Effects of prune consumption on the ratio of 2-hydroxyestrone to 16α-hydroxyestrone[1–3]

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ABSTRACT

Background: A higher urinary ratio of the biologically inactive estrogen metabolite, 2-hydroxyestrone (2OHE1), to the biologically active metabolite, 16α-hydroxyestrone (16αOHE1), may be associated with a lower risk of breast cancer. High fiber intake is also associated with decreased breast cancer risk.

Objective: We investigated the effects of prunes, which are naturally rich in both soluble and insoluble fiber, on the concentrations of 2OHE1 and 16αOHE1 and on the ratio of 2OHE1 to 16αOHE1.

Design: Nineteen healthy premenopausal women consumed their habitual diets for 3 menstrual cycles and then consumed 100 g prunes/d for the next 3 cycles. Concentrations of urinary 2OHE1 and 16αOHE1 were determined during the follicular and luteal phases.

Results: Prune supplementation increased total and soluble fiber intakes by 4 and 2 g/d, respectively ($P < 0.001$). Mean (± SEM) luteal 2OHE1 excretion decreased from 3.92 ± 0.79 to 2.20 ± 0.40 nmol/mmol creatinine during the third cycle ($P = 0.017$). Luteal 16αOHE1 excretion decreased from 1.38 ± 0.24 to 0.87 ± 0.10 and 0.87 ± 0.15 nmol/mmol creatinine during the first and third cycles, respectively ($P = 0.018$ for both values). Follicular 16αOHE1 excretion decreased significantly only during the first cycle (from 0.82 ± 0.12 to 0.45 ± 0.09 nmol/mmol creatinine; $P = 0.005$). The 2OHE1-16αOHE1 ratio did not change significantly after prune supplementation.

Conclusions: Prune supplementation significantly decreased the excretion of 16αOHE1 during the follicular phase of the first menstrual cycle and during the luteal phases of both the first and third menstrual cycles. The 2OHE1-16αOHE1 ratio did not change significantly. The potential significance of the decrease in 16αOHE1 excretion, without a change in the 2OHE1-16αOHE1 ratio, on the prevention of estrogen-dependent cancers remains to be determined. *Am J Clin Nutr* 2002;76:1422–7.

INTRODUCTION

Estrogen exposure is a well-recognized risk factor for breast cancer (1–3). Research suggests that certain estrogen metabolites may also confer a risk of breast cancer (4). Metabolism of estrogens involves conversion of estradiol to estrone, which is then hydroxylated through 2 competing pathways to either 16α-hydroxyestrone (16αOHE1) or 2-hydroxyestrone (2OHE1) and 4-hydroxyestrone (4OHE1), which are catechol estrogens (5–8). 16αOHE1 retains its biological activity and therefore is considered a risk factor. Between the 2 catechol estrogens, 2OHE1 is the major metabolite. It is biologically inactive and may even have antiestrogenic activity. Thus, 2OHE1 does not promote estrogen-dependent cancers and may possibly protect against them. Therefore, a high ratio of the inactive metabolite, 2OHE1, to the active metabolite, 16αOHE1, is considered a favorable breast cancer risk profile (9, 10).

Several modes of intervention were used to try to increase the ratio of 2OHE1 to 16αOHE1. Among these, exercise (11), brassica vegetables (12), n−3 fish oils (10, 13), flax seed (14), and indole-3 carbinol (15, 16) successfully increased the 2OHE1-16αE1 ratio. However, there are conflicting reports about the effects of soy protein and isoflavonols (17–19).

The effects of dietary fiber on the 2OHE1-16αOHE1 ratio have not been conclusively established. High fiber intake is associated with low estrogen concentrations in plasma and urine and high concentrations in stool (20–30). Furthermore, this effect may be independent of the fat content of the diet (31). Nevertheless, dietary supplementation of insoluble fiber (cellulose and wheat bran) did not change the 2OHE1-16αOHE1 ratio (10, 14).

