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Research Article

Consumption of oat β-glucan with or without plant stanols did not influence inflammatory markers in hypercholesterolemic subjects

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We have earlier demonstrated that muesli enriched with oat β-glucan effectively lowered serum LDL cholesterol. Addition of plant stanols further lowered LDL cholesterol. Besides these hypocholesterolemic effects, β-glucan and plant stanol esters (PSE) may also affect inflammatory processes. Forty-two mildly hypercholesterolemic subjects randomly consumed for 4 wk (crossover design) control muesli (4.8 g control fiber), β-glucan muesli (4.8 g oat β-glucan), or combination muesli (4.8 g oat β-glucan plus 1.4 g stanol as PSE). Changes in cytokine production (IL-6, IL-8, and TNF-α) of LPS-stimulated peripheral blood mononuclear cells (PBMC) and whole blood were evaluated, as well as changes in plasma high-sensitivity (hs)-CRP. Additionally, changes in expression profiles of 84 genes involved in atherosclerosis metabolism were assessed in isolated PBMC. IL-6, IL-8, and TNF-α production by PBMC and whole blood after LPS stimulation did not differ between the treatments. Also high-sensitivity C-reactive protein (hs-CRP) levels were similar. β-Glucan consumption did not change gene expression, while only 3 genes (ADFP, CDH5, CSF2) out of the 84 genes from the atherosclerotic risk panel were differentially expressed ($p < 0.05$) after consumption of PSE. Consumption of β-glucan with or without PSE did not influence inflammatory parameters in mildly hypercholesterolemic subjects.

Keywords: Hs-CRP / Human atherosclerosis PCR array / Oat β-glucan / Plant stanol esters / Proinflammatory cytokines

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1 Introduction

It is becoming more and more established that inflammation plays an important role in the development of atherosclerosis and cardiovascular disease (CVD). Many studies have therefore been initiated to examine the effects of diets and dietary components on inflammatory markers. In this respect, much attention is paid to functional foods. Such foods contain (or lack) one or more components and therefore provide positive health effects beyond their traditional nutritional value. Examples of such food components with approved FDA-health claims are the viscous fiber β-glucan from oats and plant sterols/stanols [1, 2]. These components were approved because of their consistent LDL cholesterol-lowering effects. We have recently demonstrated that muesli enriched with β-glucan derived from oat effectively lowered serum LDL cholesterol in slightly hypercholesterolemic subjects. Addition of plant stanol esters (PSE) to β-glucan-enriched muesli even further lowered LDL cholesterol [3].

Besides these hypocholesterolemic effects, *in vitro* and animal studies have suggested that oat β-glucan and plant sterols/stanols may also affect immune and inflammatory processes [4 – 11]. Data from human studies are however limited and inconsistent. So far, only one study has examined the effects of oat β-glucan on inflammatory parameters. In this study, Queenan *et al*. [12] could not demonstrate that dietary supplementation with oat β-glucan changed plasma concentrations of C-reactive protein (CRP) in hypercholesterolemic subjects. Like for oat β-glucan, effects of plant sterol/stanols on inflammatory markers have been hardly studied. Several studies showed no signif-

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Abbreviations: ADFP, adipose differentiation-related protein; **CSF2,** granulocyte-macrophage colony-stimulating factor; **CVD,** cardiovascular disease; **hs-CRP,** high-sensitivity C-reactive protein; **PBMC,** peripheral blood mononuclear cells; **PSE,** plant stanol esters

icant effects on markers of inflammation, such as CRP, soluble adhesion molecules, and monocyte chemotactic protein-1 (MCP1) [13 – 16]. Nevertheless, Cater *et al*. [17] showed that combined administration of PSE with a statin compared to statin-treatment alone significantly reduced CRP concentrations in patients with a positive history for CVD. On the other hand, they found no significant change in CRP levels of hypercholesterolemic subjects with PSE alone. Devaraj *et al*. [18] found a significant reduction in CRP levels after consumption of a sterol-enriched orange juice in healthy subjects. Thus, more research is needed to clarify the role of these cholesterol-lowering dietary components on the inflammatory process related to atherosclerosis and CVD.

