment. Median ages at menarche were estimated by a modified product-limit method that allowed for different entry ages and the censoring of girls who had not reached menarche at their last visit; a similarly modified log-rank test was used to compare groups (38). Serum hormone data were transformed to a log_e scale before analyses. Analysis of covariance (ANCOVA) was used to calculate the mean hormone concentrations at each visit

adjusted for age, race, and annual household income (<\$20 000, \$20 000–\$49 999, ≥\$50 000) and to test the statistical significance of differences between treatment groups. Because randomization in the DISC was almost complete when the ancillary study was initiated, few girls had baseline values, and comparisons at follow-up visits were not adjusted for baseline values. No imputations were performed for missing data. Because the time from ovulation to start of next menses is more constant than the time from start of last menses to ovulation for postmenarcheal girls, we defined menstrual cycle days in relation to start of next menses. Serum samples collected 1 through 14 days before the next menses were classified as luteal, and samples collected 15 through 33 days before or on the day of onset of the next menses were classified as follicular. Girls whose next menses started more than 33 days after blood collection were not included in hormone analyses. Separate models were fit for the follicular and luteal phases, and indicator variables for day of the menstrual cycle when blood was collected were added to the models. Statistical analyses were conducted using SAS, version 8 (SAS Institute, Inc., Cary, NC) and S-PLUS 2000 (MathSoft, Seattle, WA). All tests of statistical significance were two-sided.

RESULTS

Table 1 summarizes the characteristics of the girls who participated in the HAS. The mean ages, heights, weights, and BMIs were similar for girls in the intervention and usual care groups at each visit, except that girls in the intervention group had slightly higher mean BMIs ($P = .09$) at baseline and were taller ($P = .03$) at their year 1 visits than girls in the usual care group. Girls in the intervention group reported consuming statistically significantly less total and saturated fat than girls in the usual care group at all follow-up visits except at the last visit, when girls in the intervention group and usual care group reported mean saturated fat consumptions of 9.6% of calories and 10.6% of calories ($P = .05$), respectively, and total fat consumptions of 27.4% of calories and 28.8% of calories ($P = .21$), respectively. At year 1 and year 3 visits, girls in the intervention group reported consuming statistically significantly more dietary fiber (6.9 g/1000 calories and 6.8 g/1000 calories, respectively) than girls in the usual care group (6.3 g/1000 calories and 6.1 g/1000 calories, respectively). Energy intakes did not differ statistically significantly between the two study groups, except at

Table 1. Characteristics of the girls who participated in the Dietary Intervention Study in Children (DISC) Hormone Ancillary Study*

