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ORIGINAL ARTICLE

# Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women

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**Background/Objectives:** Dietary habits have a major role in obesity, type 2 diabetes and atherosclerotic cardiovascular diseases. In this study, we compared the effects of sea buckthorn (SB) and its fractions, and bilberries (BBs) on associated variables of metabolic diseases on overweight and obese women.
**Subjects/Methods:** In total, 110 female volunteers were recruited, and they followed four different berry diets (BB, SB, SB phenolic extract (SBe) and SB oil (SBo)) in a randomized order for 33–35 days. Each intervention was followed by a wash-out period of 30–39 days. Blood samples were drawn and physical measurements were performed after each period. Eighty volunteers completed the study.
**Results:** There was statistically significant decrease in waist circumference after BB (Δ, −1.2 cm; $P=0.041$) and SB (Δ, −1.1 cm; $P=0.008$) periods and also a small decrease in weight after BB diet (Δ, −0.2 kg; $P=0.028$). Vascular cell adhesion molecule decreased after BB (Δ, −49.8 ng/ml; $P=0.002$) and SBo (Δ, −66.1 ng/ml; $P=0.001$) periods, and in intercellular adhesion molecule (ICAM) after SBe diet (Δ, −6.1 ng/ml; $P=0.028$).
**Conclusions:** Based on the results, it can be stated that different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases.
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**Keywords:** adhesion molecules; metabolic syndrome; northern berries

## Introduction

Dietary habits have a major role in obesity, type 2 diabetes and atherosclerotic cardiovascular diseases. By dietary and other lifestyle changes and subsequent weight loss, type 2 diabetes is preventable even in many of those individuals who have already presented an impaired glucose tolerance (Lindström *et al.*, 2003; Uusitupa *et al.*, 2003).

The health promoting effects of berries have been increasingly investigated during the past few years, although direct evidence that berries could decrease the risk of type 2 diabetes is still scarce. The studies concerning the effects of sea buckthorn (SB) and/or bilberry (BB) on metabolic diseases are presented below. In animal studies SB has shown positive effects on cardiovascular health (Yang *et al.*, 2007; Pang *et al.*, 2008; Koyama *et al.*, 2009) and diabetes (Zhang *et al.*, 2010). Also, positive effects of anthocyanin extract from blueberry on vascular health have been proposed based on *in vitro* (Bell and Gochenaur, 2006) and animal trials (Cohen-Boulakia *et al.*, 2000).

Moreover, already in 1989 BB extract has been shown to reduce thrombocyte activation in humans (Pulliero *et al.*, 1989). Lately in human trials further positive indications have emerged. Homogenized SB berries, even at a low 28 g daily dose, have positive effects on the inflammation marker C-reactive protein in healthy normal-weight humans

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(Larmo *et al*., 2007). In a recent study, BB juice decreased C-reactive protein, interleukin (IL)-6, IL-15 and monokine, but had no effect on tumor necrosis factor (TNF)-α (Karlsen *et al*., 2010). SB juice seems also to have slight positive effects on cardiovascular health indicators (Ecclestone *et al*., 2002). A daily dose of 5 g sea buckthorn oil (SBo) reduces both the velocity and the total amount of platelet aggregation in healthy men (Johansson *et al*., 2000). Blueberry inhibits dietary fat-induced postprandial oxidative stress (Kay and Holub, 2002) and some indications of longer-lasting antioxidant effects in body of heavy smokers exist (McAnulty *et al*., 2005). Further, SB and blueberry concentrate tends to increase the plasma antioxidant capacity in type 1 diabetic children (Nemes-Nagy *et al*., 2008).

An earlier study of ours showed positive effects of berries and berry products on serum alanine aminotransferase (ALAT) activity, a biochemical marker of non-alcoholic fatty liver (Lehtonen *et al*., 2010). In the present clinical trial, our aim was to compare the effects of two berries used in the previous study, BB and SB, as well as of two edible fractions of SB, the phenolic alcohol extract (SBe) and SBo, on variables of overweight-associated diseases. The aim was both to assess whether either of these berries would be effective on reducing ALAT activity and to assess the possible effects on the other associated variables of metabolic diseases.

