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Short Communication

Impact of a lignan-rich diet on adiposity and insulin sensitivity in post-menopausal women

Anne-Sophie Morisset[1,2], Simone Lemieux[2,3], Alain Veilleux[1,2], Jean Bergeron[4], S. John Weisnagel[4,5] and André Tchernof[1,2,3,4]*

[1]*Molecular Endocrinology and Oncology Research Center, Laval University Medical Research Center, 2705 Laurier Blvd. T3-67, Québec, Que., Canada G1V 4G2*
[2]*Department of Food Science and Nutrition, Laval University, Québec, Que., Canada G1V 0A6*
[3]*Institute of Nutraceuticals and Functional Foods, Laval University, Québec, Que., Canada G1V 0A6*
[4]*Lipid Research Center, Laval University, Québec, Que., Canada G1V 0A6*
[5]*Diabetes Research Unit, Laval University Medical Research Center, 2705 Laurier Blvd. T3-67, Québec, Que., Canada*

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There has been a growing interest in lignans, a class of phyto-oestrogens, because of their potentially favourable effects on human health. The aim of the present study was to compare the metabolic profile of post-menopausal women consuming various amounts of dietary lignans. Phyto-oestrogen intake was assessed using a 3-d dietary record analysed with a Canadian food phyto-oestrogen content data table in 115 post-menopausal women (age 56·8 (SD 4·4) years and BMI 28·5 (SD 5·9) kg/m^2). Plasma enterolactone (ENL), the major biologically active metabolite of dietary lignans, was determined by time-resolved fluoroimmunoassay. Anthropometrics, abdominal adipose tissue areas (computed tomography), body composition (hydrostatic weighing) and insulin sensitivity (hyperinsulinaemic–euglycaemic clamp) were measured in all women. Women in the high dietary lignan intake subgroup (*n* 29) had a significantly lower BMI and total body fat mass, as well as a better glucose disposal rate (GDR; $P<0·05$), compared with women in the low lignan intake subgroup (*n* 28). The majority of women with the highest dietary lignan intake were also in the highest quartile of plasma ENL (59 %). Women in the highest ENL quartile had a significantly lower BMI (26·1 (SD 4·4) *v.* 30·4 (SD 6·9) kg/m^2, $P<0·05$), total body fat mass (24·8 (SD 9·8) *v.* 33·3 (SD 13·3) kg, $P<0·05$), 2 h postload glycaemia (5·5 (SD 0·9) *v.* 5·7 (SD 0·8) nmol/l, $P<0·05$) and a higher GDR (8·3 (SD 2·7) *v.* 5·5 (SD 2·8), $P<0·01$) compared with women in the lowest ENL quartile. In conclusion, women with the highest ENL concentrations had a better metabolic profile including higher insulin sensitivity and lower adiposity measures.

Phyto-oestrogens: Lignans: Adiposity: Insulin sensitivity

Consumption of lignan-rich diets, which contain vegetables, fruits and whole-grain products, may protect against chronic diseases[1–4]. The main dietary lignans are secoisolariciresinol, matairesinol, pinoresinol and lariciresinol, found as glycosides in food[5]. Once in the colon, dietary lignans are transformed to mammalian lignans, enterodiol and enterolactone (ENL), by intestinal bacteria before reaching the circulation[1,6].

Only a few studies in human subjects have been published addressing the effects of a lignan-rich diet on adiposity measures or insulin sensitivity[7–10]. An inverse association was reported between plasma ENL concentrations and body weight in women[8]. The present study is the first specific examination of the association between dietary lignans and adiposity or insulin sensitivity using two lignan-related measures, that is, dietary intake and plasma ENL concentrations. The objective of the present study was to compare the metabolic profile of post-menopausal women consuming various amounts of lignans. We tested the hypothesis that the majority of women consuming high amounts of dietary lignans would have higher plasma ENL concentrations as well as lower adiposity measures and higher insulin sensitivity.