Fruits and vegetables, which are natural sources of dietary fiber, contain soluble as well as insoluble fiber. Animal experiments show that soluble but not insoluble fiber has protective effects against breast cancer (32, 33). In addition, phenolic compounds and xenoestrogens in fruits and vegetables increase the 2OHE1-16αOHE1 ratio by inducing the cytochrome P450 enzyme and increasing the production of 2OHE1 (15, 16).

In this study we investigated the effects of prune intake on urinary excretion of total estrogen conjugates, pregnanediol-3-glucuronide (PdG), 2OHE1, and 16αOHE1 and on the urinary 2OHE1-16αOHE1 ratio in healthy women with normal ovarian function. We hypothesized that prune intake may alter the metabolism of estrogens because prunes are a rich source of both soluble and insoluble fiber and cinnamates (34, 35) and decrease intestinal transit time (34). Because concentrations of estrogen metabolites may be affected by the menstrual

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cycle phase (36), estrogen metabolite concentrations were measured during both the follicular and luteal phases.

SUBJECTS AND METHODS

Experimental subjects

Twenty-four healthy, premenopausal women with regular menstrual cycles were recruited from the community. All of the women signed an informed consent form that was approved by the Human Subjects Committee of the University of California, Davis. Exclusion criteria consisted of a habitual dietary intake of < 30% of energy from fat or > 20 g fiber/d; habitual use of fiber supplements (ie, psyllium) or laxatives; pregnancy; irregular menses; hirsutism; polycystic ovary syndrome; use of birth control pills during the preceding 3 mo; intention to get pregnant during the time period of the study; systemic illnesses, such as renal, hepatic, and gastrointestinal illnesses; diabetes mellitus; hyperlipidemias; hypertension that required medication; smoking; and an alcohol intake of > 2 drinks/wk.

Study design

The duration of the study was 6 mo. After consuming their habitual diets for 3 menstrual cycles (control run-in period), the participants replaced dietary simple sugars with 100 g (≈12) prunes/d for another 3 menstrual cycles (intervention period). One-hundred grams of prunes contains 1004 kJ, 63 g carbohydrates, 2.6 g protein, < 1 g fat, 7.2 g fiber (3.8 g soluble and 3.4 g insoluble), and 78 mg phenolics (68 mg cinnamates). Prunes were consumed either directly or by adding them to various food items (salads, muffins, breads, cereals, and cakes). Intakes of energy, total carbohydrate, fat, protein, and other macronutrients and micronutrients were not changed.

Data collection

Nutrition assessment

Seven-day food records were obtained during the follicular phase of each menstrual cycle and analyzed by using the updated version of NUTRITION DATA SYSTEMS 93 (University of Minnesota, Minneapolis).

Anthropometric variables

Body weight was measured monthly. At the beginning and the end of the study, waist and hip circumferences and body composition were measured; the latter was measured by bioelectrical impedance analysis (Biostat, Isle of Man, United Kingdom). Because the results of bioelectrical impedance analysis may be influenced by the water content of the body, these measurements were obtained only during the follicular phase of the cycle (37).

Sex steroid hormones

Throughout the study (6 menstrual cycles) the participants collected their first morning urine sample every day. The samples for determinations of estrogen conjugates, PdG, and creatinine were collected in prelabeled 10-mL tubes, frozen immediately, stored in the participants' freezers, and delivered to the laboratory once a month. The urine samples for the 2OHE1 and 16αOHE1 assays were collected in ascorbic acid (an antioxidant) twice a month between the 5th and 8th days and the 19th and 23rd days of the menstrual cycle (follicular and luteal phases, respectively). The samples were then kept in the refrigerator and delivered on ice to the laboratory within 8 h of collection. All the urine samples collected during the first and last menstrual cycles of the control and prune supplementation periods (cycles 1, 3, 4, and 6) were assayed for estrogen conjugates, PdG, 2OHE1, and 16αOHE1.

