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ARTICLE

A diet high in fatty fish, bilberries and wholegrain products improves markers of endothelial function and inflammation in individuals with impaired glucose metabolism in a randomised controlled trial: The Sysdimet study

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Abstract
Aims/hypothesis Low-grade inflammation and endothelial dysfunction may play a role in the pathogenesis of type 2 diabetes and cardiovascular disease. We evaluated whether a diet high in fatty fish, bilberries and wholegrain products (Healthy Diet) improves biomarkers reflecting inflammation and endothelial dysfunction in individuals with impaired glucose metabolism.
Methods We recruited individuals with impaired glucose metabolism and features of the metabolic syndrome into a 12 week, parallel design, dietary intervention trial conducted at the Department of Clinical Nutrition, University of Eastern Finland (Kuopio, Finland). Randomisation was performed by matching according to sex and medians of age, BMI and fasting plasma glucose of the study population at screening. The primary endpoint in the present study was the change in plasma inflammatory markers and the measurements were performed blinded to group assignment. High-sensitivity (hs) C-reactive protein (CRP) and E-selectin responses were also analysed separately in participants not using statins (n=76).
Results Altogether, 131 individuals were assigned to either the Healthy Diet (n=44), a whole-grain-enriched diet (WGED) (n=42) or a control (n=45) diet, and 104 participants (mean±SD: age 59±7 years; BMI 31.1±3.5 kg/m^2) who had completed the study, were analysed (Healthy Diet n=36, WGED n=34 and control diet n=34). Plasma E-selectin decreased only in the Healthy Diet group. This occurred in all group participants (p<0.05) and also after excluding participants using statins (p<0.05). Plasma hsCRP levels decreased in the Healthy Diet (median −17%, p<0.05) and WGED (median −27%, p<0.01) groups in participants not using statins. Controlling for confounding factors, including BMI or insulin sensitivity, did not alter the results. A greater increase in plasma concentration of very-long-chain n-3 fatty acids and in the intake of fibre

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during the study was associated with a greater decrease in plasma E-selectin ($p<0.05$). The intake of test breads consumed during the Healthy Diet and WGED interventions was inversely associated with the change in hsCRP levels ($p<0.001$).
Conclusions/interpretation Our results suggest that the combined effect of fatty fish, bilberries and wholegrain products may improve endothelial dysfunction and inflammation in overweight and obese individuals at high risk of developing diabetes.

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Keywords Bilberries · CRP · Diet · E-selectin · Glucose intolerance · Inflammation · Intervention studies · Metabolic syndrome · *n*-3 Fatty acids · Whole grain

Abbreviations

ALA	α-Linolenic acid
CRP	C-reactive protein
CVD	Cardiovascular diseases
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
hs	High-sensitivity
IQR	Interquartile range
SAA	Serum amyloid A
WGED	Whole-grain-enriched diet

Introduction

Individuals with impaired glucose metabolism are at increased risk of developing type 2 diabetes [1]. Low-grade inflammation and endothelial dysfunction seem to play a role in the pathogenesis of type 2 diabetes and cardiovascular diseases (CVD) [2–6]. Epidemiological studies suggest that diets that are high in saturated fat, low in fibre, rich in carbohydrates and have a high glycaemic index may increase the risk of type 2 diabetes [7, 8].

Evidence based on cross-sectional studies, meta-analyses and systematic reviews links high consumption of whole grain to a decreased risk of metabolic syndrome, type 2 diabetes and CVD in different populations [9–12], but its effects on inflammatory markers have been less consistent [12–14]. The intake of fish has been linked to the prevention of chronic diseases involving inflammatory processes [15] and to a beneficial effect on risk factors for CVD, including circulating levels of inflammatory markers [16]. Experimental studies support a role for phenolic compounds, present in bilberries, in the prevention of inflammatory diseases including CVD [17].

There are no data in the literature about dietary changes involving the combined effects of fatty fish, bilberries and wholegrain products on inflammatory markers in individuals with impaired glucose metabolism. In this context, we evaluated whether dietary modifications based on increasing these dietary components improve selected biomarkers of inflammation and endothelial dysfunction in individuals with impaired glucose metabolism and features of the metabolic syndrome.

Methods

Participants and study design Altogether 131 participants were recruited for a 12 week parallel controlled dietary intervention study from the Kuopio area, Finland (Fig. 1). Recruitment was based on the following inclusion criteria: age 40–70 years; impaired glucose metabolism (impaired fasting glucose 5.6–6.9 mmol/l or impaired glucose tolerance 7.8–11.0 mmol/l during an OGTT [75 g glucose]); and at least two of the following: BMI 26–39 kg/m^2; waist circumference ≥102 cm in men and ≥88 cm in women; serum triacylglycerol >1.7 mmol/l; HDL-cholesterol <1.0 mmol/l in men and <1.3 mmol/l in women; and blood pressure ≥130/≥85 mmHg. Individuals were excluded if they had: serum triacylglycerol >3.5 mmol/l; serum total cholesterol >8.0 mmol/l; abnormal liver, thyroid or kidney functions; a history of alcohol abuse; or were using narcoleptic or corticosteroids medication (inhaled corticosteroids allowed). Participants volunteered to the study and gave written informed consent. The study plan was approved by the Local Research Ethics Committee in accordance with the Helsinki Declaration.

The participants were randomised to a Healthy Diet, whole-grain-enriched diet (WGED) or control group. Groups were matched for sex, and for medians of BMI, age and fasting plasma glucose. Altogether 106 participants completed the study (Fig. 1). Of these, two participants, with high-sensitivity (hs) C-reactive protein (CRP) concentration >10 mg/l suggesting clinical inflammation, were excluded. Therefore, 104 individuals were analysed (Fig. 1).

