Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects

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Quantitative data on phyto-oestrogen, particularly lignan, content in edible plants are insufficient. We, therefore, measured isoflavonoids and lignans in nine edible berries using an isotope dilution gas chromatography–mass spectrometry method for foods and found substantial concentrations of the lignan secoisolariciresinol (1·39–37·18 mg/kg DM), low amounts of matairesinol (0–0·78 mg/kg DM) and no isoflavones. To determine pharmacokinetics and urinary excretion pattern of the mammalian lignan enterolactone derived from plant lignans, a study with human subjects was conducted. Five healthy women and two men consumed, after a 72 h period of a phyto-oestrogen-free regimen, a single strawberry-meal containing known amounts of plant lignans. Basal and post-meal blood and urine samples were collected at short intervals. The samples were analysed using time-resolved fluoroimmunoassay of enterolactone. The meal increased plasma concentration of enterolactone after 8–24 h and in urine in the 13–24 h and 25–36 h urine collections. High individual variability of the metabolic response was observed. Enterolactone excreted in the urine collected throughout the 48 h post-meal yielded on average 114 % of the plant lignans consumed. It is concluded that berries containing relatively high concentrations of plant lignans contribute to plasma and urinary levels of mammalian enterolactone in human subjects.

Berries: Phyto-oestrogens: Kinetics

Epidemiological investigations in human subjects have provided evidence linking diet to incidence of cancer (e.g. breast, prostate cancer) and other degenerative disorders (e.g. CHD, postmenopausal osteoporosis). The detection and identification in human subjects of lignans and isoflavonoids, plant-derived oestrogenic compounds abundant in plasma of subjects living in areas with low cancer incidence, have challenged studies on their metabolic pathways and role in human health and disease.

Despite laboratory research efforts revealing interesting properties of phyto-oestrogens as inhibitors of carcinogenic and atherosclerotic processes, there is a dearth of studies on their presence in terms of quantity and quality in the human diet. Very little is known about the fate of lignans in common foods after ingestion in human subjects. There is a single study in rats, however, in which food lignan content was measured and then urinary levels of mammalian lignans were measured following ingestion (Landström *et al.* 1998).

As early as in the middle of the 1980s, it was suggested (Adlercreutz *et al.* 1986*a*, 1987, 1988) that berries and fruits containing plant lignans may be the precursors of mammalian enterolactone (ENL) and enterodiol (END). This was based on correlations found between dietary intake of fibre from berries and fruits, and urinary excretion of mammalian lignans END and ENL. In order to verify this suggestion we developed the isotope dilution gas chromatography–mass spectrometry method (ID-GC–MS) for food samples and analysed both lignans and isoflavones in some edible berries. The present paper describes for the first time concentrations of plant oestrogens in nine different types of berries.

The urinary excretion of lignans (Fig. 1) has been the most frequently studied aspect of their metabolism (Adlercreutz *et al.* 1982, 1986*b*, 1988, 1991*a*). Urinary lignan response to consumption of linseed, which contains over 200-fold more of the ENL precursor secoisolariciresinol (SECO) (Mazur *et al.* 1996) than berries, has been investigated in some long-term studies in urine (Shultz *et al.*
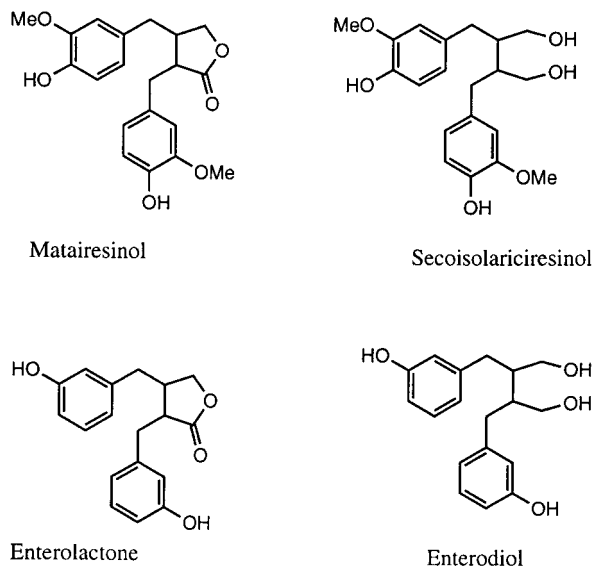
Fig. 1. Structures of plant and mammalian lignans.

