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EFFECT OF DIETARY COMPONENTS, INCLUDING LIGNANS AND PHYTOESTROGENS, ON ENTEROHEPATIC CIRCULATION AND LIVER METABOLISM OF ESTROGENS AND ON SEX HORMONE BINDING GLOBULIN (SHBG)

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Summary—A brief account of our present knowledge on the enterohepatic metabolism of estrogens and on the origin, metabolism and biological effects of mammalian lignans and phytoestrogens is undertaken. Furthermore, recently published results on the effects of dietary fiber, fat and carbohydrates on estrogen metabolism are reviewed. New preliminary results are presented on quantitative assays of lignans and phytoestrogens in urine of women belonging to various dietary and population groups and in a group of chimpanzees. The highest values of lignans and phytoestrogens were found in the non-human primates, and in macrobiotic, lactovegetarian and Japanese women, all groups considered having a low risk for the development of breast and other hormone-dependent cancer. New results on correlations between intake of various fibers, lignan and phytoestrogen excretion and plasma levels of estrogens, free testosterone and SHBG in women are presented. There is a significant positive correlation between the intake of fiber and urinary excretion of lignans and phytoestrogens, and the concentration of plasma SHBG. Fiber intake and urinary excretion of lignans and equol correlated negatively with plasma percentage free estradiol. Enterolactone excretion correlated negatively with plasma free testosterone. It is concluded that dietary macro- and micronutrients seem to play an important role in estrogen metabolism.

INTRODUCTION

Liver metabolism, biliary secretion, intestinal bacterial metabolism and reabsorption, including mucosal metabolism, are important steps in steroid metabolism in man and animals and play a significant role in the regulation of steroid levels in the organism. This is particularly true for the estrogens [1], which are excreted in high amounts in bile. In recent years evidence has suggested that dietary components like fiber and fat may play a role in the regulation of the enterohepatic metabolism of estrogens, in this way influencing the estrogen levels in the body. Furthermore, our diet contains compounds called plant lignans and phytoestrogens, which, after structural modification by intestinal bacteria to compounds with estrogenic and antiestrogenic activities, are absorbed into the circulation. These interesting compounds, when produced in sufficient amounts, may influence estrogen formation, metabolism and biological activity in the body. This brief review will be restricted to the above topics, and practically exclusively to estrogens, and will include some unpublished partly preliminary data on interrelations between dietary components, mammalian lignans and phytoestrogens, and plasma estrogens, free testosterone and sex-hormone-binding globulin (SHBG) levels.

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BILIARY EXCRETION AND INTESTINAL METABOLISM OF ESTROGENS

Between 20 and 50% of the estrogen metabolites are excreted in bile and reach the intestinal lumen practically exclusively in a biologically inactive conjugated form. The bulk of the estrogens occur in bile as polar metabolites in the form of glucuronides or sulfoglucuronides (see review in [1]). The content of the intestines is rich in β-glucuronidase mainly of bacterial origin, but some sulfatase also occurs. About 80% of the biliary conjugates are reabsorbed. However, a prerequisite for effective intestinal reabsorption of the estrogens is hydrolysis of the conjugates. Because of the abundance of hydrolytic enzymes in the intestinal lumen, usually less than 10% of estrogens produced in the body are excreted by the fecal route. The estrogens are mainly reconjugated in the intestinal mucosa, but are partly reabsorbed in the unconjugated form. These unconjugated estrogens are all biologically active. It is therefore obvious that any factors influencing the β-glucuronidase-producing bacteria and thus the concentration of β-glucuronidase in the intestinal contents may affect the reabsorption of estrogens and secondarily their level in blood.

