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Short Communication

Effects of a flaxseed-derived lignan supplement on C-reactive protein, IL-6 and retinol-binding protein 4 in type 2 diabetic patients

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Elevated C-reactive protein (CRP), IL-6 and retinol-binding protein 4 (RBP4) levels are associated with insulin resistance and diabetes mellitus. Phytoestrogens (including lignans and isoflavones) may enhance the management of diabetes and are hypothesized to act through inflammation pathways. The present study explored the effects of flaxseed-derived lignan on inflammatory factors and RBP4 concentrations in type 2 diabetics, who have higher levels of these biomarkers. Seventy community-dwelling diabetic patients (twenty-six men and forty-four post-menopausal women) with mild hypercholesterolaemia completed a randomized, double-blind, placebo-controlled, cross-over trial of supplementation with flaxseed-derived lignan capsules (360 mg/d) or placebo for 12 weeks, separated by an 8-week wash-out period. The participants maintained their habitual diets and levels of physical activity. Baseline to follow-up concentrations of CRP increased significantly within the placebo group (1·42 (SEM 0·19) *v.* 1·96 (SEM 0·22) mg/l, $P<0·001$), but were comparatively unchanged in the lignan-supplemented group (1·67 (SEM 0·19) *v.* 1·90 (SEM 0·26) mg/l, $P=0·94$); a significant difference was observed between treatments (−0·45 (95 % CI −0·76, −0·08) mg/l, $P=0·021$). This effect was confined to women ($P=0·016$), but not observed in men ($P=0·49$). No between-treatment differences were found with regard to IL-6 or RBP4; though IL-6 concentrations increased significantly from baseline to follow-up in both groups ($P=0·004$ and $P<0·001$ following lignan and placebo treatments, respectively). The study suggests that lignan might modulate CRP levels in type 2 diabetics. These results need to be confirmed by further large clinical trials of longer duration.

Lignan: C-reactive protein: IL-6: Retinol-binding protein 4: Flaxseed: Type 2 diabetes: Inflammation

Several studies suggest that chronic inflammation, as indicated by elevated levels of inflammatory factors such as C-reactive protein (CRP) and IL-6, plays an important role in the pathogenesis of diabetes mellitus[1–3]. Recently, retinol-binding protein 4 (RBP4), a protein product of hepatocytes and adipocytes, has been associated with insulin resistance, diabetes[4,5] and inflammation[6].

A growing body of evidence suggests that certain chronic disorders, including diabetes, dyslipidaemia and CVD, are responsive to dietary phytoestrogens (isoflavones and lignans)[7,8], which may modulate disease risk through inflammatory pathways[9]. Flaxseed is the richest food source of the plant lignan, secoisolariciresinol diglucoside[10]. In a recent study of twenty-two healthy post-menopausal women, Hallund *et al.*[11] observed significantly lower CRP concentrations in participants receiving a lignan complex (500 mg secoisolariciresinol diglucoside/d) for 6 weeks compared to those on placebo. Hall *et al.*[12] also detected beneficial effects on CRP with 8-week supplementation of isolated isoflavones (50 mg/d) in 170 healthy post-menopausal women. However, other studies using isoflavones have produced null findings[13–16].

We have previously reported that a flaxseed-derived lignan supplement moderately decreased glycated Hb levels in type 2 diabetics[17]. The present study is a secondary analysis, and is aimed at exploring the impact of lignan supplementation on CRP, IL-6 and RBP4 levels in an effort to better understand the effects of lignan on diabetes.

Abbreviations: CRP, C-reactive protein; RBP4, retinol-binding protein 4.

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Methods

Study design and participants

The methods of the parent study were detailed elsewhere[17]. Briefly, seventy-three type 2 diabetic patients (twenty-eight men and forty-five post-menopausal women) aged 50–79 years were consented and randomized in a crossover design to a double-blind, placebo-controlled study with 12-week supplementation of lignan or placebo capsules separated by an 8-week washout period. Randomization was performed using stratification factors of gender and tertiled LDL-cholesterol concentrations (the primary end-point of the parent study).

Intervention

The lignan capsules (LinumLife™ Extra; Frutarom Netherlands BV, Veenendaal, The Netherlands) provided a daily dose of 360 mg flaxseed-derived secoisolariciresinol diglucoside. The three capsules provided 15·5 kJ and were comprised of 20 % secoisolariciresinol diglucoside, 30 % carbohydrate, 15·6 % fat, 3·2 % protein and 2·6 % fibre. The placebo was an identical capsule of rice flour devoid of soluble fibre. The participants took three capsules per day (1·8 g) which contributed minimally to their daily energy and nutrient intake. Adherence was assessed by pill counts and urinary concentrations of lignan metabolites[17]. While on study, participants were required to take their regularly prescribed medications, and maintain their habitual diets and physical activity levels.