1991; Lampe *et al.* 1994; Cunnane *et al.* 1995) and faeces (Kurzer *et al.* 1995). Our previous investigations on lignans (and isoflavonoids) in plasma (Adlercreutz *et al.* 1993, 1994) allowed us to assess normal concentrations of these phyto-oestrogens in Finnish omnivorous and vegetarian women who consumed their habitual diets. Morton *et al.* (1994) reported plasma levels of lignans (and isoflavonoids) for postmenopausal women consuming a traditional diet supplemented with linseed, soyabean flour or clover sprouts. Brzezinski *et al.* (1997), studying the effects of a phyto-oestrogen-rich diet on menopausal symptoms in women, examined basal and end-of-study levels of phyto-oestrogens in serum without measuring the dietary content of plant oestrogens. In none of these investigations have the basal and post-meal levels of lignans in blood and urine of human subjects been measured after a challenge with food with known lignan-precursor concentration. In the present investigation we studied the kinetics of ENL for 48 h after a strawberry meal in human subjects.

Materials and methods

Subjects

Healthy subjects (*n* 7) were recruited to the experiment based on an oral interview with the project leader. All but one man (H.A.) were employed by the Helsinki University Department of Clinical Chemistry and were technicians, or medical or chemistry students. None were taking any medication and none had had any antibiotics during 3 months preceding the sampling period. The subjects' ages ranged from 23 to 55 (mean 38·1) years and BMI from 18·8 to 26·0 (mean 23·3) kg/m^2.

Berry samples

The berries studied were: strawberry (*Fragaria × ananassa*), blackberry (*Rubus fructicosus*), raspberry (*Rubus idaeus*) and cloudberry (*Rubus chamaemorus*) of the family

Rosaceae; blackcurrant (*Ribes nigrum*), redcurrant (*Ribes rubrum*) of the family *Grossulariaceae*; and cranberry (*Vaccinium oxycoccos*), lingonberry (*Vaccinium vitis-idaea*), bilberry (*Vaccinium myrtillus*) and blueberry (*Vaccinium corymbosum*) of the family *Ericaceae*. The berries were purchased from commercial sources. Strawberries for consumption by the subjects of the study (5 kg) were brought from a farm in central Finland. These strawberries were divided into ten portions of 500 g (wet weight) and frozen at −20° until consumed. Before phyto-oestrogen analysis, the berry samples were dried in the oven (45–50°, 4–5 d) and pulverized manually with a mortar. DM was determined with a Moisture Analyzer 40 (Sartorius AG, Göttingen, Germany).

Chemicals, standards and instruments

The berries were analysed using our ID-GC–MS in the selected-ion-monitoring mode (SIM) method for the determination of lignans and isoflavonoids in food samples. All chemicals and details of the synthesis of standards and the validity of the method have been presented (Mazur *et al.* 1996). Time-resolved fluoro-immunoassay of plasma and urine ENL was performed as described in detail by Adlercreutz *et al.* (1998*a*) using the following instruments, all purchased from Wallac, Turku, Finland: a Victor 1420 multilabel counter and Wallac 1420 Victor software version 1.0 for data analysis. Microtitration strips were washed and shaken with 1296-026 DELFIA platewasher and 1296-003 DELFIA plateshaker respectively. Radioactivity counting was performed with a LKB Model 1217 Rackbeta Liquid Scintillation Counter. White microtitration strips coated with goat anti-rabbit IgG were also products of Wallac.

Study design

The study design was approved by the ethics committee of the Helsinki University Central Hospital. Detailed written dietary instructions and a list of forbidden food items were given to the participants at least 1 month before starting the experiment. The subjects were requested to exclude from their diet any food items containing phyto-oestrogens for 3 d (72 h) before the consumption of the berry meal and during the collection of plasma and urine samples (the following 48 h). The participants avoided most vegetables and fruits, legumes, seeds and nuts, berries, grains, whole-grain products, and tea, beer and wine. Consumption of certain wheat products (white bread, pasta), milk products (milk, yoghurt, cheese), meat and fish, a few vegetables (e.g. potato) and fruits (e.g. an apple, a pear) was allowed so that, in principle, the intake of vitamins and macronutrients was unchanged. The subjects began this restricted (zero) diet 3 d before the experiment (D1) and followed it throughout the experiment (D5). On day 3 (D3), starting after the first morning void, participants collected one 24 h urine sample ('zero' urine), including the first morning void on day 4 (D4). Daily urine was collected into 2 litre plastic bottles containing 2 g ascorbic acid and stored at +4° until the collection was completed. Total urine volume was measured and portions were stored at −20° until analysis. On D4 at