Diets In the Healthy Diet group, participants were advised to replace their usual cereal products by products with at

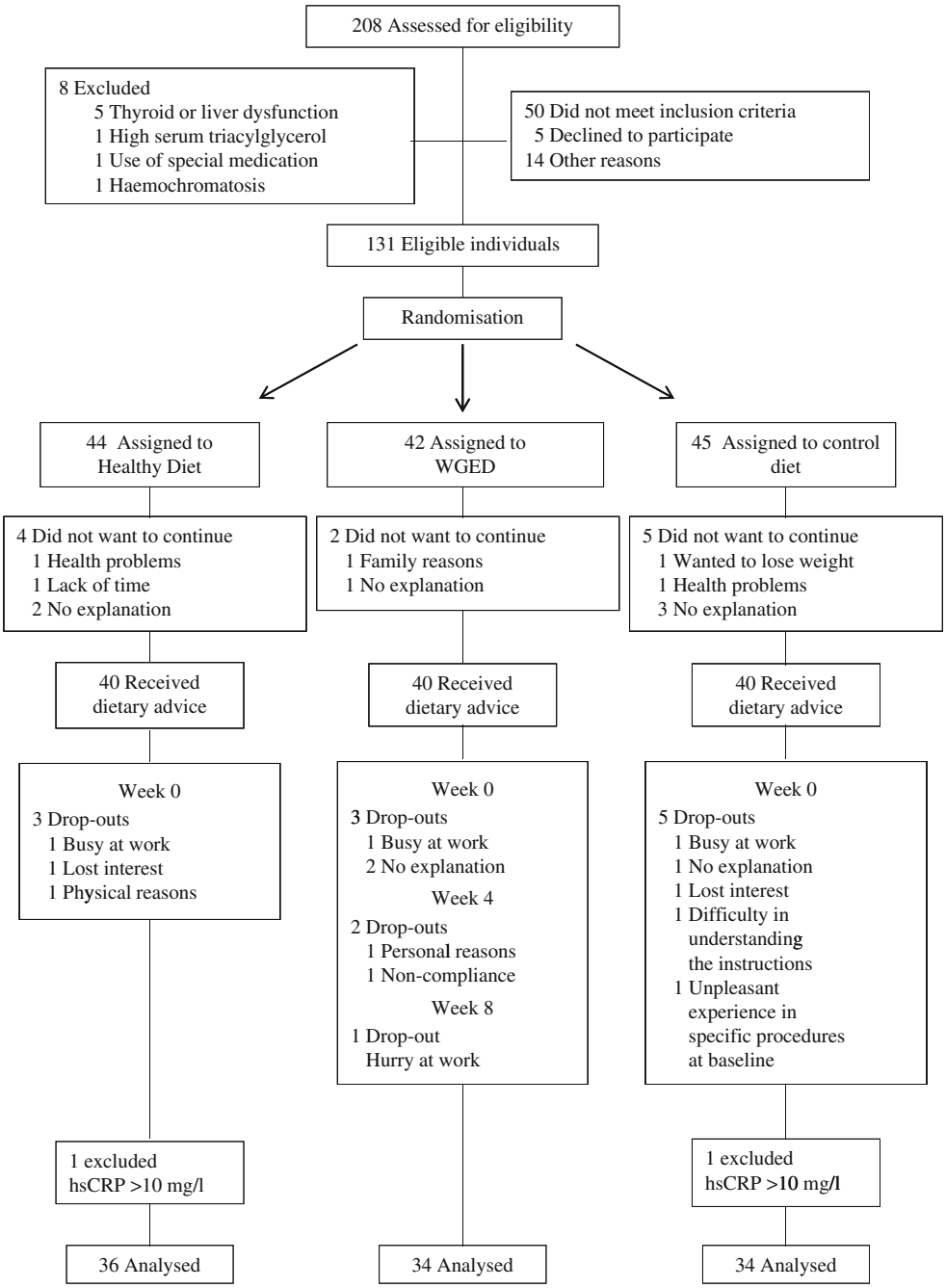

Fig. 1 Flow chart of participants

least 50% of their composition from a wholegrain source. The breads recommended, which contributed 20% to 25% of total energy intake, were: a selection of commercial rye breads (50% of bread consumption, fibre content 10.0–14.4%), sourdough wholemeal wheat bread (10%, fibre content 6.4%) and also endosperm rye bread (40%, fibre content 6.9%). The two latter breads are known to produce a low postprandial insulin response [18–20]. The recommended intake of wholemeal pasta was at least 35 g (uncooked) per week. In addition, one daily portion of another cereal product habitually consumed by the participants was allowed. Participants were instructed to eat fatty fish [21] (100–150 g fish per meal) three times per week, and to use vegetable oil and vegetable oil-based products in fish preparation. Otherwise, we did not seek to change the quality of the dietary fat, except for an increase in fatty fish consumption. Bilberries (*Vaccinium myrtillus*; frozen, pureed or dried powder) were advised to be eaten as three portions per day (equivalent of 300 g fresh bilberries per day). Participants were allowed to retain one of their habitual berry portions (e.g. raspberries) three to four times per week.

In the WGED group, participants were instructed to consume the same cereal products as in the Healthy Diet

group. In addition, they were given wholegrain oat snack bars [21] to be consumed once a day on a voluntary basis and were asked not to change their current fish and berry consumption.

In the control group, participants were asked to avoid wholegrain cereals. They were also instructed to replace the breads they usually consumed with refined wheat breads, and other cereal products, e.g. porridge or pasta, with low-fibre products [21]. The intake of bilberries was not allowed and consumption of fatty fish was allowed once a week only. Otherwise, the habitual diet and lifestyle habits were kept unchanged in all groups.

