

The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects

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The aim of the present study was to determine whether the consumption of wild blueberries (*Vaccinium angustifolium*), a concentrated source of non-nutritive antioxidant phytochemicals, would enhance postprandial serum antioxidant status in healthy human subjects. A single-blinded crossover study was performed in a group of eight middle-aged male subjects (38–54 years). Subjects consumed a high-fat meal and a control supplement followed 1 week later by the same high-fat meal supplemented with 100.0 g freeze-dried wild blueberry powder. Upon brachial vein catheterization, fasting and postprandial serum samples were taken sequentially and analysed for lipids and glucose and for serum antioxidant status. Serum antioxidant status was determined using the oxygen radical absorbance capacity (ORAC) assay and the total antioxidant status (TAS) assay. The wild-blueberry treatment was associated with a significant treatment effect as determined by the ORAC assay (water-soluble fraction ORAC_{perchloric acid (PCA)}, $P=0.04$). Significant increases in serum antioxidant status above the controls were observed at 1 h (ORAC_{PCA} (8.5 % greater), $P=0.02$; TAS (4.5 % greater), $P=0.05$), and 4 h (ORAC_{total} (15.0 % greater), $P=0.009$; ORAC_{acetone} (16.0 % greater), $P=0.007$) post-consumption of the high-fat meal. In conclusion, the consumption of wild blueberries, a food source with high *in vitro* antioxidant properties, is associated with a diet-induced increase in *ex vivo* serum antioxidant status. It has been suggested that increasing the antioxidant status of serum may result in the reduced risk of many chronic degenerative diseases.

Serum antioxidant status: Postprandial oxidation: Anthocyanins: Blueberry: Antioxidant

Epidemiological and interventional studies have suggested that plant-based flavonoids may be protective against many chronic degenerative diseases (Diplock *et al.* 1998; Liu *et al.* 2000; Middleton *et al.* 2000). Anthocyanins are a commonly consumed class of flavonoid (Middleton *et al.* 2000), having known *in vitro* antioxidant characteristics (Hertog *et al.* 1993; Satuè-Gracia *et al.* 1997). However, their function *in vivo* has yet to be proven (Rice-Evans, 2001). Lowbush ‘wild’ blueberries (*Vaccinium angustifolium*) are one of the highest fruit sources of anthocyanins (Francis, 1989) and have exhibited one of the highest recorded *in vitro* antioxidant capacities of various fruits and vegetables tested (Wang *et al.* 1996; Mazza & Oomah, 2000). The lowbush ‘wild’ blueberry as studied herein is of particular interest as it has a higher *in vitro* antioxidant capacity than the cultivated highbush blueberry (*Vaccinium corymbosum*; Kalt *et al.* 1999). Wild blueberries are relatively low in antioxidant vitamins and

minerals (Bushway *et al.* 1983); their *in vitro* antioxidant capacity has been attributed to their high concentration of phenolic compounds, particularly anthocyanins (Prior *et al.* 1998; Kalt *et al.* 1999). The purpose of the present *in vivo* study was to examine the effect of consuming freeze-dried wild blueberries rich in anthocyanins and phenolic compounds on serum antioxidant status as measured in healthy human subjects. Increasing the serum antioxidant status has been implicated as a possible preventative means to reduce the development of cardiovascular disease (Kaplan & Aviram, 1999; Salonen *et al.* 2000), diabetes (Vendemiale *et al.* 1999) and cancer (Ames *et al.* 1995; Willett, 2001).

The potential importance of biological markers of oxidative stress in disease has been recognized (Chiesa *et al.* 1998; Crews *et al.* 2001). Recently, researchers have determined a correlation between increased *ex vivo* blood antioxidant status and risk of cardiovascular disease and

Abbreviations: ORAC, oxygen radical absorbance capacity; PCA, perchloric acid; TAS, total antioxidant status; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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cancer. They found atherosclerotic patients to have significantly lower blood antioxidant status as compared with healthy controls (Durak *et al.* 2001). Furthermore, a recent Australian study has determined that increasing the serum antioxidant status is associated with a decreased risk of breast cancer (Ching *et al.* 2002). The oxygen radical absorbance capacity (ORAC) and the total antioxidant status (TAS) assays used in the following trial are recognized as accepted methods of measuring *ex vivo* antioxidant status (Cao & Prior, 1998).