08.00 hours, a vascular catheter was introduced to a median cubital (or basilic) vein and the first blood sample ('zero' plasma, four 10 ml tubes) was taken. Thereafter subjects consumed 500 g strawberries. Following the strawberry meal the 24 h urine sample was collected in four portions: 4 h + 4 h + 4 h + 12 h. Blood samples (4 × 10 ml) were taken through the catheter at the following intervals: 0·5 h, 1 h, 2 h, 4 h, 8 h after the berry meal. The last blood sample was taken on the morning of the next day (D5) (24 h after the meal). After centrifugation of the tubes, plasma (15–20 ml) was separated and stored at −20° until analysis. On D5 urine sample collection was continued in two portions (12 h + 12 h) per d. Total urine collection obtained in four (D4) and two (D5) portions was measured and stored at −20° until analysis.

The subject K.L. collected D5 urine mistakenly in one portion, 25–48 h, instead of in two portions (25–36 h and 37–48 h).

Analytical methods

Isotope dilution gas chromatography–mass spectrometry in the selected-ion-monitoring mode method for food samples. The method for the determination of lignans, isoflavones and coumestrol in berry samples is described briefly here. Dried and powdered berry samples were hydrolysed with an enzyme mixture (*Helix pomatia*; Bio Sepra Inc., Marlborough, MA, USA) and acid (HCl), extracted with diethyl ether and the extract subsequently was purified on DEAE- and QAE-Sephadex columns (Pharmacia Fine Chemicals, Uppsala, Sweden). Formononetin, daidzein, biochanin A, genistein (isoflavones) and coumestrol, as well as SECO and matairesinol (MAT) (lignans) were derivatized to form trimethylsilyl ethers and analysed by the ID-GC–MS-SIM method (Mazur *et al.* 1996) using synthesized deuterated internal standards for the correction of losses during the procedure. All assays were carried out in duplicate or quadruplicate.

Time-resolved fluoroimmunoassay methods for enterodiol in plasma and urine. The analytical procedure is briefly as follows (Adlercreutz *et al.* 1997, 1998*a,b*). After addition of the radioactive internal standard ^{3}H-oestradiol glucuronide (only for plasma), hydrolysis reagent (0·2 U/ml β-glucuronidase and 2 U/ml sulfatase in 0·1 mol/l acetate buffer, pH 5·0) was added to the plasma or urine sample which were mixed and then incubated overnight at 37°. For plasma samples diethyl ether was used to extract free (unconjugated) ENL after the hydrolysis; for urine no extraction was used. Then the hydrolysed and extracted plasma or urine samples in assay buffer (Tris-HCl buffer containing 5 g BSA/l, pH 7·8) were pipetted into prewashed goat anti-rabbit IgG microstrips. Simultaneously antiserum (dilution 1 : 250 000) and Eu-labelled ENL (dilution 1 : 400 000) in the assay buffer were added into the microstrips. After incubating and shaking the strips slowly on a DELFIA plate shaker (Wallac) at room temperature for 90 min, the strips were washed with a DELFIA plate washer (Wallac). Subsequently DELFIA enhancement solution (Wallac) was added to each well and the strips were shaken slowly for an additional 5 min. Fluorescence was read in the DELFIA Victor multilabel counter (Wallac). Results were calculated according to the formula:

Plasma ENL = concentration (read)

× 1/recovery × dilution factor,

Urine ENL = concentration (read) × dilution factor.

Statistics

Values in the text were expressed as means with pooled standard errors. After one-way ANOVA, the data were assessed using Duncan's multiple range test. Differences were considered significant at $P < 0.05$. All the statistical analyses were performed with the SPSS statistical software package (Version 6.1J, SPSS, Chicago, IL, USA).