The cereal (bread, pasta, snack bars) and bilberry products were given free of charge during the study. The cost of buying fish was reimbursed for those in the Healthy Diet group. Participants recorded daily their consumption of the test breads (all groups), pasta (Healthy Diet and WGED), oat biscuits (WGED), bilberries (Healthy Diet) and fish (Healthy Diet). Participants in all groups kept a 4 day dietary record (consecutive days, including one weekend day) during the run-in period (baseline intake) and three times (weeks 3, 7 and 11) during the intervention period. One participant from the Healthy Diet group had no baseline dietary data, therefore the changes in dietary intake for this one participant were not included in the analyses. Dietary data were analysed using Nutrica software [22]. Body weight and height were measured at baseline, and body weight again at weeks 4, 8 and 12 (end of the study). BMI was calculated as weight (kg)/height (m^2).

Biochemical analyses Blood samples were drawn at baseline and at the end of the study (week 12), through a catheter in an antecubital vein after a 12 h overnight fast. Serum total cholesterol, LDL-cholesterol and HDL-cholesterol, and triacylglycerol were analysed using commercial kits (Thermo Electron, Vantaa, Finland). Serum insulin was analysed with a chemiluminescent immunoassay (Advia Centaur, Siemens Medical Solution Diagnostics, Tarrytown, NY, USA) and plasma glucose by the glucose hexokinase method (Konelab System Reagents; Thermo Fisher Scientific, Vantaa, Finland).

The plasma fatty acids α-linolenic acid (18:3 *n*-3, ALA), eicosapentaenoic acid (20:5 *n*-3, EPA) and docosahexaenoic acid (22:6 *n*-3, DHA) were analysed by gas chromatography [21]. High-sensitivity CRP and serum amyloid A (SAA) concentrations were determined by nephelometry (Siemens, Eschborn, Germany) with an analytical range and sensitivity of 0.175 to 11.00 mg/l and 0.16 mg/l, and 1 to 200 mg/l and 0.8 mg/l, respectively. ELISA was used to measure asymmetric dimethylarginine (DLD, Hamburg, Germany), von Willebrand factor (Matched Pair Antibody Set; Affinity Biologicals, Ancaster, ON, Canada), macrophage migration inhibitory factor (DuoSet Elisa kit; R&D Systems, Abingdon, UK), soluble intercellular cellular adhesion molecule-1 (Diaclone, Besancon, France), and E-selectin, chemokine (C-C motif) ligand 5 and IL-1 receptor antagonist (Quantikine Elisa Kits; R&D Systems). High-sensitivity ELISA kits were used to measure TNF-α and IL-6 (Quantikine and Elisa Kits; R&D Systems). Measurements were done in plasma EDTA samples, except for SAA. The inter-assay CVs for hsCRP and E-selectin were 5.0% and 7.2%, respectively. The CVs of the other markers are given in the electronic supplementary material (ESM) Table 1.

Statistical analyses Variables with a skewed distribution were natural log-transformed before the analyses and are reported as median (interquartile range [IQR]). The effects of the dietary interventions (groups) on the relative change in plasma circulating markers were compared by applying general linear model univariate analysis and using group as a fixed factor, adjusted for age, sex and baseline measurement, followed by the Bonferroni correction for multiple comparisons. When a group effect or an overall change (intercept) was significant, Student's paired *t* test was used for within-group comparisons between the baseline and week 12 measurements. Because the use of statins was common in this study population (27%) and could have interfered with the effect of dietary intervention on the two inflammatory markers based on their known anti-inflammatory effects [23], we also repeated the analyses in participants not using statins (n=76). Multivariate linear regression analyses were used to test the independent effects of the changes in dietary fibre and in plasma or dietary *n*-3 fatty acids on the changes in plasma E-selectin and hsCRP. All models used for analysing the dietary data were adjusted for total energy intake. A value of $p<0.05$ was considered significant. Analyses were performed using SPSS software version 17.0 (SPSS, Chicago, IL, USA).

Results

Participant characteristics Participants in the study groups were well matched for age, sex, body composition, blood pressure, and glucose and lipid variables (Table 1). The number of participants using statins was not different among groups (Healthy Diet n=9, WGED n=10, control n=9; p=0.92) and none of the participants stopped or started this medication during the study.

Diet and body composition during the study Although at baseline the Healthy Diet group had a trend towards higher energy intake than the WGED group (p=0.06), the change in daily energy intake was similar among the groups, even after taking the baseline energy intake into account (p=

Table 1 Baseline characteristics of the participants according to study group

Characteristic	Healthy Diet	WGED	Control
n	36	34	34
Age (years)	59±7	58±8	59±7
Women (*n*)	20	17	16
Men (*n*)	16	17	18
Body weight (kg)	89.8±12.2	89.2±15.3	89.5±13.2
BMI (kg/m^2)	31.1±3.6	31.4±3.4	30.9±3.5
Waist circumference (cm)	105.7±9.6	106.3±11.1	105.7±9.9
Systolic BP (mmHg)	138±13	135±16	139±11
Diastolic BP (mmHg)	89±7	86±8	88±6
Fasting PG (mmol/l)	6.1±0.5	6.1±0.5	6.2±0.5
2 h PG (mmol/l)[a]	6.7±1.7	6.6±1.6	6.9±1.9
Total cholesterol (mmol/l)	5.1±0.9	5.1±1.0	5.4±0.9
LDL-cholesterol (mmol/l)	3.1±0.8	3.2±0.8	3.4±0.8
HDL-cholesterol (mmol/l)	1.3±0.4	1.2±0.4	1.3±0.3
Triacylglycerol (mmol/l)	1.6 (1.1, 1.9)	1.3 (1.0, 1.8)	1.3 (1.0, 1.7)

Data are mean±SD or median (IQR)

[a]From an OGTT (75 g glucose load)

PG, plasma glucose

0.18). In the within-group analyses, the reported energy intake increased in all the groups during the study as compared with baseline ([mean±SD] Healthy Diet 8,185±1,860 vs 8,980± 1,995 kJ/day, p=0.003; WGED 6,995±2,373 vs 7,655± 2,395 kJ/day, p=0.03; control group 7,244±2,028 vs 8,522± 1,718 kJ/day, p=0.05).

