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Effects of an antioxidant-rich juice (sea buckthorn) on risk factors for coronary heart disease in humans

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Abstract

There is increasing evidence to support the hypothesis that free radical-mediated oxidative processes contribute to atherogenesis. More recently the ability of antioxidant nutrients to affect cell response and gene expression has been reported *in vitro*, providing a novel mechanistic perspective for the biological activity of antioxidants. Sea buckthorn (*Hippophaë rhamnoides* L.) is a rich source of antioxidants both aqueous and lipophilic, as well as polyunsaturated fatty acids. The objective of the study was to characterize the antioxidant profile of Sea buckthorn juice (SBJ) and to evaluate its effect on plasma lipids, LDL oxidation, platelet aggregation and plasma soluble cell adhesion protein concentration. Twenty healthy male volunteers were given either a placebo or SBJ for 8 weeks. Additional daily intakes of vitamin C, α-tocopherol, β-carotene and flavonoids through SBJ supplementation were 462, 3.2, 1.0 and 355 mg respectively. There were no significant changes in plasma total cholesterol, LDL-C, platelet aggregation or plasma intercellular cell adhesion molecule 1 (ICAM-1) levels between treatment groups. Although not significant, a 20% and 17% increase in plasma HDL-C and triacylglycerol (TAG) concentrations were observed. SBJ supplementation also resulted in a moderate decrease in the susceptibility of LDL to oxidation.

Keywords: Sea buckthorn juice; CHD; Antioxidants; Plasma lipids; LDL oxidation; Platelet aggregation

1. Introduction

The contribution of free radical damage to the development of atherosclerosis is well established [1]. An imbalance in the cellular oxidant/antioxidant status can result in a pro-inflammatory response, in part mediated by cytokines which may induce the expression of adhesion molecules on the endothelial cell surface, such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) [2,3]. These molecules are centrally involved in the endothelial recruitment of mononuclear cells [4]. Adherence, followed by infiltration of mononuclear cells into the vascular wall, leads to scavenging of oxidized low-density lipoprotein (LDL), formation of lipid-laden foam cells, and development or progression of the atherosclerotic plaque. Oxidant/antioxidant status is a major determinant of LDL oxidation and therefore of its rate of accumulation by macrophages.

Platelets also play a key role in the atherosclerotic process. Platelet infiltration into the intima of arteries following endothelial damage contribute to the development of atherosclerotic lesions, and platelet aggregation at the site of atherosclerotic plaque rupture can increase the plaque size and lead to thrombus formation [5]. It is now recognized that free radical damage can impact upon the functionality of platelets and their contribution to the atherosclerotic process [6].

There is a growing interest in the utilization of antioxidant-rich plant extracts as dietary food supplements [7]. A wide spectrum of beneficial activity for human health has been advocated to these supplements due, at least in part to their strong antioxidant activity, although additional benefits are now being documented, such as a positive effect on plasma lipids [8]. Epidemiological data indicate an inverse relation between intake of fruits and vegetables, and cardiovascular risk [9], attributed in part to their relatively high

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antioxidant content. The current guidelines on fruit and vegetable intake in the UK recommend 5 portions per day. However, many people do not achieve this target, and many more may not consume fruit and vegetables in sufficient variety to obtain a wide range of antioxidant compounds. For many, the only feasible means to achieve antioxidants in the variety and concentrations required is via the use of dietary supplements. However recent attention has focused on the use of fruit juices as a concentrated source of antioxidants.

Sea buckthorn juice contains high concentrations of vitamin C [10]. However little information is as yet available on the concentration of other antioxidants such as tocopherols and tocotrienols, carotenoids, flavonoids and nutritionally important fatty acids. Furthermore the effect of SBJ on physiological determinants of cardiovascular risk has yet not been systematically investigated. The objective of this study was to characterize the antioxidant profile of SBJ. Furthermore, we investigated whether the supplementation of healthy volunteers with SBJ affects platelet aggregation, plasma lipids, LDL oxidation, and intercellular cell adhesion molecule-1 (ICAM-1) concentrations, which are recognized determinants of the pathophysiology of CHD.