Characteristic	Visit†	Intervention group			Usual care group			<i>P</i> value‡
		N	Mean	SD	N	Mean	SD	
Age, y	Baseline	34	9.1	0.7	34	9.1	0.7	.95
	Year 1	97	10.4	0.6	92	10.3	0.6	.42
	Year 3	133	12.2	0.6	119	12.2	0.6	.43
	Year 5	92	14.2	0.7	89	14.3	0.6	.69
	Last	79	16.6	0.8	79	16.5	0.9	.82
Height, cm	Baseline	34	133.0	5.7	34	134.2	6.1	.39
	Year 1	97	141.1	5.9	92	139.2	6.2	.03
	Year 3	131	153.4	7.0	119	152.3	7.1	.26
	Year 5	92	161.6	6.3	89	161.2	6.2	.72
	Last	78	162.9	6.6	79	163.7	5.8	.43
Weight, kg	Baseline	34	32.1	6.3	34	30.9	6.4	.43
	Year 1	97	36.8	7.4	92	35.4	7.3	.21
	Year 3	131	46.5	10.4	118	46.3	10.3	.85
	Year 5	92	55.4	11.2	89	55.7	11.6	.84
	Last	78	58.3	9.2	79	59.6	9.9	.41
BMI, kg/m ²	Baseline	34	18.0	2.3	34	17.0	2.4	.09
	Year 1	97	18.3	2.7	92	18.1	2.7	.63
	Year 3	131	19.6	3.3	118	19.8	3.4	.68
	Year 5	92	21.1	3.4	89	21.3	3.7	.67
	Last	78	21.9	3.0	79	22.2	3.5	.59
Energy intake, calories/day	Baseline	34	1689	361.8	34	1709	355.8	.82
	Year 1	97	1563	398.6	91	1631	404.2	.24
	Year 3	131	1574	399.0	115	1728	485.0	.008
	Year 5	88	1640	494.6	88	1671	479.6	.68
	Last	78	1704	559.4	75	1673	455.7	.71
Total fat, % of calories	Baseline	34	32.9	6.3	34	32.9	4.8	.97
	Year 1	97	28.3	5.4	91	32.9	5.7	<.001
	Year 3	131	28.7	5.6	115	33.4	5.6	<.001
	Year 5	88	27.9	6.5	87	30.7	5.2	.002
	Last	78	27.4	7.3	75	28.8	6.3	.21
Saturated fat, % of calories	Baseline	34	12.9	2.9	34	12.6	2.5	.62
	Year 1	97	9.9	2.7	91	12.2	2.6	<.001
	Year 3	131	10.3	2.6	115	12.5	2.7	<.001
	Year 5	88	10.0	2.9	87	11.2	2.4	.003
	Last	78	9.6	3.2	75	10.6	2.6	.05
Dietary fiber, g/1000 calories	Baseline	34	6.0	2.2	34	6.1	1.8	.79
	Year 1	97	6.9	1.9	91	6.3	1.8	.01
	Year 3	131	6.8	1.8	115	6.1	1.8	.003
	Year 5	88	6.8	1.9	87	6.3	1.5	.10
	Last	78	6.9	2.6	75	6.4	1.8	.16

*The value for N for a specific year differs for different characteristics because of missing data. SD = standard deviation; BMI = body mass index.

†Last visit occurred a median of 7 years after randomization in DISC.

‡*P* values from two-sided Student's *t* tests.

the year 3 visit, when girls in the intervention group reported consuming an average of 1574 calories per day and girls in the usual care group reported consuming an average of 1728 calories per day ($P = .008$).

Age at Menarche

Median age at menarche was 12.8 years (range = 9.8–16.1 years) for all girls in the intervention group and 12.9 years (range = 9.7–17.3 years) for all girls in the usual care group ($P = .74$). Median ages at menarche for girls included in the HAS were the same as for all girls in each respective treatment group. The distributions of Tanner stages of sexual maturation for all DISC girls or for DISC girls included in the HAS did not differ by treatment group at any visit (data not shown). The length of the girls' menstrual cycles at the year 3, year 5, or last visits also did not differ between treatment groups for the girls who were included in the HAS (Table 2) or for all girls in the DISC (data not shown).

Hormone Concentrations in Premenarcheal Girls

Premenarcheal girls in the intervention and usual care groups had similar serum levels of all hormones (data not shown) except at their year 1 visits, when mean estrone sulfate concentration for girls in the intervention group (30.6 ng/dL) was statistically significantly higher than that for girls in the usual care group (25.9 ng/dL) ($P = .007$; difference = 18.1%, 95% CI = 4.8% to 33.2%).

Hormone Concentrations in Postmenarcheal Girls

Girls in the HAS were, on average, 12.2 years old (95% CI = 12.1 to 12.3 years) at year 3 visits, and only 28 girls (21.0%) in the intervention group and 26 girls (21.8%) in the usual care group were postmenarcheal. The median duration since menar-

che was 0.6 years (5th–95th percentile = 0–1.7 years) for girls in the intervention group and 0.4 years (5th–95th percentile = 0–1.6 years) for girls in the usual care group ($P = .24$) (Table 2). Curves relating the serum estradiol concentrations and day of the girls' menstrual cycles for each treatment group at the year 3 visits suggested that the girls in the intervention group had a somewhat lower estradiol concentration during the luteal phase of their cycles than did the girls in the usual care group (Fig. 2). However, treatment group differences among the postmenarcheal girls at this visit were not statistically significant for estradiol, progesterone (Fig. 2), or any other hormone (data not shown).