Subjects and methods

Subjects of the study were recruited through the local newspapers of Quebec City (Canada). Inclusion criteria were: age ≤70 years; post-menopausal status (absence of menses for at least 1 year and levels of follicle-stimulating hormone ≥22 IU/l); weight stability (±2·0 kg during the 3 months

Abbreviations: ENL, enterolactone; GDR, glucose disposal rate.

* **Corresponding author:** André Tchernof, fax +1 418 654 2761, email andre.tchernof@crchul.ulaval.ca

before testing). Women using hormone replacement therapy or treatment for CHD, diabetes, dyslipidaemias or endocrine disorders (except a stable dose of thyroxin for well-controlled hypothyroidism) were excluded. Data from women who did not complete the dietary record or for whom plasma ENL measures could not be performed were not included in the study. A total of 115 women were included in the present analysis. All women signed a consent form approved by the Medical Ethics Committee of Laval University.

Body fatness and body fat distribution

Body composition was measured by the hydrostatic weighing technique[11,12]. Total, visceral and subcutaneous adipose tissue areas were assessed by computed tomography as described previously[13,14].

Glucose homeostasis

A 75 g oral glucose tolerance test was performed in the morning after a 12 h overnight fast. Blood samples were collected in EDTA-containing tubes through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min for the determination of plasma glucose[15]. Blood glucose concentration was determined using a Glucometer Elite (number 3903-E; Bayer Corporation Inc., Tarrytown, NY, USA)[15]. The inter-assay CV was 1·0 % for a basal glucose value set at 5·0 mmol/l. Insulin sensitivity was measured using the hyperinsulinaemic–euglycaemic clamp technique, as described by DeFronzo *et al.* [16]. The glucose disposal rate (GDR) corresponds to the glucose infusion rate necessary to maintain euglycaemia during the last 30 min of the clamp per kilogram of body weight. Insulin was measured using RIA with polyethylene glycol separation[17].

Plasma lipid–lipoprotein levels

Blood samples were drawn after a 12 h fast on the morning of the hyperinsulinaemic–euglycaemic clamp. Plasma lipoprotein fractions were isolated by ultracentrifugation[18,19].

Cholesterol- and TAG-level measurements were performed enzymatically, as described previously[13,14].

Food records and phyto-oestrogen intake

Dietary intake was evaluated through a 3-d food record including two weekdays and one weekend day. Food items were weighed by each participant with a scale provided by the registered dietitian. The nutrient intake was evaluated using the Nutrition Analysis Software Food Processor version 7.2 (ESHA Research, Salem, OR, USA). The phyto-oestrogen intake was calculated using the 3-d dietary record analysed with a Canadian food phyto-oestrogen content data table including lignans (secoisolariciresinol, matairesinol, pinoresinol and lariciresinol), isoflavones (genistein, daidzein, glycitein and formononetin) and coumestans (coumestrol)[5]. This database included 121 food items commonly consumed in Canada.

Plasma enterolactone

Plasma ENL concentrations were determined by time-resolved fluoroimmunoassay using the method developed and validated by Adlercreutz *et al.* [20]. Briefly, plasma samples were incubated overnight at 37°C with acetate buffer 0·1 M (pH 5·0) containing 2 U/ml sulphatase (Sigma, S9626, St Louis, MO, USA) and 0·2 U/ml β-glucuronidase (Roche, 03707580001, Mannheim, Germany). Hydrolysed ENL was extracted using diethyl ether and concentrations were measured by competitive immunoassay using a commercial kit (Labmaster, Turku, Finland). Fluorescence was quantified using a DELFIA Victor 3 multilabel counter (Wallac, Ramsey, MN, USA). To assess extraction recovery, 20 000 CPM of ^{3}H-labelled oestradiol 17β-glucuronide (American Radiolabeled Chemicals Inc., ART 1320, St Louis, MO, USA) was added to each plasma sample. All measurements were adjusted using their individual recovery percentage. The average recovery percentage was 98·6 %. The intra- and inter-assay coefficients of 3·1–6·1 and 6·1–8·6, respectively, were reported by Stumpf *et al.* [21].