2. Materials and methods

2.1. Antioxidant composition of juice

Carotenoids were extracted from homogenized seedless berries with water:ethanol:hexane (1:1:4, v/v/v), and analyzed by high performance liquid chromatography (HPLC) [11]. Tocopherols and tocotrienols were extracted from lyophilized berries using chloroform:methanol (2:1 v/v) [12], and analyzed by normal- phase HPLC with both UV and fluorescent detection, with DL-tocol as internal standard [13]. The vitamin C content of the juice was determined using the method of Washko et al. [14]. Briefly, juice was pressed from thawed berries and diluted 1:50 with water. The sample solution was analyzed by HPLC with diode array detection.

Flavonoids in the juice were identified and quantified by HPLC, as previously described [15].

2.2. Study design

The study was of double blind, placebo controlled parallel design, with an eight-week intervention period. A 300 ml portion of Sea buckthorn juice (Bayernwald Fruechteverwertung GmbH, Hengersberg, Germany) or placebo was consumed daily. The antioxidant composition of the juice is shown in Table 1, with the formulation of the placebo in Table 2. The placebo juice was developed to emulate Sea buckthorn juice in appearance and taste only. The major component of the placebo juice was water, and it contained no compounds of nutritional value.

Table 1
Antioxidant composition of sea buckthron juice

	mg/L
Vitamin E	**13.3**
α-tocopherol	10.5
β-tocopherol	0.4
γ-tocopherol	1.5
α-tocotrienol	0.5
β-tocotrienol	0.3
γ-tocotrienol	0.3
Vitamin C	**1540**
Carotenoids	**7.3**
β-carotene	3.3
Flavonoids	**1182**
Isorhamnetin-rutinoside	355
Isorhamnetin-glycoside	142
Quercetin-rutinoside	35
Quercetin-glycoside	35

Fasting blood samples were collected in the morning at baseline (t = week 0) at the mid-point (t = week 4) and the end (t = week 8) of the study.

2.3. Subjects

Recruitment for the study was aimed at male non-smokers, aged 18–55 years. Exclusion criteria included: (1) history of cardiovascular disease; (2) hematological, hepatic, renal or hormonal dysfunction (including diabetes); (3) consumption of dietary supplements including fish oils and antioxidant vitamins; and (4) use of lipid-regulating or anticoagulant drugs (including non-steroidal anti-inflammatory drugs). Thirty individuals were recruited. All subjects were normolipidaemic, with a mean-age of 35.5 ($\pm$ 2.28) and BMI of 25.7 ($\pm$ 3.34). The study was approved by the Ethics and Research Committee of the University of Reading. Subjects received oral and written information about the study and gave their written consent prior to participating. Individuals were instructed to continue with their normal diet for the duration of the intervention period, and requested not to consume alcohol in the 24-hr prior to blood sampling.

Table 2
Composition of the placebo juice

Additive	%
Fructose	7.0
Malic acid	3.1
Colour (Tartrazine & Ponceau)	0.0006
Aroma	0.2
Wheat fibre	0.6
Apple fibre	0.2
Apple pectin	0.8

2.4. Collection of fasting blood samples

Venous blood samples were obtained from each subject following a 12-hr overnight fast. Blood for LDL oxidation analysis, plasma lipid determinations and sICAM-1 measurements was collected into K_3EDTA vacutainer® tubes (Becton Dickinson, Plymouth, UK). Plasma was immediately separated by centrifugation (800 g for 20 min at 4°C), and stored at −80°C until analysis. Plasma for LDL preparation was mixed 4:1 with 50% sucrose prior to storage. Blood for platelet aggregation studies was drawn into 4.5 ml sodium citrate vacutainer® tubes (Becton Dickinson, Plymouth, UK) and kept at room temperature until analysis.