Measurements

Fasting venous blood samples were collected at the beginning and completion of each intervention period. Serum CRP was measured *via* a high-sensitive immunoturbidimetric assay on a Hitachi 7080 automatic analyser using commercial kits (Roche Diagnostics, Mannheim, Germany). IL-6 was measured by a high-sensitive ELISA (R&D Systems, Minneapolis, USA). Plasma RBP4 was measured by an in-house-developed sandwich ELISA, detailed elsewhere[5]. Fasting morning urine samples (50 ml) were collected at identical time-points using plastic jugs containing 50 mg ascorbic acid. Urinary lignan metabolites were analysed using HPLC as reported previously[17].

Statistical analyses

The power calculations of our previous study were based on serum lipids and were described previously[17]. In the present exploratory secondary analysis, a total of sixty participants provide 80 % power with an α level of 0·05 (two-sided) to detect between-treatment differences of 0·30 mg/l, 0·30 pg/ml and 14·6 μg/ml on CRP, IL-6 and RBP4, respectively.

Data were analysed in Stata 9.2 (Stata™; Texas, USA); the two-sided P value $\leq 0{\cdot}05$ was considered statistically significant. For all biomarkers, the data were natural-logarithmically transformed prior to analysis. Paired Student's t tests were used to compare differences between baseline and end of treatment. Differences between values after the 12-week intervention were analysed using a mixed model analysis of covariance with treatment and period as fixed factors, participants as random factors and baseline values as covariates. Further fixed terms corresponding to treatment–period and treatment–baseline value interactions were included. Given the weight-dependent nature of the study end-points, baseline weight and weight changes during treatment also were incorporated as covariates. The analyses were further stratified by gender to explore differential effects in males and females.

Participants with extremely high levels of CRP (≥ 10 mg/l) or IL-6 (≥ 10 pg/ml) were considered to have acute inflammation and were excluded from the final mixed models. Sixty-four participants remained for CRP analyses and sixty-seven remained for IL-6 analyses.

Results

Of the seventy-three participants consented, three participants dropped out for reasons described previously[17]. Seventy (twenty-six men and forty-four women) completed the study and were available for analyses. The mean age of the sample was 62·9 (SD 7·5) years.

Significant increases in CRP concentrations were found from baseline to follow-up within the placebo group ($P<0{\cdot}001$), while those in the lignan treatment group did not experience increases of similar magnitude ($P=0{\cdot}94$). Thus, compared to placebo, increases in CRP with the lignan supplement were significantly lower ($P=0{\cdot}021$). These differences were primarily observed in women ($P=0{\cdot}016$), and not in men ($P=0{\cdot}49$) (Table 1).

In contrast, IL-6 concentrations significantly increased from baseline to follow-up in both groups, with no between-treatment difference observed. Likewise, no between-treatment difference was detected in levels of RBP4, which remained at fairly stable levels throughout the study period. Additionally, urinary excretion of lignan metabolites increased significantly after lignan treatment. As reported previously, no significant differences were observed for energy and nutrient intake, and physical activity between treatment phases over time[17].

Discussion

The CRP concentrations in the placebo group increased significantly over the study period. This is an unexpected finding, and the exact reasons are unclear. Elevated levels of CRP, however, have been documented among diabetic patients and correlated with both glycaemic control and complications[1]. Speculation exists that increased CRP concentrations may reflect aggravated diabetes management within these individuals. Furthermore, some participants had medication changes during the study period, which also might have influenced their inflammatory status. Although CRP levels increased slightly in the lignan-treated group, this increase was not statistically significant. The present findings are similar to a recent study by Hallund *et al.*[11] which also employed a crossover design delivering placebo *v.* a lignan supplement (500 mg secoisolariciresinol diglucoside/d) among twenty-two healthy post-menopausal women. They also found greater increases in CRP during the placebo period (from 0·80 to 1·10 mg/l) than during lignan treatment (from 0·88 to 0·92 mg/l) ($P=0{\cdot}028$). Similarly, Teede

Table 1. Baseline and 12-week follow-up levels of C-reactive protein (CRP), IL-6 and retinol-binding protein 4 (RBP4) in placebo *v.* lignan-supplemented study groups†
(Mean values with their standard errors)