There was a trend towards a decrease in body weight in the Healthy Diet group and towards an increase in body weight in the control group (mean±SD: 89.8±12.2 vs 89.0±11.7 kg/m^2, p=0.08 and 89.5±13.2 vs 89.9±13.1 kg/m^2, p=0.08, respectively). These developments were also reflected in terms of BMI (mean±SD: 31.1±3.6 vs 31.0±3.4 kg/m^2, p=0.08 and 30.9±3.5 vs 31.0±3.6 kg/m^2, p=0.05, respectively). Although the reported energy intake in the WGED group increased during the study compared with baseline, body weight and BMI did not change (89.2±15.3 vs 89.1±15.3 and 31.4±3.4 vs 31.4±3.4 kg/m^2, respectively, p>0.10 for both). However, the changes in BMI and body weight between baseline and the end of the study were not significantly different between the groups (p>0.05 for all).

During the study, participants recorded a mean intake of test breads of 7.7, 7.9 and 6.8 portions per day in the Healthy Diet, WGED and control groups, respectively. In the WGED, the mean daily intake of the wholegrain oat snack bar was 13 g. In the Healthy Diet group, participants consumed a mean of 3.2 fish meals (>85% as fatty fish) per week; their intake of bilberries was 3.2 portions per day. The main source of fat used for preparing the fish meals was rapeseed oil. The change in dietary nutrient intake based on the 4 day food records is depicted in Table 2. While the change in carbohydrate consumption was similar among groups (p=0.55), fibre intake increased in the Healthy Diet and WGED groups compared with the control group (p<0.001), being even higher in the former than in the WGED group (p<0.001).

While the changes in intake of total and saturated fat were not different among groups (p>0.05), the intake of polyunsaturated fat decreased in the WGED and control groups (p=0.001 for both). In the Healthy Diet and WGED groups, the saturated fat intake slightly decreased during the intervention (p=0.002 and p=0.02, respectively). However, the magnitude of these changes was very small (Table 2). In the control group, the intake of total and saturated fat did not change (p=0.58 and p=0.71, respectively). In the Healthy Diet group, participants reported an increase in their intake of ALA and EPA+DHA during the study as compared with baseline (p<0.001), thus differing from the control group (p<0.001). In plasma, however, although the percentage of EPA+DHA (p<0.001) and ALA (p<0.001) increased in the Healthy Diet group, only the former was significantly different from the control (p<0.001) and WGED (p<0.001) groups (Table 2).

Glucose and insulin metabolism during the study As previously found [21], although there were no statistical differences in the changes of glucose and insulin metabolism between the groups (p>0.10 for all), the 2 h plasma glucose value decreased in the Healthy Diet (mean±SD: 6.7±1.7 vs 6.1±1.7 mmol/l, p=0.002) and WGED (6.6± 1.6 vs 6.1±1.9 mmol/l, p=0.009) groups; and the glucose AUC also decreased in the Healthy Diet group (mean±SD: 244±132 vs 194±121 mmol/l, p=0.007). Also in the latter we observed improvements in early-phase insulin secretion, estimated as insulinogenic index, and in the composite measure of beta cell function, estimated as the disposition

Table 2 Nutrient intake and *n*-3 fatty acids in plasma lipids at baseline and during the study according to the study interventions and their respective relative changes

Variable	Healthy Diet (*n*=35)			WGED (*n*=34)			Control (*n*=34)			*p* values *for* group effect[b]
	Baseline	Week 12	Change (%)[a]	Baseline	Week 12	Change (%)[a]	Baseline	Week 12	Change (%)[a]	
Nutrient										
Carbohydrates[e]	46.7±7.2	48.2±5.8	6 (−4, 10)	45.7±6.3	47.2±7.5	4 (−3, 15)	47.6±5.5	47.2±5.1	−1 (−7, 8)	0.55
Total fibre (g/day)[c]	29.3±8.3	36.5±6.0	28 (7, 48)	24.6±7.0	26.5±5.4	9 (−5, 25)	22.2±6.9	17.6±4.0	−16 (−30, 4)	<0.001[d]
Fat[e]										
Total fat	32.2±6.1	30.2±4.4	−5 (−14, 6)[f]	33.6±5.2	31.1±6.3	−7 (−20, 9)[f]	31.3±5.4	31.9±6.0	3 (−10, 15)[f]	0.14
Total SFAs	11.9±3.0	10.6±2.4	−8 (−22, −1)[f]	12.3±2.4	11.1±2.8	−8 (−23, 6)[f]	12.0±2.6	12.2±2.8	4 (−13, 17)[f]	0.08
Total MUFAs	10.6±2.6	9.5±1.9	−9 (−14, −3)[f]	11.3±2.1	9.8±2.7	−12 (−20, −4)[f]	10.2±2.5	9.9±2.7	−1 (−9, 7)[f]	0.43
Total PUFAs	5.6±1.5	5.4±1.2	0 (−8, 8)[f]	6.0±1.6	5.0±1.5	−14 (−24, 5)[f]	5.6±1.5	4.6±1.2	−14 (−23, −6)[f]	0.009[g]
ALA (g/day)	1.7 (1.2, 2.3)	2.4 (1.9, 3.0)	45 (10, 102)	1.5 (0.9, 2.1)	1.2 (0.9, 1.7)	−3 (−35, 28)	1.4 (1.1, 2.1)	1.5 (1.2, 2.1)	8 (−25, 35)	<0.001[h]
EPA+DHA (g/day)	0.4 (0.1, 0.7)	2.1 (1.7, 3.4)	498 (230, 1,180)	0.3 (0.1, 0.6)	1.0 (0.6, 1.7)	203 (81, 968)	0.2 (0.1, 0.4)	0.5 (0.2, 1.0)	129 (12, 533)	0.002[i]
Plasma fatty acids										
ALA (18:3 *n*-3) (%)	0.9 (0.7, 1.1)	1.1 (0.9, 1.3)	10 (−1, 33)	1.0 (0.7, 1.3)	0.9 (0.7, 1.1)	−7 (−20, 4)	0.9 (0.8, 1.2)	1.0 (0.8, 1.2)	2 (−25, 35)	<0.001[j]
EPA+DHA (%)[k]	5.3 (3.5, 6.0)	6.7 (4.9, 8.2)	37 (10, 68)	4.6 (3.8, 6.1)	4.4 (3.4, 5.5)	−12 (−24, 13)	4.8 (3.8, 5.9)	4.0 (3.2, 5.6)	−15 (−19, 22)	<0.001[h]