To explore whether the effects of oat β-glucan-enriched muesli with or without PSE not only changed LDL cholesterol, but also inflammatory processes, we first examined their effects on plasma high-sensitivity CRP (hs-CRP) concentrations. Hs-CRP is considered a marker for low-grade systemic inflammation and is positively associated with an increased risk for future cardiovascular events [19]. Since leukocytes are easily triggered by modified LDL cholesterol, potential changes in leukocyte reactivity may be more evident in hypercholesterolemic subjects. We therefore determined the effects of β-glucan with or without PSE on leukocyte function. This was studied *ex vivo* by measurement of inflammatory cytokine (IL-6, IL-8, and TNF-α) production after LPS stimulation of isolated peripheral blood mononuclear cells (PBMC) and of whole blood. IL-6, IL-8, and TNF-α are proinflammatory markers involved in the development of atherosclerotic lesions [20, 21]. Finally, we studied in PBMC the effects of these functional ingredients on the expression of 84 genes involved in atherosclerosis.

2 Materials and methods

2.1 Study population

This trial was part of a study on the simultaneous effects of oat β-glucan and PSE on the lipoprotein profile in healthy men and women with slightly elevated serum cholesterol concentrations. Details of the study have already been published [3]. All participants were given a detailed description of the experimental protocol and purpose of the study before they gave their written informed consent. They were invited for two screening visits, which consisted of measurements of body weight, height, blood pressure, serum total and HDL cholesterol concentrations, serum triacylglycerol concentrations, presence of glucosuria, and hematological parameters. In addition, all subjects had to fill in a general and a medical questionnaire.

Participants were selected for the study according to the following inclusion criteria: stable body weight (weight gain or loss <3 kg in the past 3 month); Quetelet-index <32 kg/m^2; systolic blood pressure <160 mmHg; diastolic blood pressure <95 mmHg; mean serum total cholesterol concentrations between 5.0 and 8.0 mmol/L; mean serum triacylglycerol concentrations <4.0 mmol/L; no presence of glucosuria, proteinuria, or anemia; no use of medication or a prescribed diet known to affect lipid or glucose metabolism; no history of coronary heart disease, cancer, diabetes, kidney-, liver-, pancreatic disease, or malignancies <5 years ago; no abuse of drugs and/or alcohol; no pregnant or breast-feeding women; willingness to stop the consumption of vitamin supplements, fish oil capsules or products rich in plant stanol or sterol esters 3 wk before the start of the study. Blood donation or participation in another biomedical trial was not allowed 30 day before and during the study. The Medical Ethical Committee of the University of Maastricht had approved the study and all subjects gave written informed consent.

Table 1. Baseline characteristics of the participants as measured before the start of the study[a)]

	All
Number (M/F)	42 (20/22)
Age (year)	52 ± 11
BMI (kg/m^2)	25 ± 3
Systolic blood pressure (mmHg)	138 ± 15
Diastolic blood pressure (mmHg)	86 ± 9
Total cholesterol (mmol/L)	6.62 ± 0.85
LDL cholesterol (mmol/L)	4.28 ± 0.82
HDL cholesterol (mmol/L)	1.63 ± 0.43
Triacylglycerol (mmol/L)	1.56 ± 0.75

a) All values are presented as means ± SD.

Forty-three volunteers were selected for the study. One man withdrew in the second week of the first period of the study because he started blood pressure medication. All other 42 volunteers, 20 men and 22 women, completed this study. Baseline characteristics are shown in Table 1.

2.2 Experimental design

The study had a randomized, double-blinded, controlled, multiple crossover design [3]. Briefly, subjects consumed for 4 wk in random order; control muesli (4.8 g control fiber), β-glucan muesli (4.8 g oat β-glucan), or combination muesli (4.8 g oat β-glucan plus 1.4 g plant stanols as PSE). There was a 2-wk washout period between the treatment periods.