2.5. Isolation of low density lipoprotein (LDL)

Following thawing, the density of the plasma was raised to 1.2 g/ml with KBr. LDL (density 1.019–1.063 g/ml) was separated by sequential ultracentrifugation [16], using a near-vertical rotor (NVT65, Beckman Instruments (UK) Ltd.). LDL was dialysed against phosphate buffer (in the presence of Chelex-100): (in mmol/L) NaCl 140, NaH_2PO_4 1.9 and Na_2HPO_4 8.1. LDL was then sterilized by membrane filtration (0.2μm Minisart RC4, Sartorius, UK). Protein content (μg/ml) was determined using the method of Lowry et al. [17]. Oxidative susceptibility of LDL was determined by continuously monitoring the production of conjugated diene according to the method of Esterbauer et al. [18]. LDL, adjusted to 50 μg protein/ml, was incubated with 5 μmol/L $CuSO_4$ in PBS (final volume, 2 ml) at 37°C. Conjugated diene formation during LDL oxidation was monitored by changes in wavelength absorbance at 234 nm in a spectrophotometer (Lambda Bio 20 UV/VIS Spectrometer, Perkin Elmer, Beaconsfield, Bucks, UK) equipped with a six-position automatic changer. The changes in absorbance were recorded every 1.5 min for 2 hr after initiating oxidation with copper. The lag phase (in minutes) was taken as the point of intersection of the tangents to the initiation phase and propagation phase of conjugated diene formation.

2.6. Whole blood platelet aggregation

Platelet aggregation studies were performed in a two-channel whole blood lumi-aggregometer (Model 560, Chronolog Corporation, Labmedics Ltd., Cheshire, UK). Whole blood (500μl) was diluted, 1:1, with phosphate buffered saline (PBS) and the impedance response to the following agonists measured (final concentration in whole blood): Adenosine diphosphate (ADP, 10 μmol/L), collagen (Col, 1.5 and 5.0 μg/ml) and arachidonic acid (AA, 0.75 mol/L). Maximum aggregation was measured at 6 min, with the extent of aggregation expressed in ohms of impedance using Aggrolink software (Chronolog Corporation, Labmedics Ltd., Cheshire, UK). Platelet studies were initiated 60 min after venepuncture, and completed within 180 min. Inter- and intra-assay CV's of 4–7% and 5–10% were observed depending on the agonist of interest.

2.7. Plasma lipid measurements

Plasma total cholesterol (TC), triacylglycerol (TAG), and high density lipoprotein cholesterol (HDL-C) concentrations were measured by an enzymatic method using commercially available enzymatic agents for use with the automated Monarch analyser (Instrumentation Laboratories, Warrington, UK). Concentration was determined spectrophotometrically with the cholesterol and TAG sequentially metabolized to produce quinoneimine (red) the concentration of which is proportional to the cholesterol and TAG concentration in the original sample. HDL cholesterol in whole plasma was measured after precipitation of apo B–containing lipoproteins with dextran sulfate (Dextralip 50) and magnesium chloride. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald equation [19]. The mean intra- and inter-assay CV's for TG, TC and HDL-C were 1.8, 2.0, and 3.2% and 3.2, 4.2 and 5.0% respectively.

2.8. Soluble intercellular adhesion molecule-1 (sICAM-1) measurements

sICAM-1 was measured using a commercially available sandwich ELISA kit (R&D Systems, Abingdon, UK). The assay employs an antibody specific for sICAM-1 and comparison of the intensity of the color development in the samples with known standards allows the concentration to be determined.

3. Results

3.1. Composition of sea buckthorn juice (SBJ) and placebo

The composition of the SBJ is given in Table 1. The juice providing on average 13.3, 1540, 7.3 and 1182 mg/L of vitamin E, vitamin C, carotenoids and flavonoids respectively. Over 75% of the total vitamin E was in the form of α-tocopherol, while isorhamnetin (~50% total) was the predominant form of flavonoids present in the juice.

The placebo juice contained fructose, malic acid, fiber and pectin at concentrations of 7.0, 3.1, 0.8 and 0.8% respectively and only trace concentrations of vitamin E, C, carotenoids and flavonoids.