Estrogen conjugates and PdG were measured by using competitive, microtiter solid-phase enzyme immunoassay methods (38). Urinary estrogen conjugate and PdG concentrations correlate very closely with plasma estradiol and progesterone concentrations, with a 1–2-d delay (38). Daily measurements of urinary estrogen conjugates and PdG provide more detailed information about sex steroid exposure than do measurements of plasma estradiol and progesterone once or twice a month (39). Estrogen conjugate and PdG concentrations were indexed to the creatinine concentration in the same urine sample. All of a subject's urine samples obtained during a single menstrual cycle were assayed in duplicate on the same plate. Urine samples in which the creatinine concentration was < 1.77 mmol/L were considered to be too dilute to yield accurate measurements, and these samples were considered as missing. None of the participants had more than 2 missing samples during a menstrual cycle. The sensitivity of the estrogen conjugate assay was 2.5 nmol/L, and that of the PdG assay was 0.48 μmol/L. The intraassay CVs for the high and low internal controls were 14.7% and 13.1%, respectively, for estrogen conjugates and 15.6% and 12.9%, respectively, for PdG. Cumulative estrogen and progesterone exposure was assessed by calculating the area under the curve for estrogen conjugates and PdG during each menstrual cycle by using the trapezoidal rule (40).

2OHE1-16αOHE1 ratio

2OHE1 and 16αOHE1 metabolites were measured in triplicate by using the ESTRAMET 2/16 kit (Immunacare, Bethlehem, PA). The intraassay CV was 4%, and the interassay CV was 10% (41, 42).

Statistical analysis

Of the 24 subjects who began the study, 5 dropped out because of either relocation (n = 2) or the inconvenience of daily urine collections (n = 3). Nineteen subjects with a mean (± SE) age of 40 ± 1 y provided at least 95% of the daily urine samples during the 6-mo study. Data from these subjects were analyzed by using the MIXED and CORRELATIONS procedures of SAS for TSO40 release 6.12 (43, 44). The participants served as their own controls. Data from the control months were averaged. Data from the intervention period were not averaged because the duration of intervention may affect hormonal response independently. Repeated-measures analysis of variance with an unstructured covariance matrix was used. Contrasts between the average of 2 control values and values from either the first or the last month of the study were used to test the significance of the early or late effects of the intervention. To adjust for the multiple comparisons (early and late changes from the baseline), the step-up Bonferroni procedure was used (45). A two-factor analysis of variance with time (baseline, early, and late), stage (follicular or luteal), and the interaction of time with stage was used to determine the significance of the interaction effects. The step-up Bonferroni procedure was used to determine which interaction was significant. To evaluate the relations between the change in fiber intake and estrogen metabolites, partial correlations were computed after accounting for the change in fat intake.

TABLE 1
Dietary macronutrient intakes before and after prune supplementation[1]

	t_0	t_1	t_2
Energy (MJ)	7.79 ± 0.43	7.86 ± 0.41	7.47 ± 0.26
Fat			
(g)	64 ± 6	62 ± 6	57 ± 5[2]
(% of energy)	31 ± 2	29 ± 2	28 ± 2[2]
Fiber (g)			
Total	20 ± 2	24 ± 2[3]	24 ± 2[3]
Soluble	7 ± 1	9 ± 0.4[4]	9 ± 0.4[4]
Carbohydrate			
(g)	255 ± 16	274 ± 14	266 ± 12
(% of energy)	55 ± 2	59 ± 2[3]	60 ± 2[3]
Starch (g)	111 ± 8	107 ± 9	101 ± 8
Sucrose (g)	45 ± 6	36 ± 4	35 ± 4[2]
Glucose (g)	25 ± 2	41 ± 2[4]	40 ± 2[4]
Fructose (g)	24 ± 2	29 ± 2[2]	28 ± 2[2]
Protein (g)	74 ± 5	70 ± 4	67 ± 4[2]

[1] $\bar{x}$ ± SEM; n = 19. t_0, t_1, and t_2, before and 1 and 3 mo after prune supplementation, respectively.
[2–4] Significantly different from t_0: [2] $P < 0.05$, [3] $P < 0.01$, [4] $P < 0.001$.