Experimental methods

Subjects

Eight male subjects (46.9 (SEM 1.9) years, BMI 23.8 (SEM 0.8) kg/m²) were recruited from the Guelph (Ont., Canada) area. Baseline characteristics are outlined in Table 1. Participants met all criteria as determined by a medical questionnaire. The principal criteria for eligibility were: (1) absence of clinical disease; (2) no history of renal or gastrointestinal disorders; (3) no alcoholism; (4) no smoking. Subjects taking lipid-altering or blood pressure medications were excluded. Subjects also refrained from taking aspirin or anti-inflammatory medications prior to or during the study, and discontinued all forms of antioxidant supplementation 1 month prior to the investigation. Furthermore, subjects were instructed to maintain a consistent diet throughout the study period. The present study conformed with the ethical guidelines of the University of Guelph (Ont., Canada) and was approved by the Human Subjects Committee. All subjects gave written consent in advance.

Supplementation

The freeze-dried wild blueberry powder used in this trial was produced at the University of Guelph (Department of Human Biology and Nutritional Sciences) in conjunction with the Ontario Ministry of Agriculture, Food and Rural Affairs (Guelph, Ont., Canada) and the Guelph Food Technology Center (Guelph, Ont., Canada). The powder was produced from wild blueberries of the species *Vaccinium angustifolium* (lowbush blueberry) obtained from the Sudbury area of Northern Ontario. The chemical composition of the freeze-dried wild blueberry powder and placebo are listed in Table 2. The blueberry supplement (100.0 g) contained 1.20 g total anthocyanins (42 % total phenolics) and had an ORAC value of 147 μmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents/g (as determined by Brunswick Laboratories, Wareham, MA, USA). The control supplement was matched for digestible carbohydrate and contained 76.4 g (1.28 MJ (305 kcal)) glucose (Atlantic Sugar Ltd, Toronto, Ont., Canada), 0.5 g sugar-free Kool Aid (Kraft Canada, Don Mills, Ont., Canada), and had an ORAC value of 5 μmol Trolox equivalents/g.

Experimental design

This study followed a single-blinded crossover design. Subjects were admitted to the Human Testing Lab (Department of Human Biology and Nutritional Sciences, University of Guelph) on the morning of the study in a fasted state (12–14 h, no alcohol in the previous 24 h). Anthropometric measurements, resting sitting blood pressure and heart rate were obtained before trial commencement. A cannula with an Intima 20 gauge intravenous catheter (Becton Dickinson, Rutherford, NJ, USA) was inserted into the brachial vein and

Table 1. Fasting baseline characteristics of study participants before initiation of treatment* (Mean values with their standard errors for eight subjects)

Treatment group...	Control†		Blueberry†	
	Mean	SEM	Mean	SEM
ORAC _{total} (μmol Trolox equivalents/l)	2407	105	2436	106
ORAC _{PCA} (μmol Trolox equivalents/l)	679	67	656	50
ORAC _{acetone} (μmol Trolox equivalents/l)	725	123	726	119
TAS (μmol Trolox equivalents/l)	1350	20	1270	20
Serum total cholesterol (mmol/l)‡	4.91	0.27	5.04	0.21
Serum triacylglycerol (mmol/l)§	1.00	0.13	1.20	0.18
Serum HDL-cholesterol (mmol/l)	1.00	0.06	1.05	0.08
Serum LDL-cholesterol (mmol/l)	3.45	0.30	3.44	0.21
Serum glucose	5.19	0.45	5.46	0.20
Systolic blood pressure (mmHg)	118	7	115	5
Diastolic blood pressure (mmHg)	76	5	72	4
Heart rate (beats/min)	66	3	70	4

ORAC, oxygen radical absorbance capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; PCA, perchloric acid; TAS, total antioxidant status.

* Serum lipids and glucose analyses were conducted at an Ontario Ministry of Health Licensing and Inspection Branch licensed laboratory (Guelph General Hospital Core Laboratory, MDS Laboratory Services, Guelph, Ont., Canada). Serum ORAC was determined by Genox Laboratories, Baltimore MD, USA.