Physical activity record

Women filled a validated 3-d activity diary[13,22] with a list of categorised activities graded on a 1–9 scale for each 15 min period. We considered the mean daily energy expenditure and the frequency of participation for categories 6–9 (moderate to intense exercise), which have an energy cost of ≥5 kJ/kg per 15 min (≥4·8 MET), using a previously published formula[13]. The 3-d average of moderate to intense exercise values was used.

Statistical analyses

Since lignan consumption and plasma ENL were not normally distributed and did not normalise with mathematical transformations, the analyses were performed using quartiles of each variable. The use of quartiles was based on the fact that twenty-nine women (25 % of the sample) had lignan intakes that were particularly elevated. Similar distributions were also observed for the ENL concentrations. For lignan consumption, quartile 1 was considered as the low dietary lignan intake subgroup and was compared with quartile 4, considered as the high dietary lignan intake subgroup by *t* tests. ANOVA/Tukey's honestly significant difference or Wilcoxon tests were performed to compare the means among the ENL quartiles. Adjustments for confounders were performed using least-squares means and *a posteriori* mean contrasts. The Spearman rank correlation coefficients were computed to quantify the association between lignan intakes and plasma ENL concentrations. The κ value was used to evaluate the concordance between the ENL and lignan consumption quartiles.

Results

The characteristics of the sample are shown in Table 1. Women in the high dietary lignan intake subgroup (*n* 29) had a significantly lower BMI (30·0 (SD 5·2) *v.* 26·6 (SD 4·0) kg/m^2, $P \leq 0{\cdot}01$) and total body fat mass (31·5 (SD 10·1) *v.* 26·3 (SD 9·3) kg, $P \leq 0{\cdot}05$), as well as a higher GDR (6·2 (SD 3·2) *v.* 7·8 (SD 2·4) mg/kg per min, $P \leq 0{\cdot}05$) compared with women with low dietary lignan intake (*n* 28). Dietary fibre intake (total, soluble and insoluble) was significantly higher in women in the high dietary lignan intake subgroup. However, energy intake, energy macronutrient distribution,

Table 1. Physical and metabolic characteristics of the study sample of 115 women
(Mean values and standard deviations)

Variable	Mean	SD	Range
Age (years)	56·8	4·4	46·4–68·0
Anthropometrics			
Weight (kg)	72·8	16·0	47·9–154·10
BMI (kg/m^2)	28·5	5·9	19·0–59·7
Total body fat mass (kg, *n* 111)	29·4	11·6	7·6–83·7
Waist circumference (cm)	91·0	13·4	65·9–134·7
Abdominal adipose tissue areas (cm^2, *n* 109)			
Total	508	172	166–943
Visceral	140	57	40–288
Subcutaneous	368	131	104–736
Lipid profile (mmol/l)			
Cholesterol	5·4	0·9	3·1–7·5
LDL-cholesterol	3·6	0·8	1·1–5·6
HDL-cholesterol	1·4	0·3	0·7–2·7
TAG	1·2	0·6	0·5–3·5
Glucose homeostasis			
Fasting glycaemia (mmol/l)	5·6	0·8	4·0–9·5
2 h Postload glycaemia (mmol/l)	8·0	2·9	3·2–17·0
Fasting insulinaemia (pmol/l, *n* 114)	76·0	44·0	3·0–374·0
Glucose disposal rate (mg/kg per min, *n* 110)	7·0	3·0	1·5–14·0
Energy expenditure (kJ/kg per d)			
Moderate to intense exercise	13·0	16·7	0–80·5
Energy intake (kJ/d)	8209	1904	4113–14310
Phyto-oestrogen intake*			
Total phyto-oestrogens (μg/d)	1623	+6599/−1390	50–93588
Total lignans (μg/d)	403	+3832/−243	45–92083
Total isoflavones (μg/d)	63	+1002/−30	5–29533
Flaxseed intake (g/d)	0	+0/−0	0–22·0
Plasma ENL (nmol/l)*	22·5	+18·1/−12·9	0–374·2

*Medians and interquartile ranges.

n-3 fatty acid intake and physical activity were similar in these two subgroups (data not shown).