There is at least one other factor in addition to hydrolysis of the conjugates contributing to an enhancement of the biologic activity of the biliary estrogens in the intestinal lumen. *In vitro* and *in vivo* studies have proven that a reductive metabolism is

Table 1. Estrogen mean ratios in bile and feces*

	Estrone/Estradiol		Estrone + estradiol/estriol	
	Bile†	Feces	Bile†	Feces
Young women	2.1	1.4	0.14	2.0
Postmenopausal women	3.0	1.4	0.27	1.8
Men	3.0	1.2	0.17	1.2

*Data from Refs [4, 5] and unpublished sources. Estrogens determined in bile by GC–MS (selected ion monitoring) and in feces by RIA after chromatography.
†Bile obtained by biliary T-tube drainage in cholecystectomized patients.

dominant in the intestines. Incubations of estrone (E1) and estradiol (E2) with mixed fecal flora or isolated bacteria have demonstrated that the main pathway is the reduction of E1 to E2 [2, 3], enhancing the biologic activity of E1 by a factor of about 10. In Table 1 it can also be seen that the ratios of E1/E2 and E1+E2/estriol(E3) are much higher in bile than in feces [4, 5], which indicates an intestinal reductive metabolism. This means that E1, which is one of the main metabolic products of ovarian E2 in the liver and which also is formed by aromatisation of androstenedione in peripheral tissues, is first conjugated in the liver mainly with glucuronic acid to estrone-3-glucuronide, then excreted in bile [6] and again reactivated to E2 in the gut by hydrolysis and reduction. The E2 thus formed may be reabsorbed and affect the level of E2 in blood and overall biologic activity of the estrogens in the body [7]. Evidence obtained in experiments with oral administration of antibiotics to women indicate that estrogen levels in the organism may be influenced by the activity of the intestinal microflora [7–10]. However, as we will see later, components of the diet also significantly affect the enterohepatic metabolism of estrogens.

EFFECT OF DIETARY FIBER ON ESTROGEN METABOLISM

In a collaborative study [11] with Dr Gorbachs' group at the Tufts University School of Medicine in Boston the diet was recorded 4 times in 1 yr in the midfollicular phase of 10 vegetarian and 10 non-vegetarian (omnivorous) premenopausal women living in Boston. Simultaneously, determinations of estrogens in plasma, urine and feces were carried out. The protocol for each sample period included complete 72-h collections of urine and feces, a 3-day food record, and 30 ml of blood drawn on each of the 3 days. The dietary fiber intake was only 12 g/day in the omnivorous women but 28 g/day in the vegetarians. The fat intake was slightly lower in the vegetarians but the difference was not statistically significant. The vegetarians had significantly higher fecal weight and a lower β-glucuronidase activity of fecal bacteria. We found a significant positive correlation between fecal weight and fecal excretion of estrogens. The plasma levels of E1 and E2 were negatively correlated with fecal estrogen excretion. Urinary E3 excretion was lower in the vegetarians due to a decrease in the excretion of E3-3-glucuronide, which is an almost exclusive product of the intestinal mucosal cells [1]. This demonstrates that the vegetarian fiber-rich diet partially interrupted the enterohepatic circulation of estrogens.

In another similar study carried out in Finland [12] the diet was recorded during 5 days, but this time twice yearly (winter and summer period). Thirteen urinary estrogens were measured by combined GC–MS in the selected ion monitoring mode (SIM) in 72-h urine samples in 11 lactovegetarian and 12 omnivorous women. In these groups the fiber intake differed very little from each other, the geometric mean values being 23 and 19 g/day in the vegetarian and omnivorous women, respectively. The consumption of total fiber or grain fiber per kilogram body weight correlated in the whole material (n = 23) negatively with the excretion of 10 of the 13 measured urinary estrogens [12], supporting our view that a high-fiber diet significantly affects estrogen levels in the body.

EFFECT OF DIETARY FAT ON ESTROGEN METABOLISM

In another collaborative study [13] with Dr Gorbachs' group in Boston we measured in the same way as described above twice yearly the dietary intake of nutrients and estrogen levels in plasma, urine and feces in 10 premenopausal and 12 postmenopausal Caucasian women living in Boston and 12 premenopausal and 9 postmenopausal Oriental women, who were recent emigrants from Southeast Asia to Hawaii. None of the subjects was a strict vegetarian. The main difference was in the fat intake, the Oriental women consuming only about 19–21% of their total calories as fat, which was about half that of the intake of the Caucasians. The percentage calories as carbohydrates was much higher in the Orientals. The Oriental women excreted more than twice the amount of estrogen in their feces but significantly less in their urine compared to the Boston women. The ratio of urinary to fecal estrogens was approximately 3–5 times higher in the young Caucasian women compared to the Oriental women. The main result was that in premenopausal