## Materials and methods

*Test products*

Four berry products were tested in the study. The BBs were frozen, whole berries and the SB berries of *Hippophaë rhamnoides* ssp. *turkestanica* were air dried after harvesting. Oils were extracted from berries (berry oil) and seeds (seed oil) with supercritical $CO_2$. The SBo used in the study was a standardized product containing both berry oil and seed oil. After $CO_2$ extraction, the residues were further extracted with 50% aqueous ethanol at 50 °C for phenolic compounds. After removal of ethanol, the phenolic extract was spray dried at 60–70 °C into a free-flowing powder with the aid of maltodextrin DE 6. The phenolic extract powder used in the study contained native SBe and maltodextrin DE 6 at a ratio of 1:1.

*Study subjects*

In total, 110 female volunteers were recruited through a newspaper announcement and other local advertisements. General health status and suitability for the study were checked by an interview and by biochemical laboratory tests based on the first basal sample. In total, 304 subjects were briefly interviewed for their health status and body mass index (BMI) (inclusion criteria BMI 26–34 kg/m$^2$), and 219 volunteers passed the interview and were tested for their suitability for the study by laboratory tests. Inclusion criteria were cholesterol 4.5–8 mmol/l, low-density lipoprotein cholesterol >2.5 mmol/l, triacylglycerol <4 mmol/l, glucose <6 mmol/l, insulin <25 mU/l, blood pressure <160/99 mm Hg, hemoglobin >120 g/l (anemia), thyroid-stimulating hormone 0.3–4.2 mU/l (thyroid function), ALAT <60 U/l (liver function) and creatinine <115 μmol/l (kidney function). Exclusion criteria were pregnancy, menopause, regular smoking, previously diagnosed diabetes (other than gestational), thyroid, renal, hematological, or hepatic dysfunction, previous myocardial infarction, cardiovascular medication, treatment with lipid-lowering drugs and ongoing inflammatory disease. Study subjects were also questioned about other medications used, and all the subjects using regular medication other than allergy medication or joint lubricates were excluded. Baseline characteristics of the subjects are presented in Table 1.

A study with randomized cross-over study design was created, in which the only difference between the intervention (33–35 days) and wash-out (30–39 days) periods was the berry products consumed. Each study subject consumed the four berry diets, namely BB, SB berry, SBe and SBo, in an independently randomized order.

Based on our earlier work (Lehtonen *et al*., 2010), 85 subjects formed the minimal size of study population needed to achieve sufficient statistical power. In the calculations, differences in the low-density lipoprotein cholesterol values between the intervention and the control groups were utilized. The value of $\delta$ (avg2−avg1) was 0.14 mmol/l, s.d. was 0.23 mmol/l and the level of significance ($\alpha$) used in the calculations was 0.05. The size of the study population needed to achieve statistical significance was calculated with equation $n = (2\text{s.d.}2)/(\text{avg2}-\text{avg1})2 \times f(\alpha\beta)$.

The amount of berries/berry fractions in the berry diets was equivalent to an average daily dose of 100 g fresh berries. According to a survey by National Public Health Institute,

**Table 1** Baseline characteristics of subjects ($n = 80$)

| | *Average ± s.d.* |
|---|---|
| Age (years) | 44.2 ± 6.2 |
| Weight (kg) | 81.6 ± 8.5 |
| Height (cm) | 165.7 ± 6.1 |
| BMI (kg/m$^2$) | 29.6 ± 2.1 |
| Waist circumference (cm) | 95.8 ± 7.3 |
| Systolic blood pressure (mm Hg) | 133.3 ± 14.3 |
| Diastolic blood pressure (mm Hg) | 84.2 ± 7.9 |
| Serum glucose (mmol/l) | 5.3 ± 0.4 |
| Serum insulin (IU/l) | 7.7 ± 3.5 |
| Serum cholesterol (mmol/l) | 5.7 ± 0.7 |
| Serum HDL cholesterol (mmol/l) | 1.6 ± 0.4 |
| Serum LDL cholesterol (mmol/l) | 3.5 ± 0.6 |
| Serum triacylglycerols (mmol/l) | 1.7 ± 0.8 |
| ALAT (IU/l) | 21.0 ± 9.1 |
| Number of subjects meeting MetS criteria[a] | 21 |