At year 5 visits, girls in the HAS were, on average, 14.2 years old (95% CI = 14.1–14.3 years), and 82.3% were postmenarcheal. The median duration since menarche was 1.8 years (5th–95th percentile = 0.5–3.5 years) for girls in the intervention group and 1.7 years (5th–95th percentile = 0.6 to 2.9 years) for girls in the usual care group ($P = .39$) (Table 2). Girls in the intervention group had 29.8% (95% CI = 5.4% to 47.9%) lower estradiol ($P = .02$), 30.2% (95% CI = 7.0% to 47.7%) lower non-SHBG-bound estradiol ($P = .02$), 20.7% (95% CI = 4.7% to 34.0%) lower estrone ($P = .02$), and 28.7% (95% CI = 5.1% to 46.5%) lower estrone sulfate ($P = .02$) levels during the follicular phase of the menstrual cycle than girls in the usual care group (Table 3). Furthermore, a plot of the serum estradiol concentrations at the year 5 visits as a function of menstrual cycle day suggested that the levels of this hormone began to increase later in the follicular phase for the girls in the intervention group than it did for the girls in the usual care group (Fig. 2). The luteal phase concentrations of estrogens (estradiol, non-SHBG-bound estradiol, estrone, and estrone sulfate) and progesterone did not differ by treatment group. However, girls in the intervention group had a statistically significantly higher mean luteal phase testosterone concentration (36.30 ng/dL) than girls in the usual care group (28.53 ng/dL) ($P = .01$; difference = 27.2%, 95% CI = 5.7% to 53.1%) (Table 3).

By the last visit, the average age of girls in the HAS was 16.6 years (95% CI = 16.4 to 16.7 years) and 93.7% were postmenarcheal. Their median time since menarche was 3.8 years (5th–95th percentile = 1.5–5.7 years) for girls in the intervention group and 3.7 years (5th–95th percentile = 1.8–5.6 years) for girls in the usual care group ($P = .69$) (Table 2). The mean luteal phase progesterone concentration for girls in the intervention group was 110.40 ng/dL, which was statistically significantly lower than that for girls in the usual care group who had a mean luteal phase progesterone concentration of 234.58 ng/dL ($P = .007$; difference = 52.9%, 95% CI = 20.0% to 72.3%) (Table 4, Fig. 2). There were no statistically significant differences between the two treatment groups in the concentrations of the other hormones at the last visit.

Adjustment for BMI, years since menarche, and physical activity did not substantially change the results reported in Tables 3 or 4, except that the slight differences in follicular phase androstenedione and DHEAS concentrations at last visit were no longer apparent. Leptin, which is secreted by fat cells in proportion to body fat stores and stimulates estrogen and androgen production (39), was measured only at the last visit (data not shown). As expected, serum leptin levels were highly correlated with BMI ($r = 0.72$; $P < .001$). Leptin levels did not differ by treatment group, and replacement of BMI with leptin in models did not affect the results for any hormone (data not shown).

Table 2. Years since menarche and menstrual cycle lengths at year 3, year 5, and last visit

	Visit*	Intervention group		Usual care group		<i>P</i> value†
		<i>N</i>	%	<i>N</i>	%	
Postmenarcheal girls	Year 3	28	21.0	26	21.8	.88
	Year 5	74	80.4	75	84.3	.56
	Last	73	92.4	75	94.9	.75
		<i>Median (5th–95th percentile range)</i>		<i>Median (5th–95th percentile range)</i>		<i>P</i> value‡
Years since menarche	Year 3	27	0.6 (0.0 to 1.7)	26	0.4 (0.0 to 1.6)	.24
	Year 5	74	1.8 (0.5 to 3.5)	75	1.7 (0.6 to 2.9)	.39
	Last	73	3.8 (1.5 to 5.7)	75	3.7 (1.8 to 5.6)	.69
Menstrual cycle length, days§	Year 3	28	31.5 (21 to 48)	23	29 (21 to 40)	.36
	Year 5	73	31 (22 to 50)	70	30 (21 to 49)	.24
	Last	72	29 (22 to 50)	73	30 (23 to 46)	.64