women there was a positive correlation between intake of total and saturated fat and plasma E1 and E2 concentrations. There was also a negative correlation between fiber intake and plasma E1 and E2 levels in these women, supporting the two earlier studies [11, 12] described above. The mechanism by which fat intake influences plasma estrogen levels is unknown. One possibility is increased reabsorption from the intestine because unconjugated E1 and E2 are rather non-polar fat-soluble steroids, the absorption of which may be enhanced by the presence of high concentration of fat in the intestinal content. This is supported by the observation of high excretion of estrogens in feces and low plasma estrogens in the Orientals consuming a low-fat diet [13]. In addition, the reabsorption of estrogens in the Orientals may be decreased due to a decrease in the β-glucuronidase activity of the intestinal contents because of the low-fat diet [14].

EFFECT OF DIETARY PROTEINS AND CARBOHYDRATES ON ESTROGEN METABOLISM

In a recent study by Anderson *et al.*[15] the effect of proteins and carbohydrates on estrogen 2- and 16α-hydroxylation was investigated in male subjects. When the subjects shifted from a high protein to a high carbohydrate diet (fat content was equal), 2-hydroxylation of E2 decreased significantly, but 16α-hydroxylation did not change at all. The latter result is in agreement with our observation that lactovegetarian and omnivorous Finnish women, with similar intake of fiber, had identical urinary excretion of estriol [12]. During the protein diet in the experiments of Anderson *et al.*[15], plasma half-life of antipyrine was low, indicating rapid metabolism. The oxidative metabolism of this drug is carried out by the cytochrome P-450-linked mixed function oxidases in the liver and the same seems to be true for both estrogen 2- and 16α-hydroxylations [15]. Interestingly, in contrast, the 4-ene-5α-reduction of testosterone in the liver decreases on a high-protein diet [16]. Similar metabolic alterations, as caused by the high-protein diet, can be induced in man by drugs such as phenobarbital and environmental chemicals [16].

LIGNANS IDENTIFIED IN MAN AND ANIMALS

In 1979 the cyclic excretion of two unknown phenolic compounds in animal and human urine during the menstrual cycle was detected [17–19]. In subsequent studies they were found to be hormone-like compound of plant origin structurally modified by intestinal bacteria (see below). Two groups of investigators [20, 21] simultaneously presented the structure of these compounds, now called animal or mammalian lignans, to differentiate them from plant lignans. The main compound is now named enterolactone (Enl) [*trans*-2,3-*bis*(3-hydroxybenzyl)-γ-butyrolactone] and its reduction product enterodiol (End) [2,3-*bis*(3-hydroxybenzyl)-butane-1,4-diol]. Recently also the common plant lignan matairesinol (Mat) [*trans*-2,3-*bis*(3-methoxy-4-hydroxybenzyl)-γ-butyrolactone], the immediate precursor of Enl, was identified in human urine [22]. Both Enl and Mat were identified also in cow milk and urine, in chimpanzee urine and in addition End was detected in low amounts in cow milk and high amounts in cow urine. Recently we also identified the lignans secoisolariciresinol (the immediate plant precursor of End), lariciresinaol and isolariciresinol in human urine [23–26].