3.2. Subjects

During follow-up, ten subjects requested withdrawal from the study due to gastro-intestinal upset and diarrhea attributed to consumption of both the sea buckthorn and

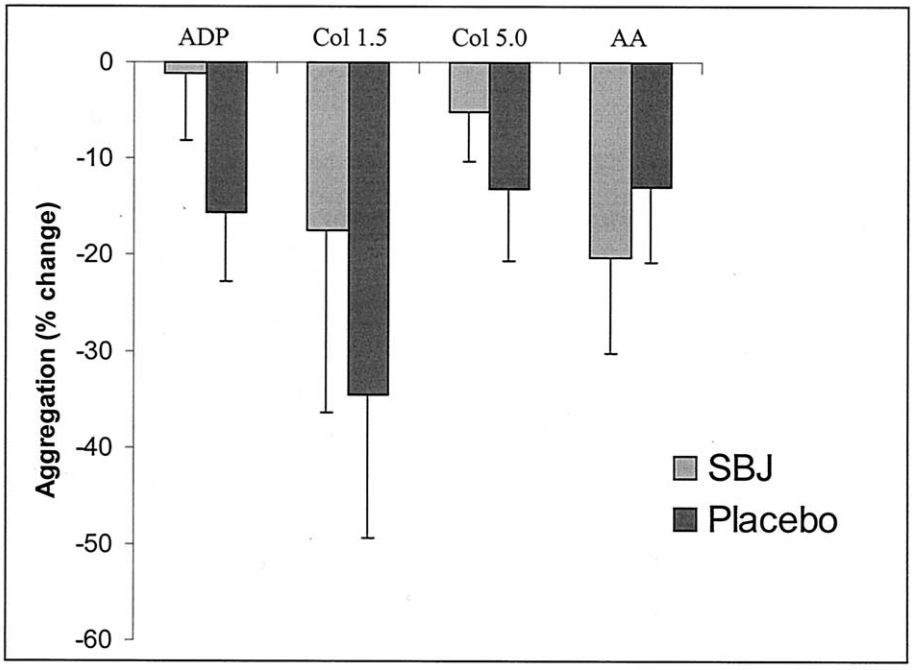

Fig. 1. Percentage change in platelet aggregation over the 8-week study period. Aggregation was measured in response to ADP (10 μmol/L), Collagen (1.5, μg/ml, Col 1.5), Collagen (5.0, μg/ml, Col 5.0) and arachidonic acid (0.75mol/L, AA). Group mean + SEM shown.

placebo juice supplements. Twenty subjects completed the 8-week study, and were included in the analysis.

3.3. Effects on platelet aggregation

In both placebo and active groups a modest decrease in platelet aggregation to all agonists used, was evident from baseline to 8 weeks. The decreases in response to ADP and collagen 5.0 μg/ml were minimal. In the active group collagen 1.5 μg/ml and arachidonic acid resulted in a decrease in platelet aggregation over the 8-week period. However, the placebo group responded in a similar manner. Therefore, there were no significant differences between placebo and active groups on platelet aggregation in response to ADP ($p = 0.56$), collagen 1.5 μg/ml ($p = 0.66$), collagen 5.0 μg/ml ($p = 0.77$) or AA ($p = 0.87$) (Fig. 1).

3.4. Effects on low density lipoprotein (LDL) oxidation

There was a small increase in lag phase in the active treatment group, with no change observed in the placebo group (Fig. 2). However, the inter-group difference failed to reach significance.

3.5. Effects on fasting plasma lipids

There was no significant change in TC or LDL-C in either treatment group in the 8 week study period ($p = 0.97$) (Fig. 3). There was an overall increase in HDL-C in the active treatment group of 14% compared with baseline over the study period. The placebo group showed a converse trend towards decreased HDL-C (−8%) in the same period. The inter-group differences however failed to reach statistical significance. Changes in the HDL-C/LDL-C ratio also failed to reach statistical significance (data not shown).

Both groups saw an initial decrease in TAG concentrations between weeks 0–4, followed by an increase to 30% and 13% above baseline values in weeks 4–8, in the active and placebo groups respectively. No significance differences were observed between treatment groups ($p = 0.12$).

3.6. sICAM-1

sICAM-1 levels were unaffected by either Sea buckthorn or placebo juice treatment (Fig. 4).

4. Discussion

Analysis of the Sea buckthorn juice showed it to be a rich source of antioxidants providing 462, 3.99, 2.19 and 354.7 mg of vitamin C, vitamin E, carotenoids and flavonoids per 300 ml. Therefore a single 300 ml portion per day would provide approximately 75 and 25% of the daily-recommended intakes for α-tocopherol and β-carotene in the UK and over 10 times the recommended intake for vitamin C [20]. The juice therefore provides a convenient means for individuals to significantly increase their antioxidant intake without the use of dietary supplements.