Abbreviations: ALAT, alanine aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and MetS, metabolic syndrome.
[a]Number of subjects meeting the MetS criteria was evaluated by International Diabetes Federation (IDF) 2005 criteria (Alberti *et al*., 2006).

in Finland, a berry consumption of over 45 g/day can be regarded as high (Similä *et al*., 2005). To remain a constant daily energy intake during all intervention and wash-out periods, the study subjects were instructed to replace part of their original diet with the berry products provided during the intervention periods. Flow chart of study design is presented in Figure 1.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki (2000), and all procedures involving the human subjects were approved by the Ethics Committee of the Hospital District of Southwest Finland. Written informed consents were obtained from all subjects. The study products used in the study were safe berry products.

*Blood samples*

Blood samples were taken after an overnight fast (12 h) between 07:00 and 11:30 h a.m. from each study subject before the trial and after each of the intervention and wash-out periods. The study subjects were instructed to avoid alcohol and all medication for 2 days before sample collection. Plasma, serum and glucose tubes were centrifuged at 2200 *g* for 10 min.

*Physical measurements and clinical analyses*

Body composition was determined by bioelectrical bioimpedance analysis. Body weight of bare-foot subjects wearing light indoor clothing was recorded to the nearest 0.1 kg by a calibrated weighing scale (Inbody 3.0, Sunborn Saga Oy, Turku, Finland) and total body fat mass, fat-free mass and fat percentage calculated from impedance values. Body height was recorded to the nearest 0.5 cm. Waist circumference was measured midway between *spina iliaca superior* and the lower rib margin. Blood pressure was measured in duplicate using the automated Omron M4-I device (Normomedical Oy, Helsinki, Finland).

Serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triacylglycerols were measured from plasma samples by enzymatic photometric methods with commercial kits (Thermo Clinical Labsystems Oy, Espoo, Finland) using the Konelab20i analyser (Thermo Clinical Labsystems Oy, Konelab, Finland). Plasma glucose was analyzed by enzymatic photometric method using Konelab Glucose HK as reagent. ALAT and γ-glutamyl transpeptidase were analyzed by kinetic enzymatic method (International Federation of Clinical Chemistry (IFCC)), and glycated hemoglobin and serum high-sensitive C-reactive protein by immunoturbidimetric method with a Konelab20i analyser and thyroid-stimulating hormone was analyzed using Axsym (Abbott Diagnostics, Espoo, Finland).

Serum insulin was analyzed by chemiluminescence-immunoassay with an Immulite 1000 analyser (Siemens Medical Solutions, Espoo, Finland). Hematological parameters were measured with a CellDyn analyser (Abbott Diagnostics).

Soluble adhesion molecules such as soluble intercellular adhesion molecule (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1) and adiponectin were simultaneously measured from the plasma samples with an Millipore's Human CVD1-kit (HCVD1-67AK) (Millipore, Billerica, MA, USA), and TNF-α as well as IL-6 with an Millipore's Human Serum Adipokine (Panel B, HADK2-61K-B) using a Bio-Rad Bio-Plex 200 System (Bio-Rad Laboratories, Espoo, Finland). Hemoglobin A1c analysis and blood cell count were performed from fresh blood collected into EDTA tubes by an immunoturbidimetric method.

*Compliance*

The study subjects were requested to write down all the subjective health symptoms 5 days before each sample collection day. During all the intervention and wash-out periods, they were asked to keep 3-day food diaries. During the whole clinical trial, the subjects filled a diary in which they wrote down possible diseases and drugs taken, berries consumed (both test products and other berries) and physical activities. In the beginning of the first intervention period, the overall health and lifestyle of the study subjects were estimated based on a questionnaire modified from the FINRISK Physical Activity Questionnaire (Laatikainen *et al*., 2007).