Seventeen women (59 %) who were in the high dietary lignan intake subgroup were also in the highest ENL quartile. A concordance between lignan consumption and plasma ENL concentrations (when comparing quartiles 1, 2, 3 *v.* 4) resulted in a κ value of 0·45, which represents a moderate agreement[23]. A significant positive correlation was observed between lignan intakes and plasma ENL concentrations ($r = 0{\cdot}30$, $P = 0{\cdot}001$).

Women in the highest ENL quartile had significantly higher dietary lignan intake compared with women in quartiles 3, 2 and 1 (28 094 (SD 31 241) *v.* 4484 (SD 10 696), 4292 (SD 11 176) and 1240 (SD 1593) μg/d, $P < 0{\cdot}01$). This was reflected by a significantly higher dietary flaxseed intake in women in the highest ENL quartile compared with women in quartiles 3, 2 and 1 (7·1 (SD 8·0) *v.* 0·8 (SD 2·7), 0·8 (SD 2·6) and 0·0 (SD 0·1) g/d, $P < 0{\cdot}0001$). Figure 1 shows a significantly lower BMI and a trend for a lower waist circumference ($P < 0{\cdot}07$) in women in the highest ENL quartile when compared with women in quartile 1. A trend for a lower total ($P < 0{\cdot}10$) and visceral ($P < 0{\cdot}10$) adipose tissue areas in women in the highest ENL quartile compared with women in the lowest ENL quartile was also observed (Fig. 1). No significant difference was observed for subcutaneous adipose tissue area. Women in the highest ENL quartile had a lower 2 h postload glycaemia, a lower fasting insulinaemia and a significantly higher GDR when compared with women in the lowest ENL quartile (Fig. 1). No significant difference was observed for fasting glycaemia, energy intake, *n*-3 intake and physical activity. Women in the highest ENL quartile had a significantly higher daily number of whole-grain product portions compared with women in quartiles 1–3 (data not shown).

The differences in GDR and 2 h postload glycaemia between women in the highest and women in the lowest ENL quartile remained significant after statistical adjustment for either BMI, total body fat mass or waist circumference (data not shown). No difference was observed in years since menopause among women in lignan intake subgroups or ENL quartiles. Also, no difference was observed in alcohol consumption in women across the ENL quartiles. Excluding smokers (*n* 7) from the analysis did not alter the results.

Discussion

We aimed at comparing the metabolic profile of post-menopausal women consuming various amounts of lignans. A majority of women consuming high amounts of lignans were also in the highest serum ENL subgroup. For a similar energy intake, macronutrient energy distribution and physical activity, women with the highest ENL levels had a better metabolic profile including lower BMI, body fat mass, 2 h postload glycaemia and a higher GDR. The higher insulin sensitivity observed in women in the highest ENL quartile was unaffected by statistical adjustments for BMI, fat mass and waist circumference.

The literature on lignan consumption, obesity and fat distribution is not abundant[24]. Milder *et al.* [25] observed that lignan intake was higher in individuals with lower BMI values. In another study, plasma ENL decreased by 6·2 %