Body weights did not differ after the control (73.8 ± 11.1 kg), β-glucan (73.9 ± 11.0 kg), and combination (73.8 ± 11.1 kg) diet periods ($p = 0.997$).

2.3 Blood sampling

The volunteers fasted overnight and were not allowed to use alcohol the day preceding or to smoke on the morning of

blood sampling [3]. Blood was collected in three 10 mL EDTA tubes (Becton Dickinson Vacutainer Systems, Breda, The Netherlands) for analysis of plasma hs-CRP concentrations and total RNA isolation. In addition, 16 mL of blood was sampled in two endotoxin-free heparinized tubes (Becton Dickinson Vacutainer Systems) for PBMC and whole blood stimulation. After sampling, all five tubes were immediately placed on ice. EDTA plasma was obtained directly by centrifugation of 1 EDTA tube at $2000 \times g$ for 30 min at 4°C. Plasma was then divided into aliquots, snap-frozen, and subsequently stored at −80°C until analysis.

2.4 CRP analysis

Hs-CRP concentrations were measured in EDTA plasma with a highly sensitive immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, WA, USA) [22].

2.5 PBMC and whole blood stimulation

Heparinized blood of 30 randomly selected subjects was used to study the effects of β-glucan and PSE on leukocyte reactivity. The baseline characteristics and responses of this subgroup were comparable to that of the whole group. Leukocyte reactivity was evaluated by stimulating both PBMC as well as whole blood with LPS as described [22]. Briefly, to examine *ex vivo* cytokine production in whole blood, four sterile 2 mL microcentrifuge tubes were each filled with 2 mL blood. Immediately, 20 μL LPS (*Escherichia coli* LPS serotype 055:B5, Sigma Chemical, St. Louis, MO, USA; final concentration 10 ng/mL in endotoxin-free buffered saline) was added to two tubes and 20 μL polymyxin B (antibiotic, Bedford Laboratories, Bedford, OH, USA; final concentration 1 mg/mL) to the remaining two tubes. Blood samples were incubated at 37°C for 3 h (determination of plasma TNF-α levels) or 21 h (determination of plasma IL-6 and IL-8 levels). After incubation, samples were centrifuged at $1000 \times g$ for 30 min and platelet-poor plasma was stored at −80°C until analysis. To examine *ex vivo* cytokine production by PBMC, cells were isolated from whole blood using Lymphoprep (Nycomed Pharma AS, Oslo, Norway) under sterile conditions. After isolation, PBMC of one subject were immediately plated in 4 wells of a 24-well flat-bottom culture plate (2.5×10^6 cells/mL *per* well; 150 – 500 μL *per* well). For each subject, PBMC of two wells were mixed with 10 μL LPS (final concentration 10 ng/mL) and the remaining two wells with 10 μL polymixin B (final concentration 1 mg/mL). RPMI-1640 was used as the culture medium, containing 1% penicillin/streptomycin, 1% sodium pyruvate, and 1% of a heat-inactivated human serum pool. The cells were incubated for 3 h (determination of plasma TNF-α levels) or 21 h (determination of plasma IL-6 and IL-8 levels) at 37°C. After incubation, the culture media were aspirated. The aspirated media were centrifuged at $1000 \times g$ for 30 min to obtain cell-free media, which were stored at −80°C until analysis.

2.6 Cytokine analysis

TNF-α, IL-6, and IL-8 concentrations in platelet-poor plasma of stimulated whole blood and cell-free media of stimulated PBMC were assessed by sandwich ELISA as previously described [22]. Briefly, plates (Greiner Bio-One, Frickenhausen, Germany) were coated with monoclonal murine antihuman TNF-α, IL-6, and IL-8 antibodies. Recombinant human TNF-α, IL-6, and IL-8 were used for their respective standard titration curves. Immobilized TNF-α was detected using a specific rabbit-anti-human TNF-α antibody, followed by the addition of goat-anti-rabbit peroxidase (Jackson ImmunoResearch, West Grove, PA, USA) and tetramethylbenzidine (TMB) substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Immobilized IL-6 or IL-8 was detected using a specific biotinylated rabbit-anti-human IL-6 or IL-8 polyclonal antibody, followed by the addition of peroxidase-conjugated streptavidin (Zymed Laboratories, San Francisco, CA, USA) and TMB substrate.