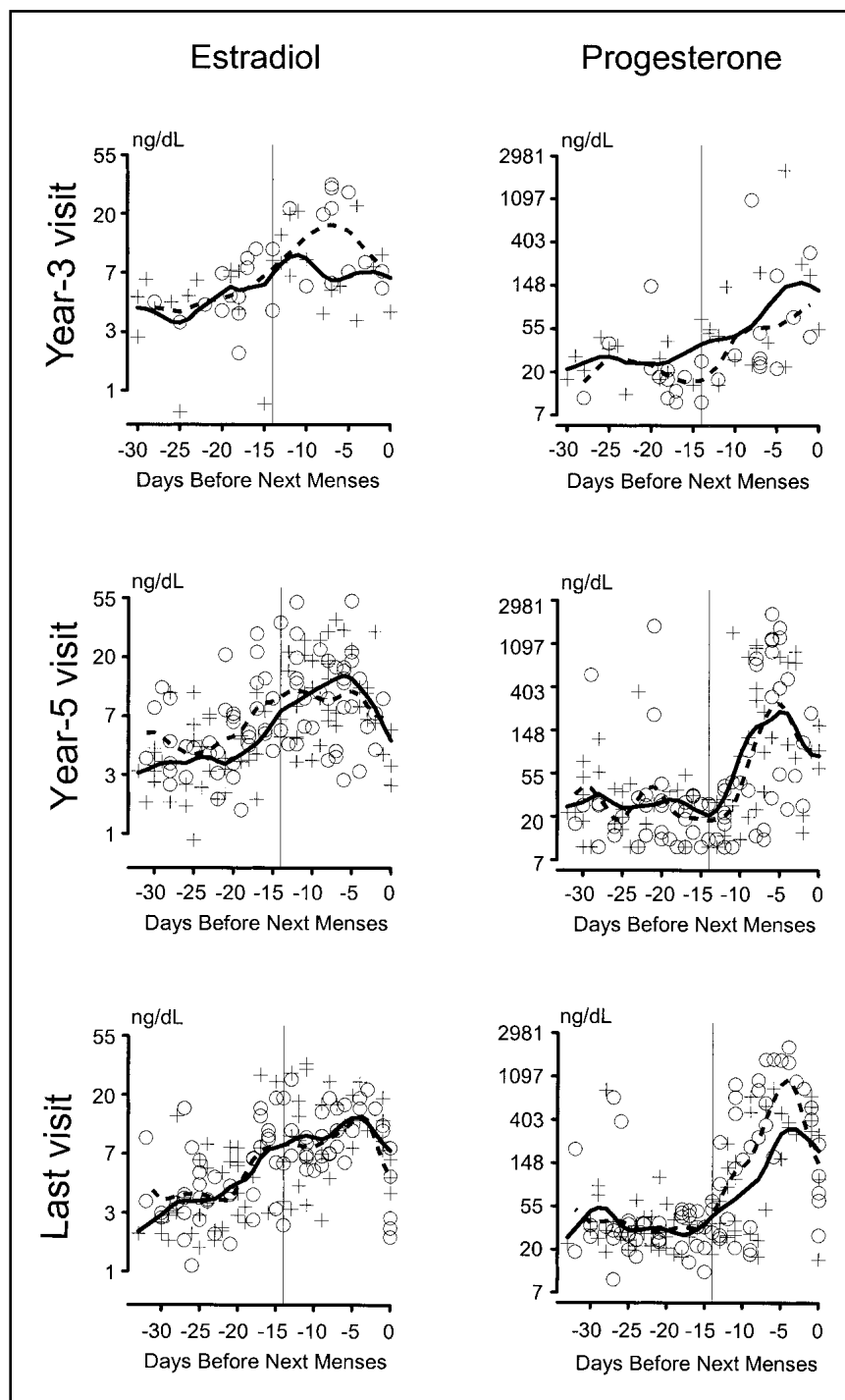
*Last visits occurred a median of 7 years after randomization in the Dietary Intervention Study in Children.

†Fisher's exact test (two-sided).

‡Wilcoxon rank sum test (two-sided).

§Calculated from menstrual calendars as the number of days before start of next menses plus the number of days since start of last menses.