Although the impact of Sea buckthorn berry fractions on cardiovascular disease progression has been well studied in Chinese populations [21,22], information on the impact of

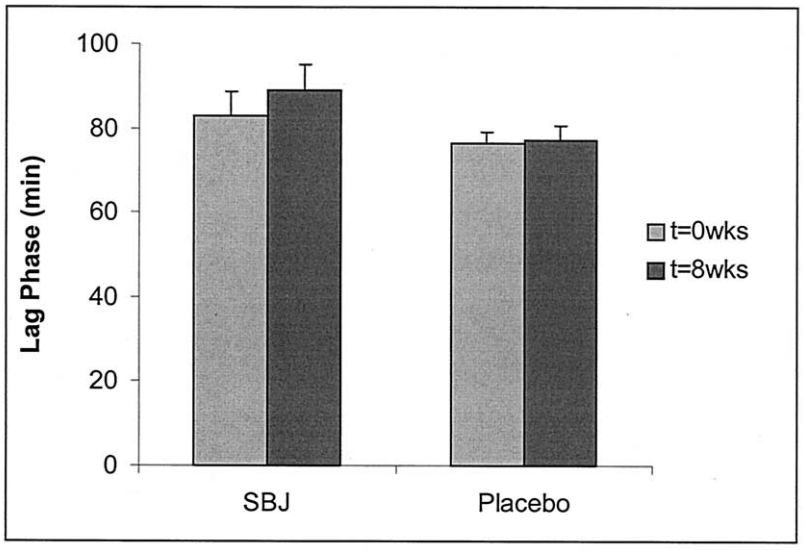

Fig. 2. Effect of supplementation with sea buckthorn juice (SBJ) on plasma low-density lipoprotein oxidation. Changes in LDL oxidation are measured by lag phase (mins) at baseline (week 0) and endpoint (week 8). Group means + SEM shown.

this intervention on atherogenesis and coronary heart disease (CHD) risk indicators in Western populations is relatively limited. In the present study the impact of Sea buckthorn juice (SBJ) on plasma lipids, LDL oxidation, platelet aggregation, and adhesion molecule concentration was investigated in a healthy UK adult population.

In general, due to its bitter and acidic nature and gastrointestinal intolerance, the juice was not well accepted, resulting in a 33% drop-out. Most of the participants who completed the study reported adding sugar (sucrose), honey or sweetened carbonated drinks in order to improve the palatability of the product.

4.1. Plasma lipids

There was no changes observed in total- or LDL cholesterol following the consumption of SBJ for 8 weeks. However, there were trends towards increases in both plasma TAG and HDL-C in the active juice group. Although the response did not reach statistical significance due to the relatively small group number and large inter-individual variability, a 22% increase in circulating HDL-C levels was evident in the SBJ compared to the control group. Dietary supplementation of CO_2–extracted oil from sea buckthorn fruit flesh and skin fraction (5g/day for four months) sig-