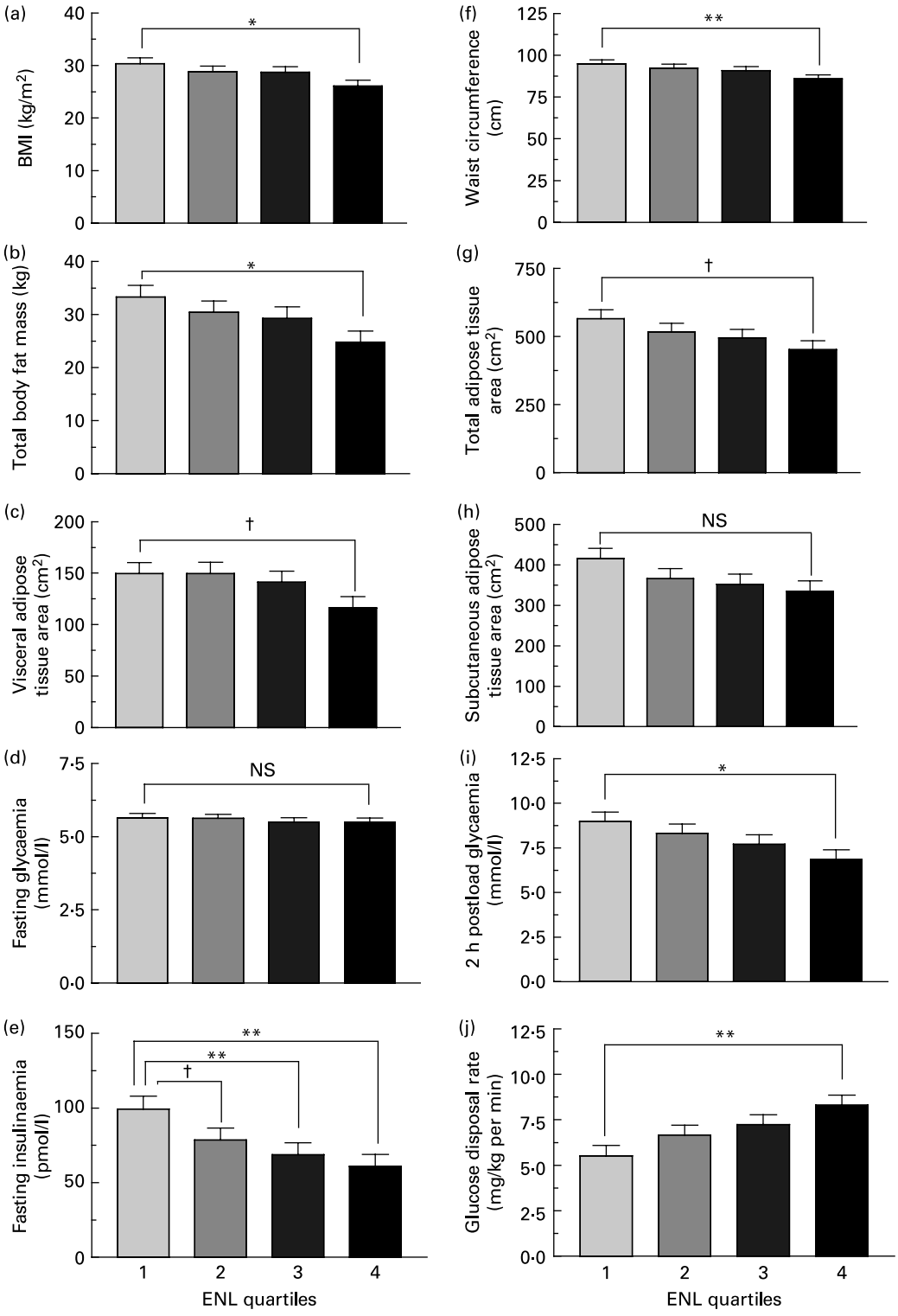

Fig. 1. Adiposity and glucose homeostasis measurements in women according to ENL quartiles. (a) Body fat mass, *n* 28 (q1), *n* 29 (q2), *n* 29 (q3), *n* 29 (q4); (b) total body fat mass, *n* 27 (q1), *n* 28 (q2), *n* 27 (q3), *n* 29 (q4); (c) visceral adipose tissue area, *n* 28 (q1), *n* 28 (q2), *n* 28 (q3), *n* 26 (q4); (d) fasting glycaemia, *n* 28 (q1), *n* 29 (q2), *n* 29 (q3), *n* 29 (q4); (e) fasting insulinaemia, *n* 25 (q1) *n* 29 (q2), *n* 29 (q3), *n* 28 (q4); (f) waist circumference, *n* 28 (q1), *n* 29 (q2), *n* 29 (q3), *n* 29 (q4); (g) total abdominal adipose tissue area, *n* 27(q1), *n* 28 (q2), *n* 27 (q3), *n* 29 (q4); (h) subcutaneous adipose tissue area, *n* 27 (q1), *n* 28 (q2), *n* 27 (q3), *n* 29 (q4); (i) 2 h postload glycaemia, *n* 28 (q1), *n* 29 (q2), *n* 29 (q3), *n* 29 (q4); (j) glucose disposal rate, *n* 26 (q1), *n* 28 (q2), *n* 29 (q3), *n* 27 (q4). *$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$, †$P \leq 0{\cdot}10$.

for each one unit increase in BMI in healthy men and women[26]. Kilkkinen *et al.*[8] reinforced this notion by observing that women with a normal BMI had significantly higher plasma ENL concentrations than obese women. These studies are concordant with the present results and suggest that a high lignan intake is associated with lower adiposity. Evidence for an effect of lignans on glucose metabolism is scarce[24]. Three randomised controlled trials have shown that flaxseed improved glucose and insulin metabolism[3,9,27]. A cross-sectional study reported that fasting insulin tended to decrease with increasing total dietary lignan intake[28], which is concordant with the present results.

Lignans are mainly produced from secoisolariciresinol found in flaxseed. In the present sample, the majority of women in the highest ENL subgroup were flaxseed consumers. *n*-3 fatty acid and/or soluble fibre found in flaxseed could partially explain the more favourable metabolic profile in women with elevated ENL. However, we found that *n*-3 fatty acid intake was similar among the ENL quartiles. On the other hand, fibre intake could have contributed to insulin sensitivity and adiposity differences in the present sample. Even if the differences in fibre intake between the highest and the