Values are mean±SD or median (IQR)

[a] Per cent change: (mean of intake at weeks 3, 7 and 11−intake at baseline)×100/intake at baseline for the dietary intake; or (week 12−baseline)×100/baseline for the plasma fatty acids

[b] General linear model univariate analysis

[c] $p<0.01$ for the difference in baseline value between the Healthy Diet and control group; [d] $p<0.001$ for all pair-wise comparisons between the groups; [e] percentage of daily energy intake; [f] mean (95% CI); [g] $p<0.01$ for Healthy Diet vs control group; [h] $p<0.001$ for Healthy Diet vs WGED and control groups; [i] $p=0.001$ for pair-wise comparison between Healthy Diet vs control group; [j] $p>0.05$ for all pair-wise comparisons between the study groups

[k] EPA (20:5 *n*-3) + DHA (22:6 *n*-3)

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

index (Table 3). No change in insulin sensitivity between and within groups was observed when estimated either by fasting insulin, HOMA of insulin resistance [21], quantitative insulin sensitivity check index (QUICKI) or by the Matsuda insulin sensitivity index (Table 3).

Changes in plasma inflammatory markers during the study The results of all 11 inflammation- and endothelial function-related markers are described in Table 3. Only E-selectin and hsCRP changed in different ways across the groups or after the 12 week study in within-group analyses.

Plasma concentrations of E-selectin and hsCRP did not differ among groups at baseline ($p>0.10$ for both) (Table 3). Plasma E-selectin concentration in the Healthy Diet group decreased significantly during the intervention ($p<0.05$), but not in the WGED or control groups ($p>0.05$). The change during the Healthy Diet intervention was significantly different from that in the control group, but it was not significant when compared with the WGED after testing for multiple comparisons ($p=0.02$ and $p=0.26$, respectively). Circulating levels of hsCRP decreased only in the WGED group ($p<0.05$), but the changes among the groups were not significantly different ($p>0.05$) (Table 2).

In the Healthy Diet group, there was a higher proportion of individuals with high-risk hsCRP levels (>3.0 mg/l) at baseline in non-statin-users than in statin-users (33.3% vs 0%, $p=0.08$). However, this difference was attenuated after the 12-week intervention (15% vs 0%, $p=0.55$). These respective changes were also seen in the median hsCRP values before (non-statin 1.9 [IQR 0.8, 3.6] vs statin 1.1 [0.7, 1.4], $p=0.10$) and after the intervention (1.1 [0.8, 2.6] vs 1.1 [0.9, 2.2], $p=0.91$).

Figure 2 displays the analyses of hsCRP after excluding participants who used statins during the study. The hsCRP concentrations at baseline were not different among the groups ($p=0.91$). Plasma hsCRP concentrations decreased in individuals following the WGED and Healthy Diet interventions ($p<0.01$ and $p<0.05$, respectively) (Fig. 2). The change in circulating levels of hsCRP in the WGED group was significantly different from that in the control group ($p<0.05$). Plasma E-selectin concentrations at baseline also did not differ among groups ($p=0.55$) in participants not using statins, and decreased only in the Healthy Diet group ($p=0.004$). The change in E-selectin also differed between the Healthy Diet and control groups (median −9% [IQR −15, 1] vs 2% [−7, 9], $p=0.02$), but not between the Healthy Diet and WGED (−2% [−7, 9], $p=0.25$) groups. Adjustment for the changes in BMI, insulin sensitivity and energy intake did not alter the results. Although an increase in energy intake was associated with a decrease in hsCRP levels, a change in BMI or insulin sensitivity was not significantly associated with either of the outcomes in each respective model (data not shown).

Association between changes in dietary intake and changes in plasma E-selectin and hsCRP Additionally, we explored whether the changes in dietary intake of fibre and in dietary intake and plasma percentages of *n*-3 fatty acids could explain the change in E-selectin and hsCRP concentrations in participants not using statins (Table 4). A higher increase in the intake of fibre was significantly associated with a greater decrease in plasma E-selectin during the study. Similarly, a greater increase in the intake and plasma percentage levels of the sum of EPA and DHA was associated with a decrease in plasma E-selectin. However, the effect of the increase in ALA intake on decreasing E-selectin was not reflected by ALA plasma proportions (Table 4).

Interestingly, belonging to the Healthy Diet group was associated with a greater decrease in E-selectin levels compared with the control group (Fig. 2), even when including the change in intake of fibre, EPA+DHA or ALA in the models ($p<0.05$ for the effect of group and for the difference between Healthy Diet and control groups in all models in participants not using statins).

In similar models, but considering hsCRP as a dependent variable, we only found a trend for an association between the increase in ALA intake and the decrease in hsCRP, and this was not confirmed at plasma levels (Table 4). Finally, and still in participants not using statins, a higher intake of test breads in the Healthy Diet and WGED interventions was strongly associated with decreased hsCRP levels (slices/day $\beta=-0.51$, $p<0.001$). Of the various breads, the endosperm rye bread was the one that was highly correlated with the change in hsCRP ($\beta=-0.59$, $p<0.001$), followed by the other commercial rye breads ($\beta=-0.37$, $p<0.01$ for all commercial rye breads).