Results

The lignan concentrations in eight types of berries grown in Finland are shown in Table 1. None of the isoflavonoids in these samples was measurable with our ID-GC–MS-SIM technique, mainly due to their low concentrations and some interfering compounds at these very low (trace) levels. However, relatively high levels of the lignan SECO were

Table 1. Phyto-oestrogen content of berries (μg/kg DM)*

Berry	Daidzein	Genistein	SECO†	Matairesinol
Rosaceae				
Blackberry (*Rubus fructicosus*)	Tr	Tr	37180	225
Strawberry (*Fragaria× ananassa*)	Tr	Tr	15046	781
Cloudberry (*Rubus chamaemorus*)	0	0	2030	0
Raspberry (*Rubus idaeus*)	0	Tr	1390	0
Ericaceae				
Lingonberry (*Vaccinium vitis-idaea*)	0	0	15100	0
Cranberry (*Vaccinium oxycoccos*)	0	0	10540	0
Blueberry (*Vaccinium corymbosum*)	0	0	8350	0
Grossulariceae				
Blackcurrant (*Ribes nigrum*)	0	0	3880	95
Redcurrant (*Ribes rubrum*)	0	0	1653	0

SECO, secoisolariciresinol; Tr, trace (present in trace amounts; over 50–60 % of detection limit).
* Analysis by isotope dilution gas chromatography–mass spectrometry in the selected-ion-monitoring mode.
† Total amount of anhydrosecoisolariciresinol and SECO.

Table 2. Enterolactone in plasma (nmol/l) and urine (nmol/l) before and after consumption of the strawberry meal

(Values for individual subjects and mean values with pooled standard errors)

Time of plasma collection (h after strawberry meal)	Urine collection Time (h)		Subject								
			A.S.	I.W.	W.M.	T.N.	H.A.	R.T.	K.L.	Mean	SEM
0		Plasma	10·6	8·3	1·7	11·0	4·7	13·5	22·4	10·3*	2·5
	Day 3; 24	Urine	686·8	849·3	303·6	608·9	2413·1	2470·3	3128·4	1494·3	429·2
0·5		Plasma	7·9	11·7	1·1	12·1	4·2	11·3	21·0	9·9*	2·4
	Day 4; 0–4	Urine	697·8	782·7	99·8	523·0	440·3	1447·2	2324·6	902·2	283·6
1		Plasma	6·9	11·3	1·5	11·5	4·0	11·7	24·1	10·1*	2·8
	Day 4; 5–8	Urine	719·3	659·0	586·5	941·8	251·6	786·7	2293·6	891·2	247·2
2		Plasma	8·8	9·1	1·5	10·4	4·2	10·3	19·8	9·2*	2·2
	Day 4; 9–12	Urine	776·5	1000·4	587·3	458·5	1049·3	1351·7	7071·6	1756·5	893·1
4		Plasma	13·5	11·3	0·9	10·4	3·2	8·6	17·4	9·3*	2·2
	Day 4; 13–24	Urine	627·2	2742·2	816·0	1438·9	1889·7	4363·6	1237·3	1873·6	493·3
8		Plasma	14·1	13·4	4·0	15·5	3·3	9·2	25·9	12·2	2·9
	Day 5; 1–12	Urine	673·0	3973·0	698·6	1339·5	771·0	2296·8	1246·6	1571·2	454·2
24		Plasma	16·5	50·0	2·4	25·9	8·1	10·8	30·2	20·6	6·2
	Day 5; 13–24	Urine	1230·2	1797·3	562·6	1238·9	1716·7	1984·6	Nd	1421·7	212·4