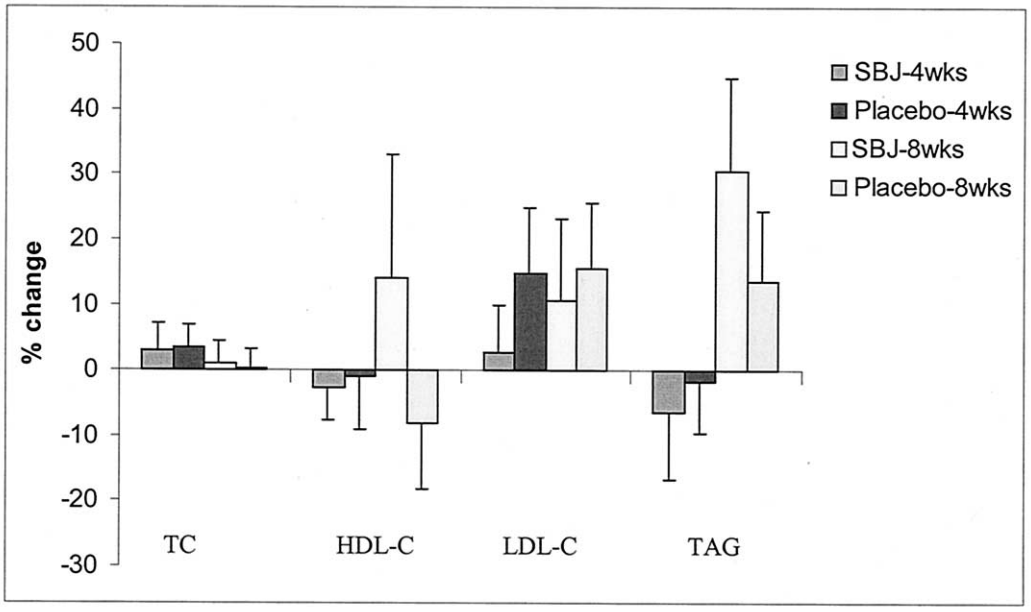

Fig. 3. Effect of supplementation with sea buckthorn juice (SBJ) on fasting plasma lipids. Results expressed as percentage change at the 4 and 8 week points. Group means + SEM shown. TC, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TAG, triacylglycerols.

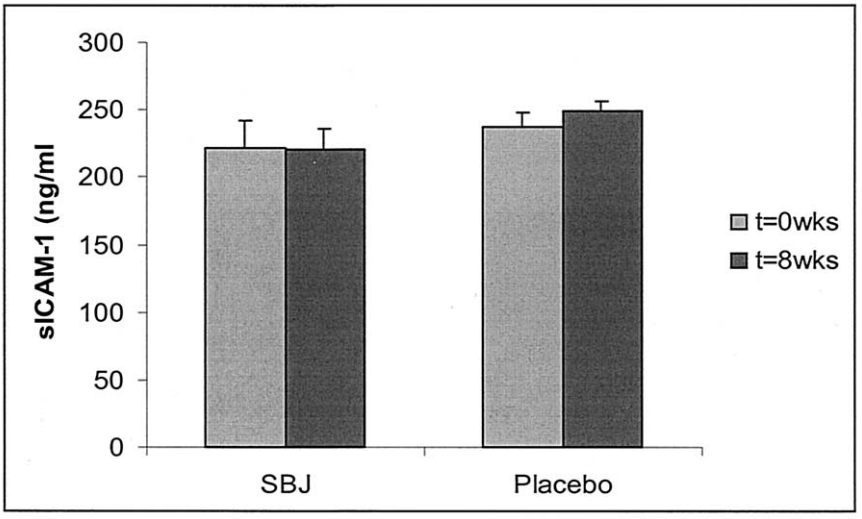

Fig. 4. Effect of supplementation with sea buckthorn juice (SBJ) on plasma levels of sICAM-1. Plasma adhesion molecule levels were measured at baseline (week 0) and endpoint (week 8). Group means + SEM shown.

nificantly increased the HDL-C levels by 11% ($p < 0.05$) in patients with atopic dermatitis [23]. Kurowska and coworkers reported a comparable 21% increase in HDL-C following the consumption of 750 ml of orange juice per day for 4 weeks in hypercholesterolaemic subjects [8]. Similarly, a non-significant 9%, 18% and 23% increase in HDL-C was observed in diabetics, following a daily consumption of 500 ml of tomato juice, 500 mg ascorbic acid, or 800 IU vitamin E respectively for 4 weeks [24]. In a study conducted by Johansson et al., no change in circulating HDL-C following Sea Buckthorn oil intervention was observed [25]. However, to the best of our knowledge, the impact of SBJ on circulating lipid levels in Western Societies has not previously been reported. HDL-C is known to be a significant and independent predictor of CHD risk [26,27] and a recent meta-analysis of four large prospective studies has quantified the associations [28]. The results indicated that for every 0.026 mmol/L increase in circulating levels (approximate 2% change in an individual with normal HDL-C levels) the incidence of coronary events decreases by 2% in men and 3% in women [28]. Further investigation is needed to establish the underlying mechanisms of these antioxidant rich fruit juices on HDL-C levels.