	Lignan treatment				Placebo treatment				Difference between treatment		
	Baseline		12 weeks		Baseline		12 weeks				
Variable	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	95 % CI	*P* for between-treatment difference‡
CRP (mg/l)											
All (*n* 64)	1·67	0·19	1·90	0·26	1·42	0·19	1·96***	0·21	−0·45	−0·76, −0·08	0·021
Male (*n* 25)	1·59	0·28	1·83	0·36	1·25	0·23	1·55*	0·23	−0·20	−0·65, 0·46	0·49
Female (*n* 39)	1·72	0·26	1·95	0·37	1·53	0·27	2·23*	0·31	−0·67	−1·02, −0·16	0·016
IL-6 (pg/ml)											
All (*n* 67)	1·72	0·11	2·03**	0·14	1·65	0·13	2·00***	0·14	−0·06	−0·32, 0·23	0·68
Male (*n* 25)	1·92	0·22	2·39*	0·30	1·70	0·22	2·09***	0·24	0·07	−0·40, 0·66	0·78
Female (*n* 42)	1·61	0·13	1·82	0·14	1·62	0·16	1·95**	0·17	−0·13	−0·51, 0·26	0·63
RBP4 (μg/ml)											
All (*n* 70)	40·2	1·20	39·2	1·14	40·3	1·16	40·2	1·21	−0·98	−3·63, 1·67	0·89
Male (*n* 26)	43·0	2·17	41·1	2·01	40·2	2·10	40·8	2·30	−1·26	−5·77, 3·25	0·63
Female (*n* 44)	38·5	1·36	38·1	1·31	40·3	1·38	39·9	1·38	−1·13	−4·43, 2·18	0·36
Urine lignans (μg/ml)											
All (*n* 70)	1·21	0·28	14·21***	2·13	1·35	0·30	2·31	0·86	13·64	8·20, 19·08	<0·001
Male (*n* 26)	1·71	0·55	16·77***	3·63	1·23	0·28	1·88	0·54	16·20	8·11, 24·29	<0·001
Female (*n* 44)	0·92	0·31	12·69***	2·62	1·41	0·44	2·57	1·34	11·67	3·73, 19·61	<0·001

Mean values were significantly different from those of the baseline: *$P<0·05$, **$P<0·01$, ***$P<0·001$.
† For details of subjects and procedures, see Methods.
‡ *P* values are shown for the treatment effect analysed using a mixed model analysis of covariance.

et al. [18] also found increased CRP levels over 3-month treatments of soya (from 1·91 to 2·33 mg/l) and placebo (from 1·39 to 1·87 mg/l) in fifty healthy post-menopausal women, however no between-treatment difference was observed.

The present findings of moderate protective effects on CRP are consistent with the results of five previous trials that either tested isolated lignans[11] or isoflavones[12], flaxseed flour[19] or soya protein[20,21]. However, the present findings differ from studies which tested isolated isoflavones[13–16] or whole flaxseed[22,23], which found no differences with respect to CRP. It must be borne in mind that the difference that we observed between placebo and lignan-treated groups in the present study was primarily due to increased levels of CRP following the placebo period, rather than a direct reduction by lignan treatment. Therefore, given the exploratory nature of the present study, it remains premature to draw the conclusion that lignan can actually lower CRP levels.

Furthermore, stratified analysis found that the significant between-treatment difference of CRP concentrations was confined to females. One potential explanation is the relatively small sample of males. For detecting a between-treatment difference of 0·20 mg/l, 126 participants would be required to have a power of 80 %. The second possibility may be that although not statistically significant, post-menopausal women had moderately higher CRP levels than men, therefore, they were more likely to be responsive to phytoestrogens. Previous studies suggest that the metabolism and excretion of phytoestrogens differ between men and women[24]; however, it is currently unknown whether gender variation in the phytoestrogen bioavailability plays a role in CRP response.

The present findings of no effect on IL-6 are consistent with data from another study in which forty-two post-menopausal women with metabolic syndrome consumed soya protein or soy nuts (30 g/d containing 84–102 mg isoflavones) for 8 weeks[21]. In contrast, Jenkins *et al.* [25] found that IL-6 levels increased significantly after 1 month of a high soya diet (containing 73 mg isoflavones/d) compared to control among eighteen hypercholesterolaemic post-menopausal women. In the present study, the results of CRP and IL-6 did not follow the same pattern, which is also found in previous reports using lignan[11] or flaxseed[23]. A possible explanation for the present finding is that although IL-6 is the major inducer of CRP in the liver, other pro-inflammatory factors could also induce CRP production in other cell types[26].

RBP4 is a newly recognized adipokine associated with obesity, insulin resistance and diabetes[4,5]. A previous study showed that RBP4 levels decreased in parallel to weight loss and increased LDL-cholesterol catabolism after a 16-week intervention of a hypoenergetic low-fat diet in men with metabolic syndrome[27]. In our previous study, we found no effects of lignan supplementation on weight, lipid profiles and insulin resistance[17]. Therefore, it is possible that RBP4 levels were not affected. Since whole or defatted flaxseed has consistently shown to be effective in improving insulin resistance, and lowering total and LDL-cholesterol[7,8], its potential impact on RBP4 levels needs to be elucidated in future.

In conclusion, flaxseed lignan may suppress CRP elevation in type 2 diabetics without affecting IL-6 and RBP4 concentrations compared to placebo. However, further studies are needed to validate the present results and explore the efficacy of phytoestrogens on inflammatory factors before consensus is achieved.

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