Discussion

Our results clearly show that a 3 month dietary intervention with an experimental diet high in fatty fish, bilberries and wholegrain products (Healthy Diet) reduced plasma E-selectin circulating levels in individuals with impaired glucose metabolism and features of the metabolic syndrome compared with a control diet that was low in fibre, fatty fish and berries, this reduction being independent of the use of lipid-lowering medication (statins) during the study. We also observed a similar decrease in hsCRP concentration in individuals on the Healthy Diet or the WGED, in the latter of which participants kept to their habitual diet, but replaced refined cereal products with wholegrain cereal products. Consistent with the main findings, greater increases in the intake of total fibre and *n*-3 fatty acids were inversely associated with greater decreases in E-selectin circulating levels.

Table 3 Indexes of insulin and glucose metabolism, and circulating levels of biomarkers related to inflammation and endothelial dysfunction before and after Healthy Diet (n=36), WGED (n=34) and control (n=34) dietary interventions and their respective relative changes

	Study stage			p values for		
Variable	Baseline	Week 12	Change (%)[a]	Group effect[b]	Overall change[b]	Within group[c]
Matsuda ISI[d]						
Healthy Diet	3.89 (2.95, 5.15)	3.44 (2.82, 5.08)	−4 (−18, 22)	0.85	<0.001	0.26
WGED	3.53 (2.55, 6.34)	4.10 (2.34, 5.66)	−1 (−19, 16)			0.84
Control	4.18 (2.44, 5.51)	4.06 (2.47, 5.27)	−1 (−25, 18)			0.58
Insulinogenic index[d]						
Healthy Diet	123 (82, 165)	142 (90, 211)	13 (−11, 86)	0.21	<0.001	0.02
WGED	123 (82, 221)	167 (83, 201)	−8 (−25, 51)			0.95
Control	129 (84, 200)	124 (88, 181)	−13 (−29, 23)			0.75
QUICKI[d,e]						
Healthy Diet	0.33±0.02	0.32±0.02	−1 (−2.5, 0.7)	0.83	<0.001	0.18
WGED	0.33±0.02	0.32±0.03	−1 (−2.4, 0.7)			0.10
Control	0.32±0.02	0.32±0.02	−1 (−1.7, 0.8)			0.39
Disposition index[d]						
Healthy Diet	41.0 (27.9, 52.6)	47.2 (29.3, 72.9)	15 (−18, −22)	0.20	<0.001	0.03
WGED	41.1 (27.0, 69.5)	54.0 (27.1, 65.5)	−5 (−19, −16)			0.99
Control	43.2 (28.7, 62.7)	40.5 (26.8, 59.6)	−13 (−25, 18)			0.68
E-selectin (μg/l)[d]						
Healthy Diet	33.2±13.8	31.5±13.3	−8 (−15, 3)	0.02	<0.001	0.04
WGED	31.7±10.7	32.3±12.4	1 (−6, 9)			0.98
Control	28.5±11.5	30.0±12.7	2 (−7, 15)			0.13
hsCRP (mg/l)[d]						
Healthy Diet	1.4 (0.7, 3.1)	1.1 (0.9, 2.5)	−10 (−37, 41)	0.20	<0.001	0.22
WGED	1.5 (0.7, 3.9)	1.2 (0.6, 1.9)	−20 (−40, 11)			0.02
Control	1.4 (0.8, 2.3)	1.3 (0.9, 2.0)	−8 (−35, 49)			0.97
TNF-α (ng/l)[d]						
Healthy Diet	0.6 (0.5, 0.9)	0.6 (0.4, 0.8)	5 (−25, 33)	0.35	<0.001	0.43
WGED	0.7 (0.5, 0.9)	0.6 (0.5, 0.8)	9 (−32, 46)			0.75
Control	0.6 (0.2, 0.9)	0.5 (0.3, 0.9)	3 (−18, 69)			0.65
IL-6 (ng/l)[d]						
Healthy Diet	1.6 (1.0, 2.6)	1.5 (1.0, 2.1)	−7 (−25, 13)	0.66	<0.001	0.29
WGED	1.4 (1.0, 2.3)	1.5 (1.1, 1.9)	3 (−15, 31)			0.97
Control	1.3 (0.8, 2.0)	1.4 (1.1, 1.8)	3 (−11, 35)			0.35
IL1RA (ng/l)[d]						
Healthy Diet	329 (210, 417)	299 (232, 447)	5 (−14, 30)	0.13	<0.001	0.43
WGED	301 (214, 468)	300 (171, 372)	−7 (−22, 16)			0.75
Control	245 (179, 348)	238 (192, 413)	7 (−6, 27)			0.65
SAA (ng/l)[d]						
Healthy Diet	3.0 (2.0, 5.1)	2.9 (2.0, 4.2)	−2 (−28, 19)	0.26	<0.001	0.29
WGED	2.9 (2.0, 4.1)	2.9 (2.1, 3.6)	0 (−19, 20)			0.97
Control	3.4 (2.0, 4.8)	3.3 (2.0, 5.2)	0 (−16, 28)			0.35
ADMA (μmol/l)[d,e]						
Healthy Diet	0.63±0.10	0.62±0.13	−1 (−7, 5)	0.15	0.32	
WGED	0.63±0.12	0.66±0.14	6 (−1, 13)			
Control	0.65±0.13	0.63±0.12	0 (−8, 7)			
vWF (%)[d,f]						
Healthy Diet (n=34)	114 (82, 164)	117 (87, 176)	16 (−18, 27)	0.26	<0.001	0.29

Table 3 (continued)