† There were no significant differences for baseline (*t* 0) measures between treatment groups (paired *t* test): *P* > 0.05.

‡ For serum cholesterol 1 mmol/l = 38.57 mg/dl.

§ For serum triacylglycerol 1 mmol/l = 88.57 mg/dl.

|| For serum glucose 1 mmol = 17.9 mg/dl.

Table 2. Composition of treatment supplements

Component	Freeze-dried blueberry supplement*	Control supplement†
ORAC (μmol Trolox equivalents/g)‡	147	5
Total phenolics (g/kg)§	27.9	0.0
Anthocyanins (g/kg)§	11.6	0.0
Vitamin C (g/kg)	0.1	0.0
Digestible carbohydrate (g/kg)	764	764
Total dietary fibre (g/kg)	178	0.0
Protein (g/kg)	27	0.0
Fat (g/kg)	24	0.0
Ash (mineral content) (g/kg)	10	0.0
Energy		
kcal/g	3.38	3.05
kJ/g	14.10	12.72

ORAC, oxygen radical absorbance capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

*Lowbush 'wild' blueberry (*Vaccinium angustifolium*); for details of preparation, see p. 390.

†The control supplement contained 76.4 g glucose (Atlantic Sugar Ltd, Toronto, Ont., Canada), 0.5 g sugar-free Kool-aid (Kraft Canada, Don Mills, Ont., Canada; For details, see p. 390).

‡Analysis conducted by Brunswick Laboratories, Wareham, MA, USA.

§HPLC analysis of phytochemicals (total phenolics and anthocyanins) within the freeze-dried blueberry supplement conducted by Brunswick Laboratories, Wareham, MA, USA.

||Chemical analyses of freeze-dried blueberry powder conducted by Maxxam Analytics Inc., Mississauga, Ont., Canada.

a fasting blood sample was drawn. Subjects received the control supplement (dissolved in 500 ml water) with a high-fat meal (3.56 MJ (853 kcal), 46.7 g fat of which 15.5 g was saturated fat, as determined by Maxxam Analytics Inc., Mississauga, Ont., Canada) consisting of one Egg McMuffin, one Sausage McMuffin and two hash brown patties (McDonald's Corporation). The macronutrient and vitamin composition of the high-fat meal is shown in Table 3. Initiation of the high-fat meal was taken as t 0; subsequent blood samples were taken at 1, 2, 3 and 4 h. Procedures were repeated (on the same subjects) 7 d later using the same high-fat meal, with 100.0 g freeze-dried wild blueberry powder (dispersed in 500 ml water) in place of the control supplement.

Sampling procedures

Blood was drawn from a brachial vein catheter into evacuated glass tubes (Vacutainer; Becton Dickinson). Samples were allowed to clot at room temperature for 25 min. Samples were then immediately centrifuged (3000 rpm,

1000 g) for 15 min at 5–15°C to recover serum. Serum was extracted and divided into portions in 2 ml vials (Cryovial; Fisher Scientific Ltd, Nepean, Ont., Canada) over an iced-water bath (3–4°C) using disposable glass pipettes. The serum was then snap-frozen in liquid N₂ and stored at –80°C. All glass was pre-washed in a 0.2 M-HCl solution and rinsed three times with distilled, de-ionized H₂O to remove possible contaminants (metal ions).

Serum antioxidant capacity

Serum antioxidant capacity was measured using the ORAC (ORAC_{total}, ORAC_{perchloric acid (PCA)} and ORAC_{acetone} fractions) and TAS assays. Serum ORAC was determined by Genox Laboratories, Baltimore, MD, USA, using the ORAC assay as outlined by Cao *et al.* (1993). Modifications to the ORAC assay allow for the isolation of lipid- and water-soluble phases of the serum sample. The ORAC_{PCA} fraction (serum treated with PCA) is a non-protein fraction that preserves the water-soluble antioxidants within the sample. ORAC_{acetone} (serum treated with acetone) is a non-protein fraction containing both water-soluble and lipid-soluble antioxidants (Cao & Prior, 1998). Briefly, the ORAC assay was conducted using the automated COBAS FARA II spectrofluorometric analyser (Roche Diagnostics, Basel, Switzerland) with fluorescent filters at an excitation wavelength of 546 nm and an emission wavelength of 565 nm. β -Phycoerythrin was used as the target molecule for free-radical attack, with 2,2-azobis(2-amidinopropane) dihydrochloride as the peroxy radical generator. Trolox, an aqueous soluble vitamin E analogue, was used as a control standard. Fluorescence of β -phycoerythrin was recorded every 55 s and expressed relative to the initial reading. Final results were calculated by using the differences of the areas under the β -phycoerythrin decay curves between the blank and the sample. ORAC readings are expressed as μmol Trolox equivalents, where 1 Trolox equivalent equals the net protection area under the curve