ISOFLAVONIC PHYTOESTROGEN METABOLITES IDENTIFIED IN MAN AND ANIMALS

Isoflavonic phytoestrogens were the reason for massive outbreaks of infertility in sheep in Australia, when grazing formononetin-containing clover [27, 28]. Formononetin (4′-methoxy-7-hydroxyisoflavone) (For) (Fig. 1) is converted by ruminal bacteria to daidzein (Da) (4′,7-dihydroxyisoflavone), equol (Eq) (4′,7-dihydroxyisoflavan), and O-desmethylangolensin (O-Dma) [1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)-propan-1-one]. Eq is the main product and seems to be responsible for the infertility syndrome. It was first identified in urine of pregnant mares [29] and later also found in the urine of many other animals [28]. Recently Eq [30, 31], Da and O-Dma [22, 32] were identified in human urine and we now have definite evidence for the presence of genistein (Ge). Furthermore, we have tentatively identified two intermediates between Da and O-Dma and Da and Eq, respectively (Intermediate O and Intermediate E in Fig. 1), and 3′,7-dihydroxyisoflavan, an isomer of Eq, in human urine [26]. Both Eq and its isomer, and a new metabolite methylequol (MeOEq) were identified in cow milk [23]. MeOEq is an intermediate metabolite between For and Eq and represents a second pathway from F or to Eq in sheep [28] (Fig. 1). It is likely that in human subjects demethylation of MeOEq occurs, e.g. in the intestinal tract, resulting in the formation of Eq. Furthermore, we have recently identified biochanin A, Ge, For, Da, MeOEq and O-Dma in cow urine by GC–MS [26].

ORIGIN AND METABOLISM OF LIGNANS AND ISOFLAVONIC PHYTOESTROGENS IN MAN

It has been shown that human diet, especially grain and other fiber-rich food [33–35], contains plant lignans, which act as precursors for the structurally modified mammalian lignans, the modification being carried out by bacteria in the intestinal tract [36–40]. Secoisolariciresinol, which we recently identified in human urine, is one of the precursors and has been found in large amounts in linseed as a glycoside [38]. If linseed is added to the diet of rats or human subjects large amounts of Enl and End are excreted in urine. When Mat, also identified in human

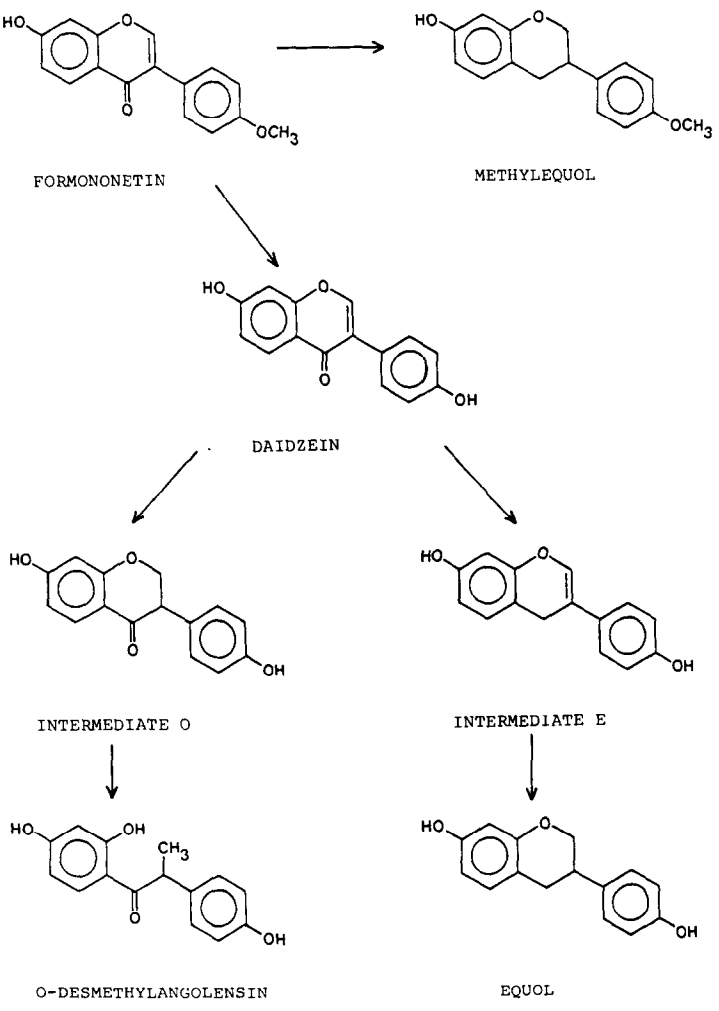

Fig. 1. Metabolism of isoflavonic phytoestrogens in man.

urine [22], is added to rat diet, Enl is excreted in urine [40]. Germ-free rats as opposed to conventional rats do not excrete any lignans in urine [37]. Administration of antibiotics to human subjects results in a dramatic decrease in the excretion of lignans in urine and feces [35, 36]. When End was administered orally to rats. Enl was excreted in urine, but if these experiments were carried out in germ-free rats or if End was administered intraperitoneally to bile-fistula rats, no Enl was detected [38].