Fig. 2. Serum estradiol and progesterone levels at the year 3, year 5, and last visits by menstrual cycle day. Intervention group data points are shown as **crosses** and **solid lines**. Usual care group data points are shown as **circles** and **dashed lines**. The **vertical line** at -14 days indicates the end of the follicular phase and the beginning of the luteal phase of the menstrual cycle. Curves were smoothed using the S-PLUS kernel smooth function (S-PLUS 2000; MathSoft, Seattle, WA) with \log_e transformed data. The y-axes show the antilog of \log_e transformed values. Number of intervention group and usual care group data points included in each panel are as follows: year 3 estradiol measurements, intervention group $n = 28$ and usual care group $n = 26$; year 5 estradiol measurements, intervention group $n = 74$ and usual care group $n = 74$; last visit estradiol measurements, intervention group $n = 70$ and usual care group $n = 75$; year 3 progesterone measurements, intervention group $n = 28$ and usual care group $n = 25$; year 5 progesterone measurements, intervention group $n = 66$ and usual care group $n = 70$; last visit progesterone measurements, intervention group $n = 73$ and usual care group $n = 76$.



DISCUSSION

The DISC HAS is the first randomized controlled clinical trial to evaluate the effect of an intervention to reduce fat intake on sexual maturation and serum sex hormone concentrations during adolescence. Girls in the intervention group reported lower energy intakes at the year 3 visits and lower total fat and saturated fat intakes at all follow-up visits than did girls in the usual care group, except at the last visit, when the intervention group reported a lower intake of saturated fat but not of total fat than the usual care group. Age at menarche and Tanner stage progression did not differ between the two groups at any visit.

However, at the year 5 visit, girls in the intervention group had statistically significantly lower estradiol, non-SHBG-bound estradiol, estrone, and estrone sulfate levels during the follicular phase of their menstrual cycles and statistically significantly higher testosterone levels during the luteal phase of their menstrual cycles than did girls in the usual care group. At the last visit, after an average of 7 years of study participation, the mean luteal phase progesterone concentration for girls in the intervention group was less than half that for girls in the usual care group, and that difference was statistically significant. These results suggest that modest reductions in total fat, saturated fat, and perhaps energy intake during adolescence may alter the function of the hypothalamic-pituitary-ovarian (HPO) axis,

Table 3. Mean serum hormone and SHBG concentrations by menstrual cycle phase in Dietary Intervention Study in Children (DISC) girls at year 5 visits*

Hormone or SHBG	Menstrual cycle phase	Intervention group		Usual care group		<i>P</i> value‡
		N	Mean (95% CI)†	N	Mean (95% CI)†	
Estradiol, ng/dL	Follicular§	37	3.74 (3.06 to 4.57)	36	5.32 (4.34 to 6.53)	.02
	Luteal	37	11.01 (8.54 to 14.21)	38	10.41 (8.10 to 13.38)	.77
Non-SHBG-bound estradiol, ng/dL	Follicular	37	1.95 (1.61 to 2.36)	36	2.79 (2.30 to 3.40)	.02
	Luteal	37	5.90 (4.64 to 7.49)	38	5.48 (4.33 to 6.95)	.69
Estrone, ng/dL	Follicular	37	3.24 (2.86 to 3.66)	36	4.08 (3.60 to 4.63)	.02
	Luteal	37	6.21 (5.17 to 7.46)	38	5.28 (4.41 to 6.33)	.25
Estrone sulfate, ng/dL	Follicular	37	85.39 (70.40 to 103.57)	36	119.83 (98.51 to 145.76)	.02
	Luteal	37	171.68 (137.56 to 214.27)	38	161.50 (129.79 to 200.95)	.72
Progesterone, ng/dL	Follicular	32	29.34 (20.01 to 43.03)	34	26.53 (18.34 to 38.38)	.73
	Luteal	34	86.81 (54.55 to 138.17)	36	76.75 (48.90 to 120.47)	.73
Androstenedione, ng/dL	Follicular	37	119.07 (105.73 to 134.08)	36	131.06 (116.17 to 147.85)	.29
	Luteal	37	146.44 (131.54 to 163.03)	38	130.91 (117.76 to 145.52)	.17
Testosterone, ng/dL	Follicular	37	26.16 (22.24 to 30.76)	36	31.34 (26.58 to 36.94)	.15
	Luteal	37	36.30 (32.06 to 41.10)	38	28.53 (25.25 to 32.25)	.01
DHEAS, µg/dL	Follicular	37	91.35 (77.21 to 108.07)	36	106.32 (89.64 to 126.10)	.24
	Luteal	37	128.26 (109.10 to 150.80)	38	104.53 (89.11 to 122.62)	.10
SHBG, nmol	Follicular	37	74.03 (64.02 to 85.60)	36	72.38 (62.46 to 83.87)	.84
	Luteal	37	74.47 (64.03 to 86.62)	39	74.19 (64.07 to 85.92)	.97