Caloric and main nutrient intakes during the intervention periods were estimated based on the 3-day food records collected during each intervention and wash-out period. Nutrient and total energy intakes during the intervention periods and an average of all wash-out periods are presented in Supplementary Material. During the SB period there was an increase in total energy, fat (both saturated and unsaturated fatty acids), carbohydrate (also sugars), magnesium, potassium and vitamin E intake. All of these changes were not due to SB intake, as the daily berry portion contained 1.04 g of sugars whereas the increase in sugar intake was 16 g/day. It can be concluded that the study subjects changed their diet during the SB period by incorporating other food items into their diet. Also during the BB period, sugar and carbohydrate intakes were higher than during the wash-out periods. During the SBe diet there

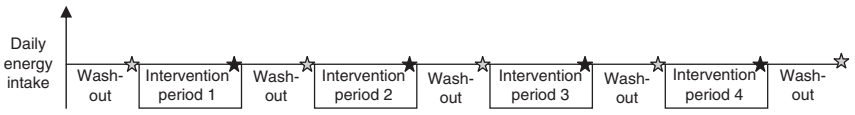


**Figure 1** Flow chart of study design. Daily energy intake was intended to be constant during the whole study period. Length of each wash-out period was 30–39 days and of each intervention was 33–35 days. Stars indicate sampling days.

were no statistically significant changes in nutrient intakes, and during the SBo diet the only difference was the increased intake of mono- and polyunsaturated fatty acids as well as fat-soluble vitamins.

*Statistical analyses*

The changes in the parameters after berry interventions were calculated by reduction of the average of two wash-out values (one of the samples taken before the berry intervention and the other one taken after another 35 days wash-out following the berry intervention) from the value obtained at the end of the berry intervention. General linear model repeated measurements variance analysis was used for all results to compare the changes in different berry interventions with the average change of all different wash-out periods. Statistical significance was indicated by $P<0.05$. Statistical analyses were performed using SPSS 14.0 (Somers, New York, NY, USA).

## Results

Observed changes in clinical parameters determined from fasting samples taken before and after each intervention and wash-out period are shown in Table 2.

**Table 2** The changes in the fasting blood samples of those measured parameters that changed statistically significantly during intervention periods

| *Parameter (fasting)* | *Bilberry* | | | |
|---|---|---|---|---|
| | *Average ± s.d.* | | *Absolute change*[a] | *Significance of change*[b] |
| | *Wash-out*[c] | *Berries* | | |
| Weight (kg) | 81.4 ± 8.7 | 81.3 ± 8.7 | −0.2 ± 1.0 | 0.028 |
| Waist circumference (cm) | 95.2 ± 7.7 | 94.0 ± 9.0 | −1.2 ± 4.5 | 0.041 |
| Insulin (mU/l) | 8.1 ± 3.1 | 8.7 ± 4.0 | 0.5 ± 3.2 | 0.048 |
| $GHbA_{1c}$ (%)[a] | 5.2 ± 0.3 | 5.4 ± 0.3 | 0.2 ± 0.3 | 0.000 |
| VCAM-1 (ng/ml)[a] | 872 ± 129 | 820 ± 155 | −49.8 ± 132.6 | 0.002 |
| TNF-α (pg/ml)[a] | 4.9 ± 1.9 | 4.7 ± 1.9 | −0.2 ± 1.6 | 0.031 |
| Adiponectin (μg/ml)[a] | 25.9 ± 12.9 | 23.3 ± 11.8 | −2.8 ± 4.6 | 0.000 |
| | *SB berries* | | | |
| | *Average ± s.d.* | | *Absolute change*[c] | *Significance of change*[a] |
| | *Wash-out*[d] | *Berries* | | |
| Waist circumference (cm) | 95.4 ± 7.2 | 94.3 ± 7.8 | −1.1 ± 3.0 | 0.008 |
| Glucose (mmol/l) | 5.1 ± 0.3 | 5.0 ± 0.4 | −0.1 ± 0.3 | 0.002 |
| $GHbA_{1c}$ (%)[a] | 5.2 ± 0.3 | 5.4 ± 0.3 | 0.2 ± 0.3 | 0.000 |
| TNF-α (pg/ml)[a] | 4.7 ± 1.7 | 4.5 ± 1.7 | −0.2 ± 1.5 | 0.023 |
| | *SB extract* | | | |
| | *Average ± s.d.* | | *Absolute change*[c] | *Significance of change*[a] |
| | *Wash-out*[d] | *Berries* | | |
| $GHbA_{1c}$ (%)[a] | 5.1 ± 0.4 | 5.3 ± 0.3 | 0.1 ± 0.3 | 0.000 |
| ICAM-1 (ng/l)[a] | 184.0 ± 29.9 | 178.3 ± 31.4 | −6.1 ± 24.6 | 0.028 |
| TNF-α (pg/ml)[a] | 4.8 ± 1.5 | 4.5 ± 1.7 | −0.3 ± 1.2 | 0.000 |
| | *SB berry oil* | | | |
| | *Average ± s.d.* | | *Absolute change*[c] | *Significance of change*[a] |
| | *Wash-out*[d] | *Berries* | | |
| Waist circumference (cm) | 95.7 ± 8.2 | 94.5 ± 7.2 | −1.2 ± 3.8 | NS (0.077) |
| $GHbA_{1c}$ (%)[a] | 5.2 ± 0.4 | 5.3 ± 0.3 | 0.2 ± 0.3 | 0.000 |
| hs-CRP (mg/l) | 2.0 ± 1.7 | 2.4 ± 2.4 | 0.5 ± 5.6 | 0.006 |
| VCAM-1 (ng/ml)[a] | 882.1 ± 128.9 | 814.8 ± 158.1 | −66.1 ± 170.0 | 0.001 |
| Adiponectin (μg/ml)[a] | 26.5 ± 13.5 | 24.2 ± 11.4 | −2.4 ± 6.5 | 0.004 |