The mode of formation of isoflavonic phytoestrogen metabolites in man seems to be similar to that in sheep. Administration of soya protein results in a marked increase in the excretion of Eq, which is a metabolite of Da found as a glycoside in soya flour [28, 41]. Some subjects may not be able to form Eq [41] and germ-free rats do not excrete Eq when given commercial pellet food [37]. O-Dma seems to be a minor metabolite in man [35]. Da and Eq are present also in cow milk [23, 24] and it therefore seems likely that the urinary isoflavonic phytoestrogen metabolites are in man, as in sheep and rat, of dietary origin. Most likely Eq is formed by intestinal bacterial action from Da and perhaps also from For present in food. However, until now we have found For only in cow urine, but not in human urine. The proposed pathways for the formation of equol and other phytoestrogen metabolites found in human urine are depicted in Fig. 1.

BIOLOGICAL EFFECTS OF LIGNANS AND PHYTOESTROGENS

The lignans and the isoflavonic phytoestrogens all have a diphenolic structure resembling those of the very potent synthetic estrogens stilbestrol and hexestrol. However, the lignans End and Enl bind only very weakly to rat uterine cytosol receptor (Ref. [42] and J.H. Clark and H. Adlercreutz, unpublished observation), and have no estrogenic activity *in vivo*

in mice [36]. However, recently *in vitro* experiments showed that Enl exhibits weak estrogenic activity and stimulates the growth and synthesis of progesterone receptors in breast cancer cells (no E2 present) [43, 44]. Da, Eq and O-Dma have been found to bind to estrogen receptors and to have weak estrogenic activity [45, 46]. As both Eq and Da as well as Enl show in some experimental conditions weak estrogenic activity they may at certain concentrations act as antiestrogens by inhibiting binding of estradiol to its receptor. In *in vitro* experiments, using stimulation of prolactin synthesis in primary rat pituitary cultures and of progesterone receptor in primary rat uterine cells, it was observed that the weak estrogenic effects produced by a concentration of 10 μmol/l of Enl and 0.3 μmol/l of Eq could be inhibited by the antiestrogen Tamoxifen [43, 44]. However, *in vivo* Enl inhibited estrogen-stimulated RNA synthesis in rat uterine tissue when administered 22 h before estradiol [47]. Furthermore, in clover disease of the sheep, the ewes are made permanently infertile by estrogenic clover containing For, which is converted to Da and Eq by ruminal micro-organisms [27, 28, 45]. The hypothalami of the affected ewes show relative insensitivity to estradiol [48]. It was shown that Eq receptor complex competes well with estradiol receptor complex for nuclear binding and yet fails to initiate the replenishment of estrogen receptors effectively in the cytoplasm [46], which suggests that this compound can act as an antiestrogen. If Eq and E2 were injected simultaneously the effect on rat uterine growth was less than that observed if E2 alone was injected [46]. These results are in concordance with those observed for Enl with regard to RNA synthesis in the uterus of rats [47]. All diphenols are weak antioxidants and some plant lignans are anticarcinogenic [34], which may have some positive anticancer effect particularly in the intestine [34]. It has been suggested that the mammalian lignans may be carcinogenic because of their phenolic structure [49]. As judged from binding inhibition studies using [^{3}H]12-0-tetradecanoylphorbol 13 acetate (TPA) with a mouse skin particulate fraction (T. Horiuchi, H. Fujiki and H. Adlercreutz, unpublished results) the mammalian lignans and phytoestrogens up to a concentration of 100 μmol/l have no tumor-promoting or -inhibiting effect. Furthermore, it has been shown that Da, Ge, For and biochanin A are all non-mutagenic when screened in the Salmonella/mammalian microsome assay [28].

Recent studies in collaboration with Dr L. Vickery (to be published) show that Enl is a moderate inhibitor of placental aromatase, can pass freely into the cell (JEG-3 human choriocarcinoma) and show intracellular aromatase-inhibiting activity. Enl binds to or near the substrate region of the active site of the P-450 enzyme and the inhibition is competitive with respect to the substrate androstenedione.