*CI = confidence interval; SHBG = sex hormone-binding globulin; DHEAS = dehydroepiandrosterone sulfate.

†Geometric means and 95% CIs adjusted for age, race, annual household income (<\$20,000, \$20,000–\$49,999, ≥\$50,000), and number of days before next menses.

‡Tested by two-sided tests from an analysis of covariance model that included terms for treatment group, age, race, annual household income, and number of days before start of next menses.

§Days 15 through 33 before and day of onset of next menses (indicator variables used in models to adjust for days before next menses: days 15 through 17, days 18 through 20, days 21 through 23, days 24 through 26, days 27 through 30, days 31 through 33, and day of onset of next menses).

||Days 1 through 14 before next menses (indicator variables used in models to adjust for days before next menses: days 1 through 3, days 4 through 6, days 7 through 9, days 10 through 12, and days 13 through 14).

which regulates ovarian hormone production. The pattern of hormonal differences could reflect delayed maturation of the HPO axis in the intervention group or, alternatively, different responses of the HPO axis to the intervention that depend on maturational stage.

Maturation of the HPO axis, which results in the transition from mostly anovulatory menstrual cycles to mostly ovulatory menstrual cycles, occurs over a period of several years during puberty (40). One possible explanation for the pattern of hormonal differences we observed in the participants in our study is that girls in the intervention group may have progressed through this process more slowly than girls in the usual care group. During the first year after menarche, approximately 80% of menstrual cycles are anovulatory, and luteal phase serum progesterone concentrations are low (41,42). At the year 3 visits, 78% and 92% of postmenarcheal girls in the intervention and usual care groups, respectively, were within 1 year of menarche, and the low luteal phase progesterone concentrations in both groups of girls suggest that many of their menstrual cycles were anovulatory. At the year 5 visits, girls in the intervention group had lower follicular phase estrogen concentrations than girls in the usual care group, and the intervention group's estradiol concentrations tended to increase later in the follicular phase than did those of the usual care group, which are typical of less mature menstrual cycles (43). Furthermore, girls in the intervention group had higher luteal phase testosterone concentrations than girls in the usual care group as well as higher testosterone levels during the luteal phase of their cycles than during the follicular phase of their cycles, which was not seen in the usual care group and which suggests that girls in the intervention group had a higher frequency of anovulatory cycles than girls in the usual care group (44–46). Progesterone concentrations were

low for girls in both groups at the year 5 visits, which suggests that many of the girls who had ovulatory cycles had insufficient luteal phases (40). Our finding—that girls in the intervention group had lower luteal phase progesterone concentrations at last visit than girls in the usual care group—suggests that the girls in the intervention group continued to have more cycles that were anovulatory or that had insufficient luteal phases than the girls in the usual care group. Treatment group differences in luteal phase progesterone, but not estradiol, concentrations at the last visit may have been related to the changing responsiveness of gonadotropin-releasing hormone to feedback by estradiol during maturation of the HPO axis (47).