RESULTS

Anthropometric variables

Comparison of the values obtained at the end of the study (sixth mo) with those obtained at the beginning of the control period showed that weight (65.2 ± 2.1 compared with 64.3 ± 2.0 kg), body mass index (in kg/m^2; 23.9 ± 1.5 compared with 23.8 ± 0.8), percentage of body fat (29.6 ± 1.5% compared with 28.9 ± 1.5%), and waist-to-hip ratio (0.76 ± 0.02 versus 0.76 ± 0.01) did not change.

Dietary changes

These changes are shown in **Table 1**. Total energy intake did not change significantly. Despite the instructions to keep dietary fat constant, the percentage of energy from fat decreased significantly during the third month of prune supplementation (from 31% to 28%; P = 0.011). Simultaneously, the percentage of energy from carbohydrates increased from 55% to 60% (P = 0.002). Total fiber intake increased from 20 to 24 g, and soluble fiber intake increased from 7 to 9 g. These increases were significant ($P < 0.004$). As could be predicted from the carbohydrate content of prunes, although sucrose intake decreased significantly, intakes of glucose and fructose increased significantly. Protein intake also decreased significantly during the third month of prune supplementation.

Changes in urinary estrogen and progesterone metabolite concentrations

Changes in the urinary excretion of estrogen conjugates, PdG, 2OHE1, and 16αOHE1 are shown in **Table 2**. Prune ingestion did not significantly alter the urinary excretion of either estrogen conjugates or PdG.

When the excretions of 2OHE1 and 16αOHE1 were compared between the follicular and luteal phases of the menstrual cycle, 16αOHE1 excretion during the luteal phase was significantly higher than that during the follicular phase both at baseline (1.38 ± 0.24 compared with 0.82 ± 0.12; P = 0.01) and during the early prune supplementation period (0.87 ± 0.10 compared with 0.45 ± 0.09; P = 0.0002). Luteal 2OHE1 excretion was significantly higher than follicular 2OHE1 excretion only during the early prune supplementation period (2.52 ± 0.35 compared with 1.67 ± 0.20; P = 0.0035). The urinary 2OHE1-16αOHE1 ratio did not differ significantly between the luteal and follicular phases.

When the changes in urinary estrogen metabolite concentrations from baseline in the early and late periods of prune supplementation were compared, urinary excretion of 16αOHE1 decreased during the luteal phase in both the first and third months of supplementation (concentrations at baseline and during months 1 and 3 were 1.38 ± 0.24, 0.87 ± 0.10, and 0.87 ± 0.15 nmol/mmol creatinine, respectively; P = 0.018 for both periods) and during the follicular phase in the first month of supplementation (from 0.82 ± 0.12 to 0.45 ± 0.09 nmol/mmol creatinine; P = 0.005). 2OHE1 concentrations decreased during the luteal phase in the third month of supplementation (from 3.92 ± 0.79 to 2.20 ± 0.40 nmol/mmol creatinine; P = 0.017). These results indicate that prune supplementation altered estrogen metabolism more consistently during the luteal phase of the menstrual cycle than during the follicular phase. Although the actual concentrations of 2OHE1 and 16αOHE1 changed significantly, no significant changes were observed in the 2OHE1-16αOHE1 ratio.

Although this study was designed to maintain a stable fat intake, dietary fat decreased significantly during the third month. To assess the relations between the changes in fiber intake and the changes in estrogen metabolite concentrations independent of fat intake, partial correlations were computed after accounting for the changes in fat intake. During the first month of the study, the change in fiber intake tended to correlate inversely with the change in follicular 16αOHE1 concentration (r = −0.529, P = 0.052) and correlated directly with the follicular 2OHE1-16αOHE1 ratio (r = 0.587, P = 0.027).

DISCUSSION

Because conventional estrogens account for < 50% of circulating estrogens (46), it is possible that biologically active estrogen metabolites also confer a significant risk of estrogen-dependent cancers. This was originally proposed almost 20 y ago as the "unconventional estrogen hypothesis" (47). In support of this, several studies showed a relation between the incidence of breast cancer and a low 2OHE1-16αOHE1 ratio (9, 10, 48).