Variable	Study stage			*p* values for		
	Baseline	Week 12	Change (%)[a]	Group effect[b]	Overall change[b]	Within group[c]
WGED (*n*=33)	138 (94, 180)	153 (112, 211)	11 (−26, 59)			0.97
Control (*n*=32)	122 (85, 155)	115 (69, 165)	−11 (−28, 38)			0.35
CCL5 (μg/l)[d,g]						
Healthy Diet	11.3 (8.2, 17.3)	9.8 (6.5, 17.1)	−7 (−46, 63)	0.45	<0.001	
WGED	12.2 (8.1, 17.3)	11.2 (6.7, 16.2)	−16 (−51, 47)			
Control (*n*=33)	11.3 (7.2, 15.2)	14.3 (7.8, 17.8)	26 (−38, 106)			
sICAM-1 (μg/l)[d,h]						
Healthy Diet	601 (521, 702)	587 (534, 676)	1 (−8, 9)	0.89	0.60	0.29
WGED (*n*=33)	602 (495, 701)	585 (443, 692)	−1 (−10, 8)			0.97
Control	596 (502, 655)	593 (528, 673)	2 (−11, 10)			0.35
MIF-1 (μg/l)[d]						
Healthy Diet	6.5 (4.9, 9.3)	7.0 (4.6, 9.6)	13 (−29, 55)	0.60	<0.001	0.29
WGED	8.1 (5.3, 11.2)	8.5 (4.5, 12.8)	−9 (−43, 46)			0.97
Control	7.3 (5.1, 10.3)	8.4 (5.5, 10.7)	26 (−30, 69)			0.35

Data are mean±SD or median (IQR)

Matsuda insulin sensitivity index (ISI) was calculated as: 10,000/√(fasting glucose×fasting insulin×average glucose at 0, 30 and 120 min×average insulin at 0, 30 and 120 min during an OGTT) [51]

Insulinogenic index was calculated as (insulin 30 min−insulin 0 min, pmol/l)/(glucose 30 min−glucose 0 min, mmol/l) during an OGTT

QUICKI (quantitative insulin sensitivity check index) was calculated as: 1/(lg10 [insulin 0 min]+lg10 [glucose 0 min]) [21]

Disposition index was calculated as a product of insulinogenic index and QUICKI

[a] Change as: (week 12−baseline)×100/baseline

[b] General linear model: univariate analysis adjusted for group, age, sex and baseline value

[c] For the difference between baseline vs week 12 after Student's paired *t* test; [d] one-way ANOVA, $p>0.10$ for the difference among the groups at baseline

[e] Data are mean (95% CI) for per cent change

[f] Five participants whose measurements of vWF (Healthy Diet *n*=2, WGED *n*=1, control *n*=2) were non-detectable (above the maximum analytical range) were excluded from the analyses

[g] One participant whose measurements of CCL5 (control *n*=1) was non-detectable (above the maximum analytical range) was excluded from the analyses

[h] One participant whose measurements of sICAM-1 in the WGED group were too low at baseline and week 12 (<0.1 μg/l) was excluded from the analyses

ADMA, asymmetric dimethylarginine; CCL5, chemokine (C-C motif) ligand 5; IL-1RA, IL-1 receptor antagonist; MIF-1, macrophage migration inhibitory factor; sICAM-1, soluble intercellular cellular adhesion molecule-1; vWF, von Willebrand factor

In our study, the Healthy Diet had a beneficial impact on endothelial function as estimated by circulating levels of E-selectin. Dysfunction of the endothelium plays an integral role in atherogenesis and CVD. E-selectin, one of the molecules produced during endothelial injury and released into the circulation, has been used as a molecular marker of endothelial dysfunction [2, 24, 25]. In epidemiological and intervention studies, a benefit of *n*-3 polyunsaturated fatty acids on cardiovascular health [26, 27] and plasma E-selectin levels [28] has been suggested. A prudent diet pattern, rich in fish, whole grains and fruit, has been inversely associated with circulating levels of E-selectin and CRP [29].

We observed that the change in the intake of fibre was a determinant of the change in E-selectin levels. The total daily fibre intake increased in the WGED intervention due to the consumption of test breads. However, the intake of fibre in the Healthy Diet was even higher due to the consumption not only of the test breads, but also of bilberries [22], which likely contributed to the observed reduction of plasma E-selectin and the association between plasma E-selectin and fibre intake in this group. On the other hand, fibre intake alone may not explain the results. Bilberries (from the *Vaccinium* family), for example, are particularly abundant in polyphenols, and anthocyanins account for most of the bilberry total polyphenol content

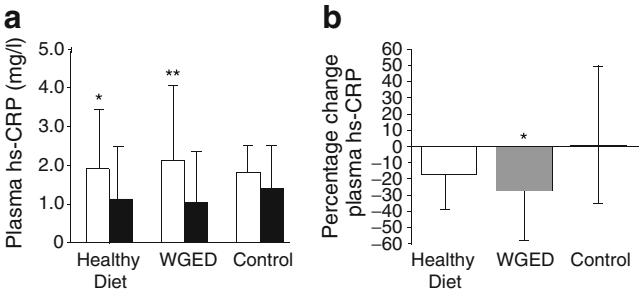

Fig. 2 Plasma concentrations of hsCRP according to randomisation group in participants not using statins during the study. **a** hsCRP before (baseline; white bars) and after (week 12; black bars) in Healthy Diet (n=27), WGED (n=24) and control (n=25) dietary interventions. *p<0.05 for baseline vs week 12 after Student's paired t test; **p<0.01 for baseline vs week 12 after Student's paired t test. **b** hsCRP relative changes ([week 12−baseline]×100/baseline) in Healthy Diet (n=27), WGED (n=24) and control (n=25) dietary interventions. p=0.91 for the difference among groups at baseline after one-way ANOVA; p=0.04 for the group effect in general linear model univariate analysis; *p<0.05 for the difference between WGED and control groups after Bonferroni correction for multiple comparisons. Values (**a**, **b**) are median and IQR

[30]. Bilberry anthocyanins have been shown to exert a wide range of biological effects, including antioxidant and vasodilatory actions [17]. Moreover, these anthocyanins and other phenolic compounds also found in bilberries may provide substantial antioxidant protection [31], which could benefit endothelial function and decrease CVD risk [17, 32].