Table 3. Composition of high-fat meal*

Component	Amount	% Energy
Digestible carbohydrate (g)†	75.2	35.4
Protein (g)†	32.4	15.3
Fat (g)†	46.7	49.3
Total dietary fibre (g)†	4.5	
Ash (mineral content) (g)†	9.1	
Vitamin C (mg)	6.0	
Vitamin E (mg)	2.1	
Energy†		
kcal†	853	
kJ†	356	

*The meal consisted of one Egg McMuffin, one sausage McMuffin and two hash brown patties (McDonald's Corporation).

†Chemical analyses of the high-fat meal conducted by Maxxam Analytics Inc., Mississauga, Ont., Canada.

provided by 1 μ mol Trolox. TAS was measured on an automated Hitachi 911 Biochemical Analyzer using the Randox-TEAC assay (Randox Laboratories, Mississauga, Ont., Canada). Briefly, the assay is based on the inhibition by antioxidants to absorb free radicals. Plasma samples were added to a 2,21-azinobis-(3-ethylbenzthiazoline-6-sulfonate) radical solution. The change in radical concentration over a 10 min incubation period was monitored spectrophotometrically (600 nm) and compared with the decrease of a known standard (Trolox). Specifics of the ORAC and comparisons between the ORAC and TAS assays have been outlined by Cao & Prior (1998).

Blood lipids

Serum lipid and glucose analyses were conducted at an Ontario Ministry of Health Licensing and Inspection Branch licensed laboratory (Guelph General Hospital Core Laboratory, MDS Laboratory Services, Guelph, Ont., Canada). Total cholesterol, HDL-cholesterol, triacylglycerol and glucose were analysed on a Synchron CX DELTA automated sample processor (Beckman Coulter Inc., Fullerton, CA, USA) with the appropriate reagent systems. LDL-cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972).

Vitamin and phytochemical analysis

Total vitamin E concentration was analysed after saponification followed by isoctane extraction and separation by normal-phase chromatography (Liu *et al.* 1996). Vitamin C was determined by HPLC following deproteinization (Speek *et al.* 1984). Both vitamin C and E were quantified by fluorescent detection. Total anthocyanin and total phenolic concentrations (within the treatment supplements) were determined by Brunswick Laboratories (Wareham, MA, USA) using automated HPLC technologies (ESA 582 HPLC (binary pump) with u.v.-visible twelve channel CoulArray detectors) and quantified via u.v. visualization. Total anthocyanin concentration was determined using cyanidin-3-glucoside as a standard.

Statistical analysis

Results are expressed as mean values with their standard errors. Baseline characteristics of the treatment groups were compared using independent paired *t* tests. The effects of diet treatment (freeze-dried wild blueberry powder) and postprandial times, as well as interactions between them, were determined by repeated measures ANOVA using the Statistical Analysis Systems statistical software package (version 6.1; SAS Institute Inc., Cary, NC, USA). Linear regression analysis was also conducted using SAS. Post-hoc tests using the least squared difference test and unpaired *t* tests were conducted to determine differences between individual group means.

Results

Blood lipids and glucose

The baseline characteristics of the eight subjects are given in Table 1. No significant differences in the fasting serum values were observed between the two treatment groups ($P > 0.05$). Results of supplementation on postprandial serum lipid and glucose concentrations are shown in Table 4. There were no significant time or treatment effects with respect to postprandial serum cholesterol concentrations.