Nd, not determined (the sample was not collected).
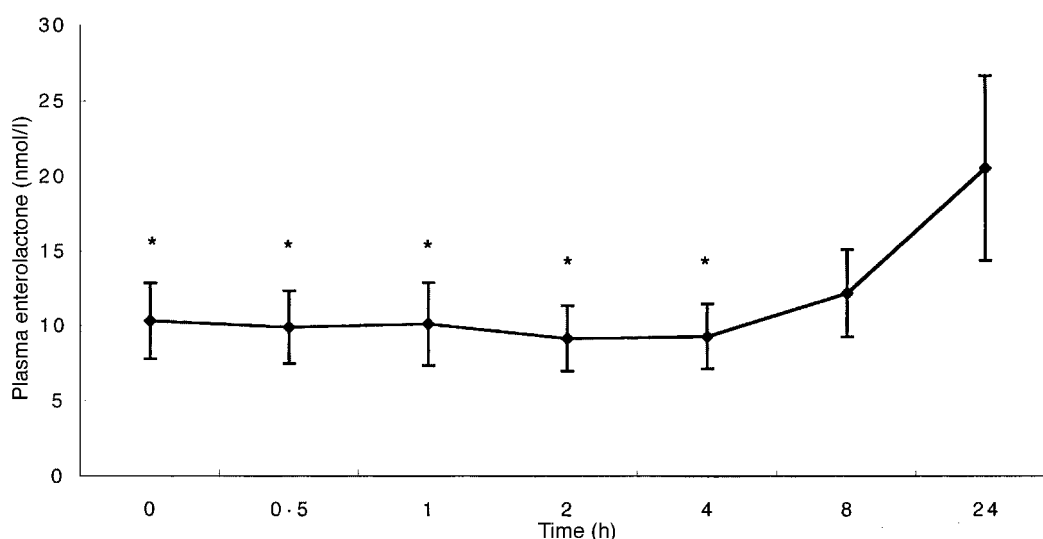Mean values were significantly different from that of the sample taken 24 h after the strawberry meal: * $P < 0.05$.

found in all the berries, ranging from 1·39 mg/kg (raspberry) to 37·2 mg/kg (blackberry) (3·9 and 104·3 μmol/kg DM respectively). Blackberry contained the highest concentrations of SECO and total plant lignans. Strawberry (the most common Finnish variety) contained 15·1 mg/kg DM, (42·4 μmol/kg). MAT, the immediate precursor of ENL, was found only in three berries (strawberry, blackberry and blackcurrant) at low concentrations. The strawberries used in the study (500 g, 77·5 g DM) contained 3·3 μmol (11·7 mg on a DM basis) of SECO and 0·17 μmol (0·61 mg on a DM basis) MAT.

The results for ENL concentrations in plasma and urine of each subject are shown in Table 2. Mean plasma ENL values before and during the experiment are displayed in Fig. 2.

In the seven subjects studied, ENL plasma concentrations recorded in the basal plasma samples taken before consumption of strawberries ranged from 1·7 to 22·4 nmol/l (mean 10·3 (SEM 2·5) nmol/l). At the four next measurements, 0·5, 1, 2 and 4 h after the berry meal, plasma ENL levels slightly decreased, ranging from 1·1 to 21·0 nmol/l (mean 9·9 (SEM 2·4) nmol/l), 1·5 to 24·1 nmol/l (mean 10·1 (SEM 2·8) nmol/l), 1·5 to 19·8 nmol/l (mean 9·2 (SEM 2·2) nmol/l) and 0·9 to 17·4 nmol/l (mean 9·3 (SEM 2·2) nmol/l) respectively. The analysis of plasma ENL in two subsequent plasma samples taken on D4 and D5 indicated an increase (statistically significant for values at 24 h; 8 h: 4·0–25·9 nmol/l, mean 12·2 (SEM 2·9) nmol/l; 24 h: 2·4–30·2 nmol/l, mean 20·6 (SEM 6·2) nmol/l). ENL concentrations recorded in the subject K.L.'s plasma (all collections but at 24 h) were higher than those of the other subjects.

The urinary excretion of ENL was calculated for 4 h collections (nmol/4 h). A 24 h urine collection on D3, corrected for 4 h period, yielded results for the subjects ranging from 70·8 nmol/4 h (subject W.M.) to 1053·2 nmol/4 h (subject K.L.) (mean 517·5 (SEM 132·3) nmol/4 h). ENL

Fig. 2. Enterolactone concentrations in plasma of the subjects (*n* 7) after consumption of a strawberry meal. Values are means with standard errors indicated by vertical bars. Mean values were significantly different from that of the sample taken 24 h after the strawberry meal: * $P < 0.05$.