Abbreviations: CRP, C-reactive protein; $GHbA_{1c}$, glycated hemoglobin A1c; ICAM, intercellular adhesion molecule; NS, non-significant; SB, sea buckthorn; TNF, tumor necrosis factor; and VCAM, vascular cell adhesion molecule.
Average values, their standard deviations, average absolute changes, their standard deviations and statistical significances are given.
[a]Change between berry intervention and two wash-out periods, one before and the other after the berry intervention.
[b]Statistical significance calculated between the absolute change (described above at superscript a) and the average change of all four wash-out periods (superscript d).
[c]Average of two wash-out periods, one before and the other after the berry intervention.

In fat percent, blood pressure, fasting plasma cholesterol, triacylglycerol, ALAT or IL-6 levels, there were no statistically significant changes due to any of the berry interventions when compared to the wash-out periods.

Statistically significant changes in waist circumference after BB (Δ, −1.2 cm; $P=0.041$) and SB (Δ, −1.1 cm; $P=0.008$) periods were observed. A decreasing but non-significant trend was also observed after the other berry interventions (SBo (Δ, −1.2 cm ± 3.8, NS; $P=0.077$) and SBe (Δ, −0.1 cm ± 4.1, NS)). Accordingly, there was a small decrease in weight after BB diet (Δ, −0.2 kg; $P=0.028$), but after the SBe and SBo interventions the decrease was statistically non-significant (Figure 2). Graphics of the non-significant fat percent changes follows the common trend of the other parameters in Figure 2. These changes were small, but indicative of positive trend.

Statistically significant decrease in VCAM after BB (Δ, −49.8 ng/ml; $P=0.002$) and SBo (Δ, −66.1 ng/ml; $P=0.001$) periods was found, and in ICAM after SBe diet (Δ, −6.1 ng/ml; $P=0.028$). In other interventions the changes were not significant, but never showed an increasing trend (Figure 3). Decrease in VCAM concentrations was higher in subjects with higher BMI after SBo intervention. There was no change in the study subjects with BMI 26.6–27.7. The highest reduction in VCAM was observed in the study subjects with baseline BMI 29.5–31.2. During BB diet the change was approximately the same in different BMI quartiles, except in the fourth quartile (BMI 31.2–39.7) where the change was minor. After SB diet only the most lean subjects (BMI 26–27.7) had reduction in VCAM values, whereas after SBe diet the reduction was highest for the most overweight/obese subjects (BMI 31.2–39.7).