2.7 Total RNA isolation

Blood of 8 subjects, randomly selected from the subgroup of 30 subjects, was used to examine the changes in expression of 84 genes related to atherosclerosis in PBMC. This subgroup of eight subjects was a representative sample of the original group. Blood was processed immediately after collection as gene expression might change very rapidly. PBMC were first isolated from 20 mL EDTA blood using Lymphoprep density gradient centrifugation (Nycomed Pharma AS) according to instructions of the manufacturer. After isolation, RNA was purified form the cells using 1.5 mL Trizol (Invitrogen Corporation, Carlsbad, CA, USA). Subsequently, samples were stored at −80°C until RNA isolation.

After Trizol extraction, total RNA was further purified using RNAeasy columns (with on-column-DNAse treatment) according to the manufacturer's protocol (Qiagen Benelux BV, Venlo, Netherlands). Total RNA yield and integrity was assessed using the Agilent 2100 bioanalyzer. Only samples with an RNA integrity number (RIN) >7.0 were used. Samples were stored at −80°C until further analysis.

2.8 Gene expression analysis

The expression of 84 genes related to atherosclerosis was profiled using the RT^2 ProfilerTM PCR Array (Superarray, Bioscience Corporation, Frederick, USA) according to the manufacturer's protocol. Genes involved in the processes of blood coagulation and circulation were included as well as genes involved in cell-adhesion and lipid transport and

metabolism. Genes involved in the stress response, cell growth and proliferation, and apoptosis were represented as well. The expression of the 84 genes of interest plus 5 housekeeping genes (human 18S ribosomal RNA, hypoxanthine phosphoribosyltransferase 1, ribosomal protein L13a, glyceraldehydes-3-phosphate dehydrogenase, β-actin) was monitored. The 96 (SYBR Green) reactions were all carried out under the same cycling conditions in the same run. Data analysis was based on the $\Delta\Delta C_t$ method. Briefly, the ΔC_t for each pathway-focused gene in each treatment group was calculated first. The β-actin gene was used to calculate the ΔC_t values. Next, the $\Delta\Delta C_t$ was calculated for each gene across two groups, and used for statistical analyses. All three samples of one subject were analyzed in three runs on the same day. The correlation coefficient and efficiency of amplification were similar for all 24 standard curves of β-actin. The mean correlation coefficient for all standard curves was 0.995 ± 0.009. The mean slope value was –3.543 ± 0.263 and the mean efficiency of detection was 89.716 ± 0.023%.

2.9 Statistics

Normality was tested by the Shapiro – Wilk test. Hs-CRP concentrations were log-transformed to achieve normality. Log-transformed hs-CRP and cytokine concentrations were analyzed by ANOVA using the general linear model procedure. Differences in effects on the parameters of interest were examined with diet and period as fixed factors and subject number as a random factor. When a significant diet effect was found, the three treatments were compared pairwise using a Tukey *post hoc* test for multiple comparisons of observed means. Values are presented as means ± SD. Gene expression levels were analyzed with the nonparametric Friedman test. When a significant diet effect was found, the three treatments were compared pairwise using the Wilcoxon test. $\Delta\Delta C_t$ are presented as medians with ranges. Differences were considered significant at a p value <0.05. Statistical analyses were performed using SPSS 11.0 (version 11.0.3) for MacIntosh OS X (version 10.3.9).