Table 3. Individual urinary excretion (nmol/24 h) and recovery (%) of enterolactone (ENL)

| | Time of urine collection | | | | |
| | Day 3; 24 h | Day 4; 1–24 h | Day 5; 25–48 h | Total ENL excreted | Recovery |
Subject	(pre-challenge)			post-challenge	(%)
A.S.	1339	1844	1817	2322	56·4
I.W.	2820	6600	14265	18045	438·0
W.M.	425	920	785	1280	31·1
T.N.	1796	2558	2745	3507	85·1
H.A.	4344	1664	1732	−948	−23·0
R.T.	4694	4839	4792	4947	119·8
K.L.	6319	6558	3491	3730	90·5
Mean					114·0

excretion measured in the urine collected in portions within the four next 4 h periods (D4: 0–4 h, 5–8 h, 9–12 h and 13–24 h) after the strawberry meal varied individually between the subjects (range 83·8–906·6 nmol/4 h, 105·6–1032·9 nmol/4 h, 114·5–1909·4 nmol/4 h and 205·4–903·2 nmol/4 h respectively); however, there was a tendency for an increase (mean 551·9 (SEM 132·2) nmol/4 h, 434·1 (SEM 103·1) nmol/4 h, 643·3 (SEM 233·8) nmol/4 h, and 646·6 (SEM 189·9) nmol/4 h respectively). In the next collection, 25–36 h, mean ENL excretion increased and was the highest recorded during the experimental period (range 139·7–3125·5 nmol/4 h, mean 926·3 (SEM 390·5) nmol/4 h). ENL excretion decreased in the urine collected at 37–48 h in all the subjects except for K.L. (the sample not collected) and A.S. (range 121·9–1629·6, mean 543·0 (SEM 227·7) nmol/4 h).

Urinary ENL excretion per d (nmol/24 h) differed among the subjects (Table 3). The recovery of plant lignans SECO and MAT determined as urinary ENL varied from −23 to 438 % (mean 114 %) for the subjects. Two subjects, however, showed very different yields: H.A. excreted 23 % less ENL in urine than calculated by the intake, whilst I.W.'s ENL recovery in urine was almost 5-fold higher (438·0 %) than judged by the amount of plant lignans ingested.

When the ENL values in plasma with respect to sampling periods were analysed statistically, the 24 h mean ENL concentration was significantly higher than the mean concentrations at 0, 0·5, 1, 2, 4 h ($P < 0·05$). With respect to the ENL values in urine, no significances were found between the different periods due to high interindividual variations.

Discussion

The quantitative results for isoflavones in berries, showing very low content of these phenols in berries, are consistent with our earlier findings in various fruits and vegetables (Mazur, 1998; Mazur & Adlercreutz, 1998) as well as with earlier reports by others (Price & Fenwick, 1985; Jones et al. 1989; Dewick, 1993; Reinli & Block, 1996). With regard to lignans, our present study is the first describing plant lignan SECO and MAT levels in berries. The results for the three berry families *Rosaceae, Ericaceae* and *Grossulariaceae* varied. Blackberry and strawberry of the *Rosacea* family contained the highest amounts of lignans whereas in another

member of this family, the raspberry, the lowest amount of SECO was detected. When compared with other edible plant foods analysed until now (Mazur, 1998; Mazur & Adlercreutz, 1998), berries are an important source of the lignans in our diet.

The present pilot study was primarily designed to evaluate whether and how rapidly a single meal, relatively rich in lignans, would affect plasma levels and urinary excretion in subjects consuming a phyto-oestrogen-free diet. In order to follow lignan metabolic changes in the biological fluids immediately after a berry meal we collected short-time urine portions and took blood at short intervals within the first 48 h post-challenge. We chose to determine plasma and urinary ENL because ENL is the chief mammalian lignan in human subjects and we have developed specific, sensitive and economical time-resolved fluoroimmunoassays for it. Strawberry was selected for the experimental meal due to its popularity in the Finnish diet, and its relatively high lignan content.

The results of our present study led to certain observations on lignan metabolism in human subjects. The first observation is that a single strawberry meal, demonstrated to contain relatively high amounts of plant lignan SECO, contributed to elevated plasma ENL levels and increased the urinary excretion of this mammalian lignan (statistically significant in 24 h post-meal plasma). The metabolic response, however, was different in different subjects. Maximal mean ENL value measured in the 24 h plasma post-challenge was accompanied by the highest mean urinary ENL in D5 25–36 h urine of the subjects. From the data it is obvious that it takes about 8 h for the lignan precursors to reach the colon where conversion to ENL takes place, reaching its maximum about 25–36 h after the meal.