Our results suggest that the increase in dietary and plasma n-3 fatty acids plays a role in the increase in E-selectin levels. However, data from intervention trials on the beneficial effects of fish intake on surrogate markers of endothelial function are controversial [33]. The source of EPA+DHA may also be important, as fish-based diets have been proposed to have a more beneficial effect than fish oil supplements on circulating inflammatory markers [34]. Although a greater increase in the intake of ALA was associated with an increase in E-selectin levels, this association was not reflected at the plasma level, so this result must be treated with caution.

Overall, the Healthy Diet and WGED interventions had an anti-inflammatory effect as estimated by within-group changes in hsCRP. A Mediterranean-style diet, in which whole grains and fibre are highly consumed, has been shown to ameliorate circulating levels of CRP and to reduce the risk of type 2 diabetes mellitus [35, 36]. Fish intake and n-3 fatty acids have been shown to decrease CRP levels [37], albeit not in all studies [38, 39]. Based on our study design, we hypothesised that there would be a greater decrease in this inflammatory marker with the Healthy Diet than with the WGED. However, we did not find any significant association between the changes in dietary n-3 fatty acids and circulating levels of hsCRP during the study. Moreover, data on the beneficial effect of bilberry intake on hsCRP levels are lacking, suggesting that other nutrients or phytochemical compounds could also have contributed to the decrease in CRP levels.

One of the most interesting findings in our study is that a higher intake of test breads (90% of which were rye breads) by participants in the Healthy Diet and WGED interventions was associated with a greater decrease in hsCRP levels. Previous work conducted by our colleagues has shown that rye breads, regardless of fibre content, have beneficial effects on insulin metabolism by lowering postprandial insulin response and increasing early-phase insulin secretion [19, 20]. Hypo-

Table 4 Regression coefficients (β) for the effect of the change in the intake of fibre, and of the changes in plasma and dietary ALA and EPA+DHA on the changes in plasma E-selectin or in hsCRP in participants not using statins during the study

Variables	Change in E-selectin		Change in hsCRP	
	β	p value	β	p value
Nutrients, change in[a]				
Fibre intake	−0.26	0.03	−0.05	0.63
ALA intake	−0.31	0.03	−0.21	0.08
EPA+DHA intake	−0.34	0.05	−0.19	0.21
Plasma fatty acids (change in)[b]				
ALA (18:3 n-3)	−0.07	0.63	0.00	0.99
EPA (20:5 n-3)+DHA (22:6 n-3)	−0.27	0.03	−0.16	0.13

n=75

[a] Multiple linear regression model where each of the independent variables listed in the table was analysed separately in a model containing the respective variable at baseline, the change and baseline value of energy intake, and the dependent variable at baseline

[b] Multiple linear regression model where each of the independent variables listed in the table was analysed separately in a model containing the respective variable at baseline and the dependent variable at baseline

thetically, increased early postprandial hyperinsulinaemia can also be pro-inflammatory [40]. In this context, it has also been shown that consumption of a diet (rye bread and pasta) geared to low insulin response might have anti-inflammatory properties [41].

Other factors, such as changes in body weight, insulin sensitivity, saturated fat and energy intake, could also have affected the decreases observed in inflammatory and endothelial markers after the dietary interventions [37, 42–45]. However, in multivariate analyses taking into account the minor changes in BMI or the recorded saturated fat or energy intakes during the study the results were not altered with respect to E-selectin and hsCRP. Furthermore, our results suggest that insulin sensitivity also does not explain the beneficial effects of the dietary interventions on these inflammatory markers.

We selected individuals at increased risk of CVD and type 2 diabetes for this study [46, 47]. Elevated circulating levels of E-selectin, among other markers of endothelial dysfunction, and high hsCRP levels have been found to predict type 2 diabetes mellitus [4–6, 24] and CVD [3, 25, 48]. Moreover, lifestyle interventions involving dietary changes, which are able to reduce the risk of diabetes, have also improved low-grade inflammation as measured by CRP [36, 43]. Therefore, the anti-inflammatory effect of the intervention diets in the present study may be important in the long-term prevention of type 2 diabetes mellitus and CVD.

Our observations also hint at a potential role of the Healthy Diet in lowering hsCRP close to the levels seen in statin users, as also previously shown when statin therapy was compared with cholesterol-lowering diets [49]. Taking this together with the beneficial effect of the above diet on E-selectin concentrations, it seems possible that the potential anti-atherogenic effect of a Healthy Diet could be through other mechanisms than the lowering of LDL-cholesterol, including, for example, HDL-cholesterol metabolism, and anti-inflammatory and antioxidant properties [50].

In this study, the individuals from the control group decreased their fibre intake by replacing their usually consumed breads with refined wheat breads and by limiting their consumption of bilberries. Considering the normal eating habits in Eastern Finland, where consumption of rye bread and berries is common, these dietary instructions needed to be given in order to determine the effects of the experimental diets. The high intake of fibre at baseline in the Healthy Diet group could also have biased our findings. However, adjustments for this variable in the models did not alter the main findings on the changes in E-selectin and hsCRP levels after the interventions.

In conclusion, a diet high in fatty fish, bilberries and wholegrain products may decrease inflammation and endothelial dysfunction as measured by hsCRP and E-selectin, an effect that occurs independently of insulin sensitivity, in overweight and obese individuals at high risk of developing type 2 diabetes and CVD.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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