RESULTS OF QUANTITATIVE ASSAYS OF LIGNANS AND PHYTOESTROGENS IN DIFFERENT POPULATION GROUPS AND IN BREAST CANCER

In man, lignans and phytoestrogens have been assayed mainly in urine, and some few assays of lignans were also carried out in blood (Refs [50, 51] and unpublished), semen [50] and feces (Ref. [36] and unpublished). In connection with the large studies described above on the effect of diet on estrogen and androgen metabolism carried out in Boston and Helsinki some of the urine samples were also used for the assay of lignans and phytoestrogens. It was demonstrated that in postmenopausal breast cancer patients the mean lignan and Eq excretion was lower (not statistically significant for Eq) compared to control omnivorous and vegetarian women [31]. Figure 2 shows some preliminary data with regard to

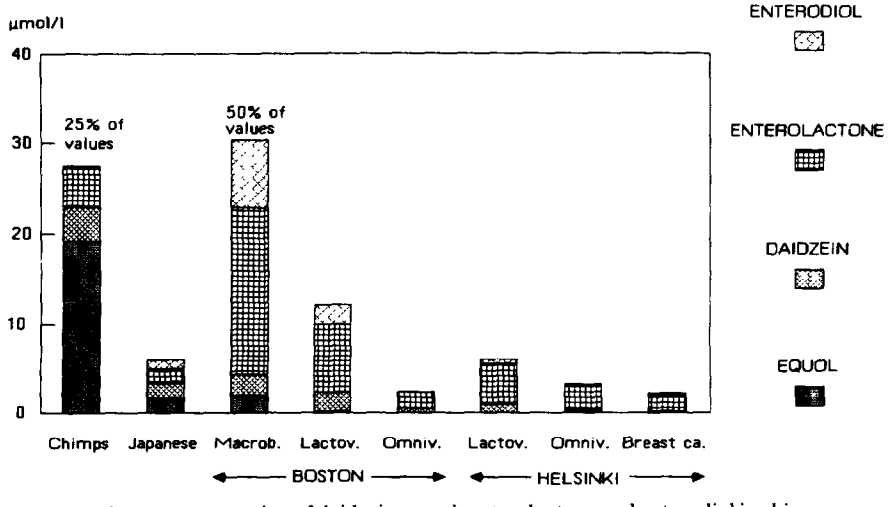

Fig. 2. Mean urinary concentration of daidzein, equol, enterolactone and enterodiol in chimpanzees, and in young women on various habitual diets. The values shown for chimpanzees are 25% of the actual values and those for the macrobiotics are 50% of the actual values.

urinary concentration of total lignans and phytoestrogens in young macrobiotic ($n = 13$), lactovegetarian ($n = 11$) and omnivorous ($n = 10$) Boston women and young lactovegetarian ($n = 12$) and omnivorous ($n = 12$) women living in the Helsinki area, including also a group of breast cancer patients ($n = 12$). Furthermore, a group of young women ($n = 7$) living in Japan and consuming their traditional diet (studied in collaboration with Dr H. Honjo, to be published) and a group of male chimpanzees ($n = 6$) in captivity consuming their normal diet (Ref. [25] and to be published) are included. Male chimpanzees were chosen to eliminate menstrual cycle effects on the excretion of the diphenols [17–21]. The chimpanzees were included because nonhuman primates appear to be remarkably resistant to the carcinogenic effect of estrogens [25]. The results were expressed in nmol/l because 24-h collections could not be carried out for the chimpanzees.

Very high urinary concentrations, particularly of the phytoestrogens, were observed in the chimpanzees, but also in the macrobiotics. The Japanese women had frequently high concentrations of Eq and Da and the lactovegetarians in Boston and Helsinki excreted relatively much Da. The highest concentration of Enl and End was found in the macrobiotic women living in Boston followed by the lactovegetarians in Boston, the chimpanzees and the Finnish lactovegetarians. The lowest concentrations of all compounds were found in the Boston omnivorous and Finnish breast cancer women. These two groups had also the lowest mean intake of total fiber in diet, 11 g/day and 14.5 g/day, respectively. Thus both young breast cancer patients in Helsinki and old ones in Boston [31] excrete lower amounts of these diphenols in urine compared to controls.