There was a small decrease in fasting plasma glucose (Δ, −0.1 mmol/l; $P=0.002$) after the SB diet. TNF-α levels decreased with BB (Δ, −0.2 pg/ml; $P=0.031$), SB (Δ, −0.2 pg/ml; $P=0.023$) and SBe (Δ, −0.3 pg/ml; $P=0.000$) interventions.

In contrast, a small but statistically significant increase in the proportion of glycated hemoglobin A1c ($GHbA_{1c}$) was observed after each berry period (Δ, 0.2% after BB, SB and SBo interventions, and 0.1% after SBe period), and in fasting plasma insulin after BB (Δ, 0.5 mU/l; $P=0.048$). A statistically significant decrease in TNF-α values was found after BB (Δ, −0.2 pg/ml; $P=0.031$), SB (Δ, −0.2 pg/ml; $P=0.023$) and SBe (Δ, −0.3 pg/ml; $P=0.000$) diets. There was a decrease in adiponectin after BB (Δ, −2.8 μg/ml; $P=0.000$) and SBo (Δ, −2.4 μg/ml; $P=0.004$) diets.

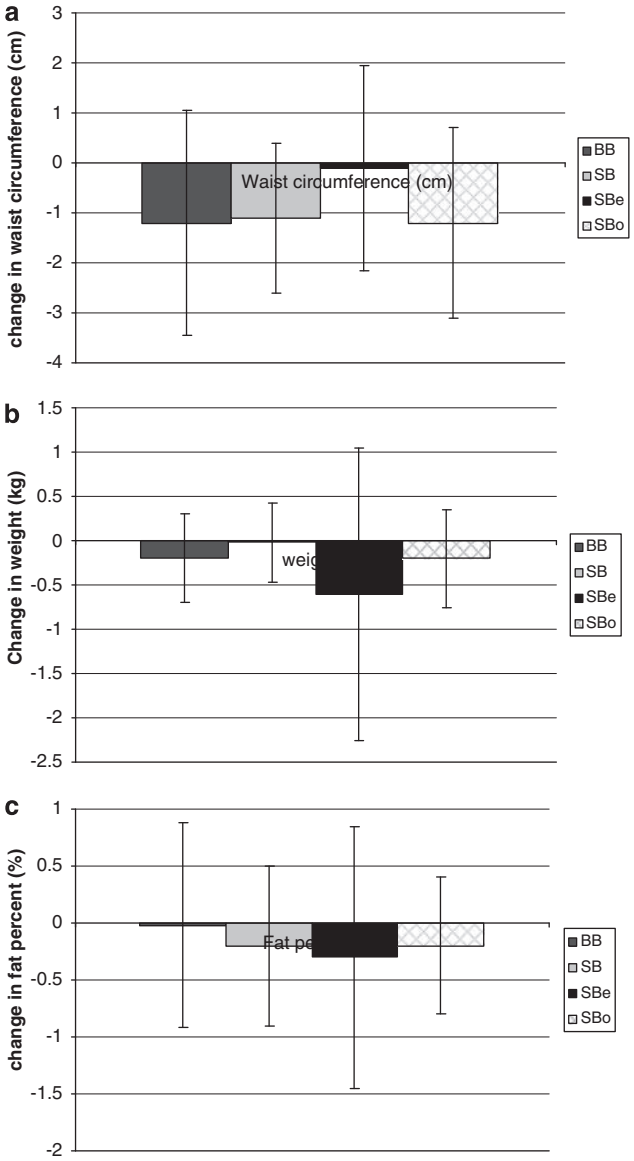


**Figure 2** Average changes and standard deviations in waist circumference (cm, **a**), body weight (kg; **b**) and fat percent (%, **c**) during each intervention period (BB (weight Δ, −0.2 kg ± 1.0; waist Δ, −1.2 cm ± 4.5; fat percent Δ, −0.0% ± 1.8); SB (weight Δ, −0.0 kg ± 0.9; waist Δ, −1.1 cm ± 3.0, fat percent Δ, −0.2 cm ± 1.4); SBe (weight Δ, −0.6 kg ± 3.3, waist Δ, −0.1 cm ± 4.1; fat percent Δ, −0.3% ± 2.3); and SBo (weight Δ, −0.2 kg ± 1.2; waist Δ, −1.2 cm ± 3.8; fat percent Δ, −0.2% ± 1.1)). Average change of waist circumference during wash-out periods was 0.7 cm. Changes are absolute differences between the value after berry intervention and the average value of two wash-out periods, one before and one after the berry intervention.