3 Results

3.1 Hs-CRP

Mean hs-CRP concentration at the end of the control diet was 2.59 ± 3.57 mg/L, 2.24 ± 4.84 mg/L for the β-glucan diet, and 2.07 ± 3.04 mg/L for the combination diet. After log-transformation, these values were not significantly different from each other ($p = 0.622$). After exclusion from the analysis of six subjects with values higher than 9 mg/L [23], values at the end of the treatment periods were 1.56 ± 1.80 mg/L, 1.35 ± 1.31 mg/L, and 1.59 ± 1.79 mg/L for the control, β-glucan, and combination diet, respectively. Again, values did not differ significantly between the diets ($p = 0.933$).

Table 2. Effects of 4-wk daily intake of β-glucan with or without PSE on cytokine concentrations in platelet-poor plasma of stimulated whole blood[a)]

	Control	β-Glucan	Combination	p for diet effects[b)]
TNF-α (ng/mL)	2.8 ± 1.8	2.9 ± 2.0	2.8 ± 1.5	0.905
IL-6 (ng/mL)	28.5 ± 15.4	24.8 ± 16.9	27.5 ± 17.8	0.510
IL-8 (ng/mL)	33.8 ± 16.3	34.1 ± 16.3	32.2 ± 15.9	0.533

a) All values are presented as means ± SD; $n = 30$.
b) Cytokine concentrations were analyzed by ANOVA using the general linear model procedure with subject number as a random factor and diet and period as fixed factors. Differences were considered significant at a p value <0.05.

Table 3. Effects of 4-wk daily intake of β-glucan with or without PSE on cytokine concentrations in cell-free media of stimulated PBMC[a,b)]

	Control	β-Glucan	Combination	p for diet effects[c)]
TNF-α (ng/mL)	0.9 ± 0.8	1.1 ± 1.5	1.0 ± 1.1	0.300
IL-6 (ng/mL)	50.9 ± 37.2	70.1 ± 43.6	71.8 ± 62.7	0.130

a) All values are presented as means ± SD; $n = 30$.
b) Because of a small volume of supernatant, only TNF-α and IL-6 were determined in cell-free media of PBMC.
c) Cytokine concentrations were analyzed by ANOVA using the general linear model procedure with subject number as a random factor and diet and period as fixed factors. Differences were considered significant at a p value <0.05.

3.2 Proinflammatory cytokine production

Effects of the interventions on IL-6, IL-8, and TNF-α production in whole blood and PBMC after LPS stimulation are shown in Tables 2 and 3. Because of a small volume of supernatant, only TNF-α and IL-6 concentrations were determined in the cell-free media of PBMC. In whole blood, Il-6, IL-8, and TNF-α production did not differ significantly between the three dietary periods. Also in PBMC, IL-6 and TNF-α production did not differ significantly between the three intervention groups.

3.3 Gene expression

Thirteen genes were not detectable (defined as requiring >35 cycles to obtain a measurable C_t) in PBMC: apolipoprotein B (APOB), collagen type-3-α1 (COL3A1), fatty acid binding protein-3 (FABP3), fibrinogen (FGA), fibrinonectin-1 (FN1), IL3 (CSF), kinase insert domain receptor (KDR), laminin-α1 (LAMA1), Lp(a) (LPA), matrix metalloproteinase-3 (MMP3), neuropeptide Y (NPY), peroxisome proliferative activated receptor γ (PPARG), and secreted phosphoprotein-1 (SPP1). Among the remaining 71 detectable genes, only 3 genes were differentially expressed ($p < 0.05$). These effects were only evident for

Table 4. Effects of 4-wk daily intake of β-glucan with or without PSE on PBMC gene expression[a,b)]