CORRELATIONS BETWEEN INTAKE OF VARIOUS DIETARY COMPONENTS, LIGNANS AND PHYTOESTROGENS AND PLASMA LEVEL OF STEROIDS AND SHBG

In previous studies in women living in Boston it has been found that there is a correlation between fiber intake and excretion of lignans in urine [33] and between the intake of grain calories and urinary Enl excretion [31, 35]. Furthermore, it is known that intake of soya products increases excretion of equol in urine in most individuals [30, 41]. In the same Finnish women used in the earlier study [12], including also a group of breast cancer patients (totally 34 subjects), the intake of fiber from berries and fruits correlated with lignan excretion ($P < 0.01$) and that of vegetable fiber with excretion of Da ($P < 0.05$). The intake of grain fiber was much greater than in the women living in Boston and the differences between the individuals were much smaller. In the Finnish women the intake of total fiber correlated positively with urinary excretion of Enl, End, Da and total lignans ($P < 0.05$–< 0.01). The lowest fiber intake was found in the breast cancer group (14.5 g/day) and it differed almost significantly from the intake of total fiber in the omnivorous women (18.5 g/day; $P < 0.07$) and highly significantly from that of the lactovegetarians (23.1 g/day; $P < 0.002$). In Table 2 some correlations obtained in the same material between excretion of lignans and phytoestrogens and plasma estrogens and SHBG are shown. In these young women we found positive correlations between the excretion of Enl, total lignans and total diphenols, with plasma level of SHBG, and negative correlations with percentage free E2 (% Free E2). End correlated negatively with free E2 concentration in plasma.

The above study in young Finnish women is not yet completed, because only two of the four collection periods have been analysed with regard to lignans and phytoestrogens using a highly specific GC–MS method [35]. We have, however, analyzed almost the whole material using a less sensitive capillary GC method measuring only the two lignans and Eq, and values have also been obtained for old women. These results have never been published, because the new methodology gives much more information. The total material consists of 62 women studied 4 times during 1 yr. Each time diet was recorded for 5 days and 72-h urine collections were made and 3 plasma samples were taken on 3 consecutive days. Multiple correlation analysis showed some interesting preliminary results, supporting those obtained with the more sophisticated method in the young women (Table 3). The correlations shown are Pearson's simple correlations and partial correlations after removing the linear effect of age and weight. Intake

Table 2. Partial correlations* found between the excretion of lignans in urine, and plasma estrogens and SHBG. Preliminary results in young omnivorous and lactovegetarian women including breast cancer patients living in the Helsinki area ($n = 34$)

Compound	SHBG		% Free E2		Free E2	
	r	P	r	P	r	P
Enterolactone	0.389	< 0.05	−0.395	< 0–05		
Enterodiol					−0.355	< 0.05
Total lignans	0.404	< 0.05	−0.404	< 0.05		
Total diphenols	0.458	< 0.01	−0.463	< 0.01		

*The linear effect of body weight was eliminated.

Table 3. Preliminary results of multiple correlation analysis of relations between intake of various fiber, urinary excretion of lignans and phytoestrogens and plasma steroids and SHBG in pre- and postmenopausal women living in the Helsinki area. Each subject was studied 4 times during 1 yr and the geometric mean values were used for calculations. Dietary records were obtained each time during 5 days and each time 3 plasma samples were taken during 3 consecutive days and 72-h urines collected