## Discussion

The clinical study was technically successful and the compliance was good. The study population achieved was large enough to enable the statistical power even though the aimed number of study subjects was not quite reached (*n* was 80 instead of 85).

VCAM concentrations decreased after BB and SBo diets, and ICAM after SBe diet. Also, the values not reaching statistical significance tended to show a decreasing trend after other berry intervention periods in VCAM and ICAM values. Although the absolute changes were small, the results are indicative of slight positive effects of berries. ICAM and

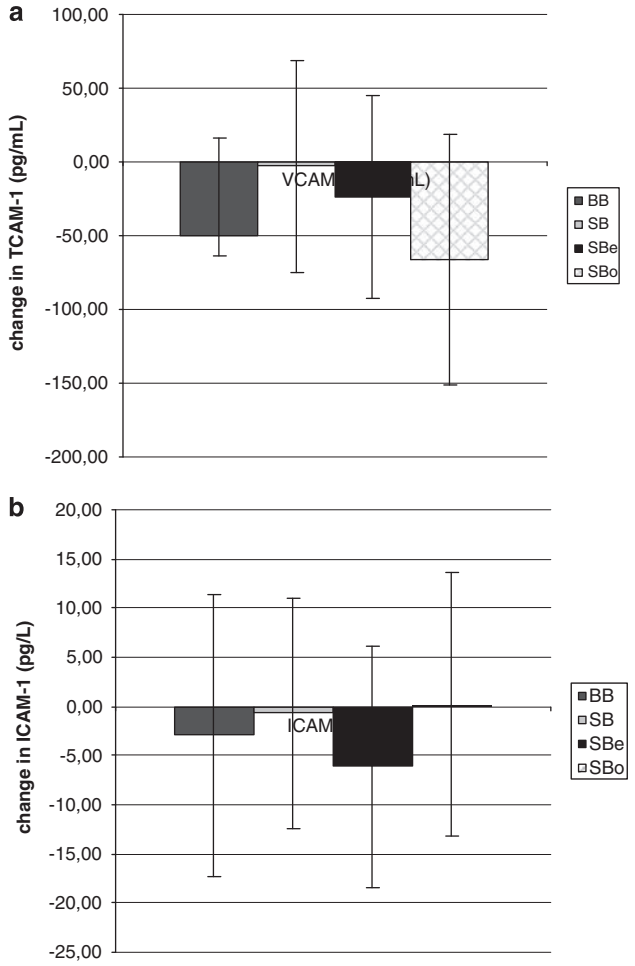


**Figure 3** Average changes and standard deviations in fasting plasma VCAM (**a**) and ICAM (**b**) concentrations during each berry intervention period (BB (VCAM Δ, −49.8 ng/ml ± 132.6; ICAM Δ, −2.9 ng/ml ± 28.7); SB (VCAM Δ, −3.0 ng/ml ± 144.5; ICAM Δ, −0.7 ng/ml ± 23.5); SBe (VCAM Δ, −23.5 ng/ml ± 137.9; ICAM Δ, −6.1 ng/ml ± 24.6); and SBo (VCAM Δ, −66.1 ng/ml ± 170.0; ICAM Δ, 0.2 ng/ml ± 26.9)). Average changes during wash-out periods were 6.5 and 1.5 ng/ml; respectively. Changes are absolute differences between the value after berry intervention and the average value of two wash-out periods, one before and one after the berry intervention.

VCAM belong to the immunoglobulin super-family, and they are involved in the process of tethering leukocyte to and transmigration across the endothelium (Konstantopoulos and McIntire, 1996). Firm adhesion and transmigration of leukocytes across the endothelium require ICAM and VCAM, and this step is the earliest stage in the development of the atherosclerotic lesion. Thus, these endothelial markers are implicated in the pathogenesis of atherosclerosis and cardiovascular diseases (Hope and Meredith, 2003), and they are also elevated in obesity and have been shown to decrease with weight loss (Keogh *et al*., 2007). Major change observed in present study, both statistically significant and more profound in subjects with greater baseline BMI, was the decrease in VCAM levels during SBo intervention, indicating that berry oil may be slightly more effective than other berry fractions on adhesion modulation.