In the present study we investigated the effects of a natural food that provides both soluble and insoluble fiber. Earlier studies showed that the amount of prunes used in the present study also increases stool volume and decreases intestinal transit time (34). Because estrogen metabolites are subject to enterohepatic circulation (28–30), we postulated that prune supplementation may increase excretion of estrogen metabolites in the stool and therefore decrease them in the plasma and urine. Consistent with our hypothesis, urinary 16αOHE1 concentrations during the first month of prune supplementation decreased significantly during both the luteal and follicular phases of the menstrual cycle. After 3 mo, the decrease in urinary 16αOHE1 persisted only during the luteal phase. Urinary 2OHE1 concentrations also decreased significantly during the luteal phase in the third month of prune supplementation. The persistence of the decreased 16αOHE1 excretion during the luteal phase but not during the follicular phase may be related to the variance in their concentrations during a menstrual cycle. In agreement with a previous report (36), the 16αOHE1 concentrations of the subjects at baseline were higher during the luteal phase than during the follicular phase. Prune supplementation probably increased the intestinal clearance of

TABLE 2
Urinary excretion of estrogen conjugates (EC), pregnanediol-3-glucuronide (PdG), 2-hydroxyestrone (2OHE1), and 16α-hydroxyestrone (16αOHE1) and the ratio of 2OHE1 to 16αOHE1 before and after prune supplementation[1]

	t_0	t_1	t_2
EC_{AUC} (nmol/mmol creatinine)	577 ± 65	601 ± 72	543 ± 51
PdG_{AUC} (μmol/mmol creatinine)	2520 ± 256	2221 ± 197	1943 ± 161
2OHE1 (nmol/mmol creatinine)			
Follicular	2.20 ± 0.27	1.67 ± 0.20	2.03 ± 0.47
Luteal	3.92 ± 0.79	2.52 ± 0.35[2]	2.20 ± 0.40[3]
16αOHE1 (nmol/mmol creatinine)			
Follicular	0.82 ± 0.12	0.45 ± 0.09[4]	0.76 ± 0.14
Luteal	1.38 ± 0.24[2]	0.87 ± 0.10[2,3]	0.87 ± 0.15[3]
2OHE1:16αOHE1			
Follicular	2.83 ± 0.29	7.61 ± 2.00	3.44 ± 0.67
Luteal	3.21 ± 0.48	3.00 ± 0.29	3.31 ± 0.63

[1] $\bar{x}$ ± SEM; n = 19. AUC, area under the curve for one menstrual cycle; t_0, t_1, and t_2, before and 1 and 3 mo after prune supplementation, respectively.
[2] Significantly different from the follicular phase, $P < 0.05$, after a significant interaction of time and menstrual phase was found by using two-factor ANOVA and adjusting for multiple comparisons with the step-up Bonferroni procedure.
[3,4] Significantly different from t_0: [3] $P \leq 0.018$, [4] $P = 0.005$.

16αOHE1 throughout the menstrual cycle. This may have caused a compensatory increase in estrogen metabolism. This increase may have been adequate to negate the effects of prune supplementation during the follicular phase when estrogen concentrations are low. However, during the luteal phase, when plasma estrogen concentrations are higher, this compensatory increase may not have been adequate to restore urinary excretion of the estrogen metabolites to their baseline values.

Despite the decreases observed in 16αOHE1 and 2OHE1 excretion, there was no significant change in excretion of total estrogen conjugates. This may be because 2OHE1 and 16αOHE1 account for only 10–40% of total estrogen metabolites (46).

Despite the changes in the urinary concentrations of 2OHE1 and 16αOHE1, there was no significant change in the 2OHE1-16αOHE1 ratio. The lack of an increase in the 2OHE1-16αOHE1 ratio was due to the proportional changes in both metabolites. An increase in the 2OHE1-16αOHE1 ratio requires preferential hydroxylation of the A ring of estrone instead of the D ring. All the intervention studies to date showed that dietary fiber does not affect the relative efficiency of these 2 competing hydroxylation pathways (10, 14, 20, 21). Studies using insoluble fiber did not even show a decrease in the urinary excretion of either 2OHE1 or 16αOHE1 (9, 10). In the present study we were able to show decreases in the excretion of both of these metabolites possibly because we used a food item that contains soluble fiber and accelerates intestinal transit. Although our intervention was designed to increase fiber intake by 7 g/d, we achieved only a 4-g/d increase, which was divided equally between soluble and insoluble fiber. Our subjects had a relatively high baseline fiber intake (20 g/d) and apparently reduced their fiber intake from other sources.