The SBJ also resulted in a non-significant 17% increase in circulating TAG, compared to the control group. A hypertriglyceridaemic impact of fruit juice was also reported by Kurowska et al., who observed a 30% ($P < 0.02$) increase in TAG following the orange juice intervention (750 ml/day) [8]. The authors attributed the effect to the additional 69 g per day of simple sugars provided by the juice [8]. The impact of increased carbohydrate intake on TAG metabolism has been widely studied [29,30], as it is a primary concern in the recommendation of lower fat-higher carbohydrate diets at a population levels as a means of reducing the incidence of CHD [31]. Chronic increases in carbohydrate feeding are known to increase hepatic *de novo* lipogenesis by increasing the flux of glucose and fructose through the fatty acid synthesis pathway [32]. In addition, carbohydrate feeding has been shown to up regulate key enzymes involved in fatty acid synthesis in rats [33]. This increase in the liver fatty acid pool stimulates VLDL-TG production, thereby increasing overall circulating TAG levels. In an acute meal study where the postprandial response of a fat-only test meal was compared with the same meal consumed with either 50 g fructose, 50 g glucose, or 100 g sucrose, Cohen & Schall concluded that the glucose increased circulating TAG levels [34]. However, 50 g fructose caused a more significant rise in the lipaemic response, which was comparable to the increase observed following the 100 g-sucrose dose [34]. Although the SBJ sugar content is comparatively low (10.1 g/day) in the current study, the addition of sucrose and fructose as table sugar/honey/soft drinks, as a sweetening agent, may have contributed to the hypertriglyceridaemic effects.

4.2. LDL oxidation

In addition to the concentration of LDL-C in the plasma, both the size/density profile and the susceptibility of the particle to oxidation are important determinants of the recognition and accumulation of LDL by the macrophages, and therefore the development of the atherosclerotic plaque [35, 36]. The levels of vitamin E in the LDL particle is thought to be an important factor determining its oxidisability [37]. Although there was a slight increase in the lag phase time, indicating a potential reduction in the susceptibility to oxidation following SBJ, the change did not reach significance. This is in agreement with other investigators who have observed that much larger doses of α-tocopherol are needed to positively impact on LDL oxidation [38–40]. For example, in a study by Jialal et al. (1995), it was found that 400 IU (268 mg)/day α-tocopherol was required to decrease the

oxidisability of LDL, and that lower doses of 60IU (40 mg)/day or 200 IU (134 mg)/day, were ineffective [39]. In the current study, the SBJ provided on average 3.2 mg α-tocopherol per day. In addition to providing vitamin E, the SBJ is also a rich source of ascorbic acid and flavonoids, whose antioxidant potential has been demonstrated repeatedly in the *in vitro* setting [41,42]. Both these nutrients are however aqueous antioxidants and scavenge free radicals in the plasma environment of the LDL particle. Ascorbic acid, along with acting as a plasma radical scavenger is thought to have the ability to act on LDL associated tocopherol radicals [41,42] and therefore has a α-tocopherol recycling benefit. However, it is unlikely than any benefit of these aqueous antioxidants would be realized using the current methodology, as the conjugated-diene method considers LDL in isolation, with all aqueous components removed. Therefore in the *in vivo* situation, the high levels of ascorbic acid and flavonoids in the SBJ may contribute to an improved LDL oxidation status.

4.3. ICAM-1

The recruitment of mononuclear cells into areas of inflammation is mediated by interacting sets of cell adhesion molecules. Intercellular adhesion molecules (ICAMs) are structurally related members of the immunoglobulin supergene family and are ligands for the beta2 integrin molecules present on leukocytes. Of the five ICAMs identified, ICAM-1 is the most extensively studied. In atherosclerosis, focal expression of ICAM-1 particularly triggered by plasma atherogenic lipoproteins has been detected, and cell adhesion proteins may mediate the recruitment of mononuclear cells to the plaque. The expression of ICAM-1 and other cell adhesion proteins can be significantly increased in the presence of proinflammatory molecules such as TNF-α, IL-1 and reactive oxygen and nitrogen species [43]. The ICAM-1 gene has regulatory sites for the redox sensitive transcription factor NF-kappa B [44]. Several in vitro studies clearly indicate that enriching cultured endothelial cells with antioxidants such vitamin E and vitamin C significantly decreases the expression of proinflammatory cytokines and cell adhesion proteins induced by native or oxidized LDL [45]. However, under the conditions investigated, sICAM-1 concentration remained largely unchanged by SBJ supplementation. The median concentration of sICAM-1 among all subjects in the present study (232 ng/ml) compares with mean concentration of 240 ng/ml reported for control subjects in the USA [46]. Similar to our data circulating levels of cell adhesion proteins did not change after a short-term dietary supplementation with tomato juice (250 ml), vitamin E (800 IU) or vitamin C (500 mg/day) over 4 weeks [24]. It should be taken into account that in studies with cultured endothelial cells the expression of cell adhesion proteins is often down regulated only with relatively high concentrations of antioxidants, which cannot be realistically achieved in human intervention trials.