It must be noted, that energy intake during SB diet was higher than in wash-out periods, mainly from other sources than the berry itself. It seems that the study subjects increased the use of sugar- and fat-rich foods possibly because of the strong taste of the berry, although the energy intake was intended to be similar. This might have hindered some effects of SB diet and explains why SBo and SBe diets had more impact on VCAM and ICAM values than SB diet.

Adiponectin is a newly identified adipose-secreted cytokine, which is present at decreased plasma level in subjects with obesity (Arita *et al*., 1999) or type 2 diabetes (Hotta *et al*., 2000). According to a recent review, experimental and epidemiological studies have provided abundant evidence that adiponectin improves insulin sensitivity, and has potent antiatherosclerotic effects (Stefan and Stumvoll, 2002). In our earlier study adiponectin increased during mixed berry diet, although the difference between berry and lifestyle groups was not statistically significant (Lehtonen *et al*., 2010). In the present trial adiponectin was decreased more or less during all berry interventions. This contradiction seems less confusing in the light of the knowledge that adiponectin values (Arita *et al*., 1999), similarly to $GHbA_{1c}$ (American Diabetes Association, 2003), change more slowly than for example ICAM and VCAM values. The rate of formation of $GHbA_{1c}$ is directly proportional to the ambient glucose concentration. As erythrocytes are freely permeable to glucose, the level of $GHbA_{1c}$ in a blood sample reflects the glycemic control of the previous 120 days, the average erythrocyte life span (American Diabetes Association, 2003). As the duration of intervention periods was only 33–35 days, the changes observed in $GHbA_{1c}$ and adiponectin values may reflect more the effect of wash-out periods than the berry intervention and vice versa. When considering the nature of the formation of $GHbA_{1c}$, intervention periods in this study were quite short (5 weeks) to make strong conclusions.

Changes in ICAM and especially VCAM have been very modest in recent weight loss studies. In a recent study a weight loss of 15 kg did not result in significant change in VCAM (Wycherley *et al*., 2010). Similarly, there was no change in VCAM in a study with a modest weight loss of 3.4 kg (4.5%), and also the observed change in ICAM was quite modest (−0.02) (Ata *et al*., 2010). It seems that VCAM and ICAM do not change very radically in relatively short (<1 year) intervention studies. Changes in cytokine levels have been somewhat more significant. Moschen *et al*. (2010) found that 8.6 kg weight loss resulted in a decrease of −2.57 in IL-6, whereas TNF-α was below detection limit both before and after the weight loss. Lee *et al*. (2010) have reported an increase of 3.3 μg/ml in adiponectin in a 12-week weight loss study with an average weight loss of 5.3 kg.

Based on the results, it can be stated that different berries and berry fractions have quite different effects on the associated variables of metabolic syndrome and type 2

diabetes. It also seems that either lingonberry and/or black currant modulate the fat metabolism of the liver into positive direction or the positive effect requires simultaneous incorporation of different berries into the diet. It must be noted that there were also other differences between the two trials as intervention periods in the present trial were shorter (5 weeks instead of 20) and the daily amount of berries less (100 g instead of 160 g). However, sampling was also performed at 10 weeks time point in the previous clinical trial, when the change in ALAT values was already clear. This implies that some trend could be expected already after 5 weeks also. Another notion should be made about SB varieties, as in the first trial berries were consumed frozen and were organically cultivated Ljubitelskaja variety grown in Finland, whereas in the second trial SB berries were dried *Hippophaë rhamnoides* ssp. *turkestanica* berries grown in China.

Despite the lack of decline in ALAT values, the clinical trial showed other positive effects of berries on the associated variables of obesity-related diseases. An alarming trend in developed countries is that obesity, metabolic syndrome and type 2 diabetes are developing at increasingly younger ages (Franks *et al*., 2010). The potential of berries to decrease the risk of these ailments could be of primary importance for children and adolescents, as berries are safe and readily accepted by them.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

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