The second comment concerns the high individual variation of ENL values in plasma and urine, before and during the experiment. Although all the subjects ingested a fixed dose of the lignans, there were substantial variations between the subjects in the post-challenge plasma levels and urinary excretion of the mammalian ENL. The between-individual variability of ENL levels in plasma and urine, before and during the experiment, is probably due to differences in individual lignan metabolism and absorption depending on the gastrointestinal microflora (Adlercreutz, 1998). The gut microflora produces enzymes catalysing

metabolic conversion of SECO, the major lignan in strawberry, to END through dehydroxylation and then demethylation (Borriello *et al.* 1985). END is then oxidized by facultative bacteria to yield ENL. ENL also can be synthesized directly from MAT, however, strawberry (and other berries) contains minimal amounts of this lignan. The conversion of plant lignans to mammalian lignans depends on the presence of facultative bacteria in the intestine, as evidenced by studies with rats and human subjects (Axelson & Setchell, 1981; Setchell *et al.* 1982; Pettersson *et al.* 1996).

There is a large body of evidence that our diet influences our gastrointestinal microflora (Rowland *et al.* 1985). The Finnish (except for W.M.) participants of the study have been consuming a lignan-rich diet for many years. Their everyday diet consists predominantly of cereals and grain (e.g. rye bread), milk and yogurt products, vegetables, seeds, and berries (all these are sources of lignans). This dietary pattern must therefore affect their microflora and influence mammalian lignan production from plant precursors (Adlercreutz & Mazur, 1997; Adlercreutz, 1998; Mazur & Adlercreutz, 1998). Daily intake of these foods is a natural stimulation of the production of mammalian lignans; the fibre increases the bacterial mass. Interestingly the subject K.L., a vegetarian, does not consume any meat and meat products at all; she had the highest ENL concentrations in pre- and particularly post-meal plasma and urine which could reflect her high metabolic response to the berry meal. High individual variation in phyto-oestrogen excretion in response to isoflavonoid- or lignan-rich plant food consumption has been reported by other investigators (Adlercreutz *et al.* 1991*b*; Lampe *et al.* 1994; Morton *et al.* 1994; Kelly *et al.* 1995; Karr *et al.* 1997; Watanabe *et al.* 1998). The subjects in these studies, however, had been treated with a much greater dose of phyto-oestrogens for a longer period of time.

The urinary recovery of ENL differed only slightly from amounts estimated by its precursors in the strawberry meal. Considering lignan metabolism and ENL recovery one should bear in mind that substantial amounts of lignans are excreted by the faecal route and the amounts are similar or only slightly lower in faeces than in urine (Bannwart *et al.* 1987) (e.g. faecal excretion of lignans in nine omnivorous women: ENL 1510 nmol/24 h, END 147·7 nmol/24 h and MAT 22·3 nmol/24 h (Adlercreutz *et al.* 1995)). This would mean that the total recovery of lignans in urine and faeces is probably higher than the intake of lignans from the strawberries. Furthermore, the time-resolved fluoroimmunoassay for urinary ENL gives about 30 % higher values than if assayed by GC–MS (H Adlercreutz and M Uehara, unpublished results), probably due to the presence of other lignan metabolites cross-reacting with the antiserum. As microflora is an indispensable factor involved in the constant process of mammalian lignan production, the bacteria seem to be loaded with lignans and could themselves supply 5–10 % ENL recovered in urine (basal plasma and urinary ENL levels, even after a long period of restricted diet, did not reach 0 value). Xu *et al.* (1995) in their study on bioavailability of soyabean isoflavones concluded that the breakdown of isoflavones by the microflora in the gut determines the recovery of the compounds.

In summary, berries contain different, relatively high amounts of the lignan SECO, but not isoflavones, and therefore contribute to elevated plasma levels and increased urinary excretion of mammalian lignan ENL. A supplement of 500 g lignan-rich strawberries increased plasma ENL concentrations 24 h post-challenge (observed as higher urinary excretion in D5 25–36 h collection) (statistically significant for mean values in plasma). Our pilot study showed clearly high individual variability of metabolic response to a single dose of lignan-rich berries.

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