Gene	$\Delta\Delta C_t$ (β-glucan − control)	$\Delta\Delta C_t$ (combination − control)	$\Delta\Delta C_t$ (combination − β-glucan)	*p* for diet effects[c)]
ADFP	−0.28 (−0.70, 0.32) $p = 0.123$	0.34 (−0.03, 0.65) $p = 0.017$*	0.73 (−0.35, 1.10) $p = 0.036$*	0.021*
CDH5	0.21 (0.00, 0.86) $p = 0.123$	0.10 (0.00, 0.86) $p = 0.068$	0.00 (−0.30, 0.00) $p = 0.180$	0.024*
CSF2	0.00 (0.00, 0.71) $p = 0.123$	−0.07 (−0.58, 0.00) $p = 0.043$*	−0.42 (−0.79, 0.00) $p = 0.043$*	0.010*

a) The expression of 84 genes related to atherosclerosis was profiled using the RT^2 Profiler™ PCR Array; $n = 8$.
b) Values are $\Delta\Delta C_t$ values and presented as medians with ranges.
c) Gene expression levels (ΔC_t values) were analyzed with the nonparametric Friedman test. When a significant diet effect was found, the three treatments were compared pair wise using the nonparametric Wilcoxon test.
* Differences were considered significant at a *p* value <0.05.

the combination muesli, since supplementation with the β-glucan muesli gave no differential gene expression as compared to the control muesli. The combination muesli significantly increased expression of granulocyte-macrophage colony-stimulating factor (CSF2) gene as compared both to the control ($p = 0.043$) and the β-glucan ($p = 0.043$) muesli (Table 4). Compared to the control muesli, expression of adipose differentiation-related protein (ADFP; $p = 0.017$) gene was significantly decreased by consumption of the combination muesli. This combined intake also effectively lowered expression of ADFP ($p = 0.036$) gene compared to the β-glucan muesli (Table 4). Gene expression of VE-cadherin (CDH5) gene differed among the three treatment groups ($p = 0.024$). However, between-diet comparisons were not significant.

4 Discussion

Several epidemiological studies have shown a negative association between the intake of dietary fiber and CVD risk. Viscous fiber, such as β-glucan, may improve CVD risk, at least partly, through improvements in serum LDL cholesterol [12]. β-Glucan may also mediate immune and inflammatory processes [5–9]. Data from human studies are however scarce. Our results showed no effect of daily consumption of muesli enriched with 4.8 g oat β-glucan on plasma hs-CRP concentrations. Consumption of oat β-glucan had also no influence on *ex vivo* LPS-stimulated cytokine (IL-6, IL-8, TNF-α) production in whole blood and PBMC. It is not likely that the lack of effect relates to the experimental approach used. In addition, previous studies have shown that effects of dietary components on cytokine production by PBMC can be detected in relatively small groups of subjects. In a study by Han *et al*. [24], in which only 19 subjects participated, IL-6 and TNF-α production by PBMC was significantly increased after consumption of a soybean oil-based stick margarine compared with soybean oil. So far, only one human study has examined the effects of oat β-glucan on inflammatory parameters. Queenan *et al*. [12] found, except for a significant reduction in LDL cholesterol, no significant changes in plasma CRP levels. In this study, 75 hypercholesterolemic subjects consumed either 6 g/day concentrated oat β-glucan or 6 g/day dextrose (control) for 6 wk. We further found that the addition of 1.4 g plant stanol as their fatty acid esters to the β-glucan-enriched muesli did not change the outcomes. Like for oat β-glucan, limited information exists on the effects of plant sterols/stanols on inflammatory markers in human volunteers. In line with our results, the majority of these studies showed no significant effects on markers of inflammation, such as CRP, soluble adhesion molecules, and MCP1 [13–16]. Nevertheless, Cater *et al*. [17] found in 13 hypercholesterolemic patients with a positive history for CVD no significant effect of PSE on CRP levels, but the combined administration of plant stanol esters with a statin significantly reduced CRP concentrations (−42%) as compared to statin-treatment alone ($n = 10$). In contrast, De Jong *et al*. [15] found no effects of PSE as add-on treatment in 45 statin-users on inflammation markers. Devaraj *et al*. [18], however, found in 72 healthy subjects, a significant reduction in CRP levels (−12%) after consumption of an orange juice enriched with plant sterols (2 g/day). Taken together, these studies suggest that the lack of effect on inflammation markers in the present study is not related to the number of subjects, the "health status" of the subjects, the dose administered, or the magnitude of the decrease in cholesterol. Using a within-subject variability of 20%, it can be calculated that the statistical power of our study was 80% to detect a true difference of 10% in CRP concentrations between the treatments. Thus, no clear explanation can be given to explain these inconsistent effects of plant sterols/stanols on parameters of inflammation. In addition, a combination of dietary factors, such as in the Mediterranean diet [25], the Portfolio diet [26], or a prudent diet [27] effectively reduced biomarkers of inflammation. These studies were however not designed to determine the impact of individual dietary factors and the possibility of synergy between dietary factors needs to be taken into consideration. Except for diet, also lipid-lowering drug interventions