lowest ENL quartiles were not significant, women in the highest quartile consumed an average of 5 g more total fibre daily, which is considerable. Such fibre consumption is reflected by a significantly higher number of wholegrain product portions. Several studies have shown that dietary fibres reduce postprandial glucose levels and improve insulin sensitivity[29–32]. Even if our differences remain significant after statistical adjustment for fibre intake, we suggest that this variable may still contribute to the association between the total lignan intake and the metabolic profile in post-menopausal women.

A limitation of the present study is that a single measurement of ENL may have overlooked significant within-day and day-to-day intra-individual variations in the ENL concentrations[33,34]. However, both dietary and plasma lignan-related measures were relatively concordant in their link with the metabolic profile. Another limitation is the use of a quartile approach, which could increase the risk of type 1 error. However, obtaining a rather consistent pattern of differences using various measures reinforces the present findings. Simple non-parametric correlations also generated a pattern that supports the findings obtained in the subgroup analysis.

Several factors may influence mammalian lignan production such as antibiotic use, obesity, smoking and fat intake[1,35,36] as well as frequency of defecation[36]. Smoking was not a confounding factor in the present study. Antibiotic use may also affect lignan metabolism for months, with the plasma ENL levels being drastically reduced for periods up to 1 year[1]. Unfortunately, we did not have information on antibiotic use over the previous year. We only excluded antibiotic use at the time of testing. These factors may have contributed to weaken the correlation between dietary lignan intake and plasma ENL. It is expected that the plasma ENL concentration will not be a perfect indicator of dietary lignan intake, but considering the relative concordance between the two measures, especially in their link with metabolic alterations, we suggest that they are relevant in assessing the association of a high lignan intake or high serum ENL with lower adiposity and insulin sensitivity.

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