Although our intervention was designed to keep dietary fat unchanged, we observed a gradual decrease in fat intake. Most of the interventions that showed decreased estrogen concentrations with increased fiber intake simultaneously reduced the fat intake (22–27). Furthermore, cross-sectional data showed that ratios of dietary fat to total fiber and to soluble fiber correlate directly with 2OHE1-16αOHE1 ratios (49). However, other research provides evidence that dietary fiber may have independent effects on estrogen metabolism (31). In our study, changes in estrogen metabolite concentrations preceded the decrease in dietary fat intake. In addition, during the third month of the study when the decrease in dietary fat became significant, estrogen metabolite concentrations did not decrease any further. Finally, during the first month of prune supplementation, the increase in fiber intake correlated with the decrease in follicular 16αOHE1 concentrations and with the increase in the 2OHE1-16αOHE1 ratio independent of the changes in fat intake. These observations suggest that the changes in estrogen metabolite concentrations were not due to decreased fat intake. One caveat remains, however: we did not determine fat absorption and thus cannot rule out the possibility that prune intake may have decreased the intestinal absorption of fat.

Certain foods that contain phenolics, indole glucosinolate, or lignans (ie, brassica vegetables and flax seed) seem to shift estrogen metabolism in favor of 2-hydroxylation and increase the 2OHE1-16αOHE1 ratio. Prunes are a rich source of cinnamates (35). Clearly, cinnamate intake did not influence these hydroxylation pathways. It should be noted, however, that in some studies the diet-induced increase in the 2OHE1-16αOHE1 ratio was very small (12), and whether an increase of this magnitude provides protection against breast cancer is not clear. In addition, hydroxylation of the A ring also results in the production of another catechol estrogen (4-hydroxyestrone) with carcinogenic potential (6–8). Thus, the overall consequence of inducing the hydroxylation of the A ring is not yet known.

Another puzzling finding of the clinical studies on the relation between breast cancer and the 2OHE1-16αOHE1 ratio is the variability of this relation. For example, in one study the 2OHE1-16αOHE1 ratio related to breast cancer risk directly in premenopausal women but inversely in postmenopausal women (50). Importantly, in the same study, urinary concentrations of both 2OHE1 and 16αOHE1 correlated directly with breast cancer risk regardless of menopausal status. In another study, the 2OHE1-16αOHE1 ratio was related to breast cancer risk only in postmenopausal women (48). Yet, in 2 other reports in postmenopausal women, breast cancer risk was not related to the 2OHE1-16αOHE1 ratio but was directly related to urinary concentrations of estrone, estradiol, and estriol (51, 52). Thus, it is not yet clear whether the concentrations of the individual estrogen metabolites or the 2OHE1-16αOHE1 ratio is a better predictor of breast cancer risk. If the former is a better predictor, the dietary intervention that we used may have potential preventive value.

Because our intervention was most effective during the luteal phase, we speculate that premenopausal women may benefit more than would postmenopausal women. However, if the 2OHE1-16αOHE1 ratio is a better predictor, then our intervention is not likely to be beneficial.

In conclusion, we showed that a natural food item that contains both soluble and insoluble fiber and accelerates intestinal transit reduces the urinary excretion of both 2OHE1 and 16αOHE1, especially during the luteal phase of the menstrual cycle, in healthy premenopausal women. We observed this effect even with a relatively high preintervention fiber intake and with relatively low fiber supplementation. Whether such an intervention would be more effective in women with lower habitual fiber intakes remains to be seen. The potential clinical significance of the decreased concentrations of individual estrogen metabolites without a change in the 2OHE1-16αOHE1 ratio needs to be determined in large prospective studies.

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