can lower CRP levels. In fact, a recent meta-analysis concluded that most of the anti-inflammatory effects of cholesterol-absorption and cholesterol-synthesis inhibiting drugs were related to the magnitude of LDL reduction [28]. It was estimated that a decrease in LDL cholesterol of 1 mmol/L was associated with a decrease in CRP of 0.89 mg/L. If these results can be extrapolated to diet-induced changes in LDL cholesterol, this would have meant that we could have expected a decrease of 0.18 mg/L (7%) in the β-glucan group and of 0.37 mg/L (14%) in the combination group. The statistical power of our study was sufficient to detect this effect at least in the combination group. Whether this means that the relationship between diet-induced changes in LDL cholesterol and CRP is weaker than that for drug-induced changes warrants further study.

Recent studies have shown that the RT^2 PCR array is a sensitive platform to detect differential expression of "thematic" genes in different human cell types, such as alterations in genes related to drug metabolism in primary hepatocytes [29] and genes related to Th2 immune response in lymphocytes isolated from allergic subjects [30]. More detailed characterization of diet-induced differential expression of genes involved in atherosclerosis may enhance the understanding of the effects of oat β-glucan and PSE on CVD risk. PBMC may be a surrogate target cells to monitor effects in target tissues [31]. Daily consumption of muesli enriched with 4.8 g oat β-glucan did however not change gene expression of an atherosclerotic risk panel of 84 genes as measured in PBMC. Addition of 1.4 g PSE increased expression of CSF2 gene (proinflammatory effect), and decreased expression of ADFP gene (anti-inflammatory effect) in PBMC as compared to both the control and the β-glucan muesli. The CSF2 gene encodes granulocyte-macrophage colony-stimulating factor (GM-CSF). CSF2 plays an important role in smooth muscle cell-dependent monocyte activation [32]. The ADFP gene encodes adipose differentiation-related protein (ADRP). ADRP is a membrane-associated protein whose mRNA levels are induced rapidly and maximally after triggering adipocyte differentiation. ADFP is highly expressed in advanced lipid-enriched human atherosclerotic plaques [33]. However, giving functional significance to changes in gene expression level alone requires caution, as it provides no information on post-translational modifications or the rate of protein degradation. In addition, mRNA levels do not always correlate with protein synthesis. It should be noted, however, that the alterations found in gene expression after addition of plant stanols as their fatty acid esters may have occurred by chance. If 84 genes are tested with a probability threshold of 0.05, 4 genes (84×0.05) are expected to be significant by chance alone. Nevertheless, it has been shown that gene expression in PBMC can change after consumption of PSE [34]. In that study, a daily consumption of 4 g PSE increased LDL receptor mRNA concentrations in mononuclear blood cells by 43% as measured by competitive RT-PCR. A reason for our lack of effect on LDL receptor gene could be the difference in the dose administered and the concomitant reduction in LDL cholesterol.

In conclusion, the current level of supplementation of β-glucan and plant stanols as their fatty acid esters in the present study effectively lowered LDL cholesterol in slightly hypercholesterolemic subjects, but seemed to have no effects on inflammatory markers related to atherosclerosis and CVD.

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The authors have declared no conflict of interest.

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