4.4. Whole blood platelet aggregation

Changes in platelet responsiveness to all agonists used were observed in the present study. However, the placebo treatment group in all cases mirrored these effects. Therefore, once the data was corrected for the placebo response, supplementation with juice produced no significant changes to platelet aggregation. The variety of potentially 'active' compounds in the sea buckthorn supplements and the use of whole blood platelet aggregometry make comparisons between this work and previous studies difficult. The method of whole blood aggregometry is not widely used, primarily due to concerns over blood stability [47] and the interference of other blood components such as white blood cells. For this reason, much of the previous work in this field has been conducted using platelet preparations, such as platelet rich plasma (PRP) and washed platelets. This was the case in a previous study on sea buckthorn oils and platelet aggregation conducted by Johansson et al. [25]. A mixture of sea buckthorn seed and pulp oil (SBO) was used in a small-scale preliminary crossover study of 12 subjects. Supplementation comprised 5g/day for 4 weeks of SBO with fractionated coconut oil as a placebo. A significant ($p = 0.05$) reduction in aggregation in response to ADP in washed platelets was shown. The disparity between the results of Johansson's study and the current study possibly illustrate the difference that preparation can make to platelet aggregation response. In addition, the relatively high levels of vitamin E and carotenoids in the pulp oil combined with the n-3 fatty acids in the seed oil may produce a potent anti-platelet mixture. Previous studies have reported the benefits of n-3 fatty acids on platelet aggregation, attributed to a replacement of arachidonic acid with EPA in platelet membranes. This promotes the formation of TXA_3, which has a much weaker pro-aggregatory action than TXA_2. Pignatelli et al. [48] reported an inhibition of collagen-stimulated platelet aggregation with vitamin E supplementation. However, the supplementation level (600 mg/day) was 60-fold higher than that found in the sea buckthorn products. Therefore, the effects of n-3 fatty acids and vitamin E in the supplements would have been minimal when compared to previous studies.

Inhibition of platelet aggregation by polyphenolic compounds, particularly flavonoids, is widely reported. However, no evidence of this was seen in this study, despite a relatively high daily intake (355 mg/day). Many previous studies use in vitro methods, as opposed to ex vivo. This involves incubation of the platelets with high concentrations of pure flavonoids prior to agonist stimulation [49,50] and is therefore not comparable with supplementation studies, but is useful for investigation of possible mechanisms of action. One study, which did use human supplementation, was that of Keevil et al. [51]. Ten subjects consumed 5–7.5 ml/kg/day of purple grape, orange or grapefruit juice for 7 days. Purple grape juice inhibited collagen induced platelet aggregation in whole blood by 77%. No effect was observed

with orange or grapefruit juice. However, a point of concern is that Keevil took the endpoint aggregation measurements 2 hr following ingestion of the final juice dose. It is therefore possible that the results obtained were due to an acute event, rather than a sustainable response.

It is clear from the previous platelet studies conducted that many factors can influence the observed results. It is possible that the low subject numbers in the present study, combined with the large intra-individual variation and relatively low intakes of the potentially active component may have contributed to the lack of platelet response to the treatments. Bioavailability of the compounds in sea buckthorn has not yet been quantified, and may be a consideration in future work.

In conclusion, sea buckthorn juice is a rich source of antioxidant nutrients. Further work is merited in order to quantify fully the positive impact on circulating HDL-C levels and to gain an understanding of the underlying mechanisms. In addition the measurement of circulating antioxidant levels in response to sea buckthorn supplementation will provide a greater understanding of its role in contributing to the 'antioxidant network'.

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