An alternative explanation for the pattern of hormonal differences between girls in the intervention and usual care groups is that the effects of the intervention changed with maturation of the HPO axis. In the Canadian Diet and Breast Cancer Prevention Study (48), premenopausal women who were assigned to 2 years of a low-fat, high-carbohydrate dietary intervention had 35% lower serum progesterone concentrations than women in the control group who received only general dietary advice. We observed a similar difference in luteal phase serum progesterone levels between girls in the intervention group and usual care group only at the last visit, when girls had been menstruating for an average of almost 4 years and exhibited a more mature pattern of changes in progesterone concentration over their menstrual cycles compared with earlier visits. At earlier visits, when many of the girls had low luteal progesterone levels that were probably related to the immaturity of their HPO axes, the intervention did not appear to affect progesterone levels. Lower follicular phase levels of estradiol, non-SHBG-bound estradiol, estrone, and estrone sulfate and higher luteal phase levels of testosterone in girls in the intervention group compared with girls in the usual care

Table 4. Mean serum hormone and SHBG concentrations by menstrual cycle phase in Dietary Intervention Study in Children (DISC) girls at last visit*

Hormone or SHBG	Menstrual cycle phase	Intervention group		Usual care group		<i>P</i> value‡
		N	Mean (95% CI)†	N	Mean (95% CI)†	
Estradiol, ng/dL	Follicular§	39	4.14 (3.39 to 5.06)	43	4.30 (3.56 to 5.21)	.79
	Luteal	31	10.47 (8.42 to 13.03)	32	10.02 (8.06 to 12.47)	.79
Non-SHBG bound estradiol, ng/dL	Follicular	39	2.12 (1.75 to 2.57)	43	2.26 (1.88 to 2.71)	.66
	Luteal	31	5.48 (4.38 to 6.85)	32	4.72 (3.77 to 5.90)	.37
Estrone, ng/dL	Follicular	39	3.20 (2.82 to 3.62)	43	3.74 (3.32 to 4.21)	.09
	Luteal	31	5.79 (4.85 to 6.92)	32	6.05 (5.06 to 7.23)	.74
Estrone sulfate, ng/dL	Follicular	40	76.10 (64.33 to 90.01)	43	89.35 (76.00 to 105.06)	.19
	Luteal	32	146.35 (115.50 to 185.45)	33	150.93 (119.56 to 190.54)	.86
Progesterone, ng/dL	Follicular	41	36.12 (28.16 to 46.33)	43	38.30 (30.05 to 48.82)	.75
	Luteal	32	110.40 (76.31 to 159.71)	33	234.58 (163.10 to 337.38)	.007
Androstenedione, ng/dL	Follicular	41	130.15 (118.77 to 142.62)	43	145.83 (133.39 to 159.44)	.09
	Luteal	32	149.54 (134.73 to 165.98)	33	139.66 (126.04 to 154.75)	.37
Testosterone, ng/dL	Follicular	40	27.55 (24.57 to 30.89)	43	31.96 (28.62 to 35.69)	.08
	Luteal	32	34.13 (30.06 to 38.75)	33	32.37 (28.57 to 36.68)	.57
DHEAS, µg/dL	Follicular	41	142.52 (126.63 to 160.40)	43	167.24 (149.04 to 187.67)	.07
	Luteal	32	153.33 (135.72 to 173.22)	33	141.62 (125.60 to 159.68)	.38
SHBG, nmol	Follicular	41	55.36 (47.13 to 65.02)	43	54.50 (46.59 to 63.76)	.90
	Luteal	32	57.68 (48.07 to 69.22)	33	62.43 (52.17 to 74.70)	.56

*Last visits occurred a median of 7 years after randomization in the DISC. SHBG = sex hormone binding globulin; CI = confidence interval; DHEAS = dehydroepiandrosterone sulfate.

†Geometric means and 95% CIs adjusted for age, race, annual household income (<\$20,000, \$20,000–\$49,999, ≥\$50,000) and the number of days before next menses.

‡Tested by two-sided test from an analysis of covariance model that included terms for treatment group, age, race, annual household income, and number of days before start of next menses.

§Days 15 through 33 before and day of onset of next menses (indicator variables used in models to adjust for days before next menses: days 15 through 17, days 18 through 20, days 21 through 23, days 24 through 26, days 27 through 30, days 31 through 33, and day of onset of next menses).

||Days 1 through 14 before next menses (indicator variables used in models to adjust for days before next menses: days 1 through 3, days 4 through 6, days 7 through 9, days 10 through 12, and days 13 through 14).

group at the year 5 visit could reflect an altered susceptibility of the immature HPO axis to environmental influences such as diet.