Food component or diphenol	Hormone or diphenol	No. of pairs*	R†	P†	No. of pairs*	R‡	P‡
Vegetable fiber	U-Enterolactone	50	0.334	< 0.05	48	0.324	< 0.05
Vegetable fiber	U-Enterodiol				48	0.287	< 0.05
Vegetable fiber	P-SHBG	62	0.255	< 0.05	48	0.393	< 0.01
Vegetable fiber	P-% Free estradiol				48	−0.405	< 0.01
Berries and fruit fiber	U-Enterolactone	50	0.472	< 0.001	48	0.508	< 0.001
Berries and fruit fiber	U-Enterodiol				48	0.375	< 0.01
Berries and fruit fiber	P-SHBG	62	0.270	< 0.05	48	0.391	< 0.01
Legume fiber	U-Enterolactone				48	0.309	< 0.05
Legume fiber	U-Enterodiol				48	0.349	< 0.01
Legume fiber	P-Free testosterone				48	−0.288	< 0.05
Total fiber	U-Enterolactone	50	0.480	< 0.001	48	0.527	< 0.001
Total fiber	U-Enterodiol				48	0.414	< 0.001
Total fiber	P-SHBG	62	0.281	< 0.01	48	0.362	< 0.01
Total fiber	P-% Free estradiol				48	−0.364	< 0.01
U-Enterolactone	P-SHBG	54	0.356	< 0.01	48	0.316	< 0.05
U-Enterolactone	P-Free testosterone	54	−0.477	< 0.001	48	−0.335	< 0.01
U-Enterolactone	P-% Free estradiol				48	−0.433	< 0.001
U-Equol	P-SHBG				48	0.296	< 0.05
U-Equol	P-% Free estradiol				48	−0.350	< 0.01
Grain fiber/kg body wt	Total lignans	50**	0.333	< 0.05			
Grain fiber/kg body wt	U-E-lactone + U-equol	50**	0.331	< 0.05			
Total fiber/kg body wt	Total lignans	50**	0.707	< 0.001			
Total fiber/kg body wt	P-SHBG	62**	0.427	< 0.001			
U-Enl + U-equol	P-SHBG	54**	0.700	< 0.001			

*Calculations made with the logarithmic values. **Calculations made with arithmetic values.
†Simple Pearson's correlations.
‡Partial correlations eliminating the linear effect of age and body weight.
Abbreviations: U, urine; P, plasma; EH-lactone, Enterlactone; SHBG, sex-hormone-binding globulin.

of total fiber, vegetable fiber, fiber from berries and fruits and legume fiber correlated with urinary Enl and End and plasma SHBG (not for legume fiber). Intake of vegetable fiber and total fiber showed inverse correlations with plasma percentage free estradiol and intake of legume fiber an inverse correlation with plasma free testosterone. Urinary Enl and Eq excretion correlated positive with SHBG and negatively with percentage free E2. Furthermore, there was a highly significant negative correlation between urinary Enl excretion and plasma free testosterone. Some other interesting correlations were almost significant ($0.05 < P < 0.10$). Of these may be mentioned negative correlations between lignan and phytoestrogen excretion, and plasma E1 and E2 levels.

DISCUSSION AND CONCLUSIONS

Previous and recent studies thus suggest that fiber-rich low-fat food in addition to its reducing effect on estrogen levels in blood and urine [11–13], at least partly due to interference with reabsorption of biliary estrogens, may have some other effects on estrogen metabolism. Due to the presence of lignan precursors and phytoestrogens in fiber-rich vegetables, legumes and grain, a diet rich in fiber may, via production of mammalian lignans, Eq and other weak estrogens in the intestinal tract, stimulate SHBG synthesis in the liver and may in this way reduce the levels of free estradiol and testosterone in plasma. It is well known that oral estrogens in contradiction to parenterally administered ones markedly stimulate SHBG synthesis [52, 53] and it is therefore not unlikely that these diphenolic weakly estrogenic compounds entering the portal circulation in very high amounts have such a stimulatory effect. This may explain the higher SHBG-values seen in vegetarians consuming fiber-rich food. Furthermore, high concentrations of diphenols in peripheral tissues like fat tissue may inhibit the aromatase enzyme reducing the conversion of androgens to estrogens. In addition to the reduced levels of free hormones, suggested to be caused by the mechanisms described above, diphenolic weak estrogens may compete with endogenous E2 at the cellular level [28, 46]. We suggest that at least some of these mechanisms may be responsible for the lower risk for hormone-dependent cancer observed in vegetarian or semivegetarian populations and in non-human primates. Further studies are necessary in order to obtain more evidence with regard to possible cause-effect relationships.

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