Few studies have evaluated the effect of diet on serum hormones during adolescence. Persky et al. (49) reported that vegetarian girls consumed less fat and had statistically significantly higher follicular phase estradiol and luteal phase DHEAS levels than nonvegetarian girls. By contrast, girls in the DISC intervention group had follicular phase estradiol levels that were the same or lower than those of the girls in the usual care group, and DHEAS levels did not differ between the two groups. However, fat intake among girls in the study by Persky et al. was higher than fat intake among girls in the DISC, which could have contributed to discrepancies between the two studies in the observed associations between diet and serum hormones. Differences in the populations studied and the study designs could also have contributed to the disparate results. Gray et al. (50) reported no differences in hormone concentrations between vegetarian and nonvegetarian girls. However, in that study, the fat intakes between the two study groups did not differ. Finally, Wilson et al. (51) reported that Thai girls have lower progesterone levels than British girls, which the authors speculated could potentially be related to dietary differences.

At the year 1 visits, premenarcheal girls in the DISC intervention group had statistically significantly higher estrone sulfate concentrations than premenarcheal girls in the usual care group, although these two groups did not differ in their levels of other hormones. Because estrone sulfate levels were not measured at baseline for any of the girls in our study, we cannot rule out the possibility that this difference in estrone sulfate levels at year 1 between treatment groups pre-existed at enrollment into the DISC. Nevertheless, this difference was not sustained, and it reversed direction after menarche.

Onset of menses and Tanner stages are clinical signs of sexual maturation that reflect underlying changes in sex hormone concentrations. In contrast to the differences that we observed in serum hormone concentrations between girls in the intervention group and those in the usual care group, age at menarche and distribution of Tanner stages at each visit did not differ between the treatment groups. Dietary differences between the groups may not have been sufficient, or the treatment groups may not have been large enough, to allow us to observe delays in sexual maturation or menarche. Alternatively, the intervention may have been initiated when the girls were too old for it to affect onset of menses. In the Harvard Longitudinal Studies of Child Health and Development (52), age at menarche was associated with diet at 3–8 years of age.

The HAS had several strengths. First, it was conducted as part of a randomized, controlled clinical trial of children who were prepubertal at randomization. Second, it included almost 300 girls. Third, the day of the menstrual cycle when blood was collected was timed in relation to start of next menses on the basis of menstrual cycle calendars completed by girls. Fourth, hormone assays were performed using highly specific radioimmunoassays, and estradiol, estrone, estrone sulfate, and testosterone radioimmunoassays were preceded by a chromatographic purification step. Fifth, data were collected by trained personnel who were blinded to treatment assignment.

The HAS also has several limitations. First, girls were selected to participate in the DISC because they had elevated levels of LDL cholesterol, which could limit the generalizability of our results. Second, DISC girls were somewhat heavier than girls in the general population. Third, the median age at menarche of the girls enrolled in the DISC was slightly older than what we expected (53). However, this difference was likely due to the

eligibility criterion that girls be 8–10 years of age and prepubertal, which would have eliminated girls who matured early. Fourth, because blood was collected in the DISC without regard to day of the menstrual cycle, a cross-sectional analysis was conducted at each visit instead of conducting a longitudinal analysis across all visits. Fifth, a high frequency of missing data, particularly at baseline, further limited our study. However, because the primary reason for the missing baseline data was that most of the girls had completed their baseline visits before the HAS was initiated, we expect that missing baseline data decreased the power of our study but did not bias the results. Finally, because we performed multiple comparisons of numerous hormone measurements, it is possible that some statistically significant differences between treatment groups were due to chance alone. Alternatively, within-person variation in hormone levels, particularly the levels of estrogens and progesterone over the menstrual cycle, could have caused us to miss some “true” associations.

In summary, findings from the DISC HAS suggest that modest reductions in total fat, saturated fat, and possibly energy intake during adolescence result in differences in serum sex hormone concentrations that suggest alterations in the function of the HPO axis. Whether these differences ultimately influence breast cancer risk is currently unknown.

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NOTES

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