The consumption of the treatment meals was associated with a significant time effect ($P = 0.001$) for the appearance of triacylglycerol (125 % greater than baseline). The rise in triacylglycerol following consumption of a high-fat meal is consistent with results in the literature (Ryu *et al.* 1992). There were no significant differences between the two groups for the postprandial appearance of serum triacylglycerol ($P > 0.05$) at any time point. The increase in serum triacylglycerol over time was positively correlated with an increase in ORAC_{total} ($P = 0.0001$).

The blueberry treatment was associated with a significant time ($P = 0.002$) and treatment ($P = 0.002$) effect for the appearance of serum glucose, however, there was no treatment \times time interaction and the time and treatment effects did not correlate with changes in ORAC or TAS ($P > 0.05$). Serum glucose concentrations were significantly higher in the blueberry-treatment group at 3 ($P = 0.03$) and 4 h ($P = 0.03$).

Serum antioxidant status

The blueberry treatment was associated with a significant treatment effect as represented by an increase in serum antioxidant status above the control group for the ORAC assay, PCA fraction (ORAC_{PCA} $P = 0.04$) (Fig. 1(a)). Furthermore, the consumption of both of the treatment meals was associated with a significant time effect, which was a result of a progressive increase in serum ORAC_{total} ($P < 0.001$) and ORAC_{acetone} ($P < 0.001$) (Fig. 2 (a and b)) over time.

The ORAC_{PCA} fraction revealed a peak maximal water-soluble response occurring at 1 h ($P = 0.02$) yielding an 8.5 % increase in serum antioxidant status over the control (Fig. 1(a)). This was supported by a significant increase (4.5 %) in TAS at 1 h ($P = 0.05$) (Fig. 1(b)). The protective effects witnessed at 1 h in both ORAC_{PCA} and TAS returned to baseline values ($P > 0.05$) for the 2–4 h sample points (Fig. 1 (a,b)). In contrast, the blueberry treatment was associated with a significant increase in serum total and lipid-soluble antioxidant status (15.0 % increase in ORAC_{total}, $P = 0.009$; 16.0 % increase in ORAC_{acetone}, $P = 0.007$) above the control at 4 h (Fig. 2 (a and b)).

Discussion

Diet and oxidative stress have been implicated in the development of cardiovascular disease (Castelli, 1998; Kaplan & Aviram, 1999), diabetes (Georgopoulos, 1999; Vendemiale *et al.* 1999) and cancer (Ames *et al.* 1995; Willett, 2001). Increasing the serum antioxidant status

Table 4. Effects of nutritional supplementation on serum lipid and glucose concentrations§
(Mean values with their standards errors for eight subjects)

	Time (h)	Treatment group‡				Statistical significance of effect (ANOVA): <i>P</i>	
		Control		Blueberry		Time	Treatment
		Mean	SEM	Mean	SEM		
Triacylglycerol (mmol/l)	0	1.00	0.13	1.20	0.18	< 0.0001	
	1	1.22*	0.15	1.27*	0.21		
	2	1.50*	0.20	1.57*	0.27		
	3	1.87*	0.29	2.10*	0.38		
	4	2.12*	0.36	2.62*	0.47		
Glucose (mmol/l)	0	5.19	0.20	5.46	0.15	< 0.005	< 0.005
	1	5.36	0.66	6.40	0.55		
	2	4.41*	0.22	5.01	0.27		
	3	4.01*	0.22	4.79*†	0.22		
	4	4.42*	0.23	5.00*†	0.11		
Total cholesterol (mmol/l)	0	4.91	0.27	5.04	0.21		
	1	4.96	0.31	5.03	0.26		
	2	4.98	0.32	5.07	0.23		
	3	4.86	0.30	5.03	0.28		
	4	4.84	0.32	5.03	0.29		
LDL-cholesterol (mmol/l)	0	3.44	0.30	3.44	0.21		
	1	3.37	0.32	3.41	0.25		
	2	3.29*	0.30	3.32*	0.21		
	3	3.03*	0.27	3.07*	0.23		
	4	2.91*	0.31	2.86*	0.24		
HDL-cholesterol (mmol/l)	0	1.00	0.06	1.05	0.08		
	1	1.03	0.08	1.04	0.09		
	2	1.00	0.06	1.02	0.09		
	3	0.97	0.07	0.99	0.08		
	4	0.95	0.08	0.96	0.08		

Mean values were significantly different from those at *t* 0 (paired *t* test): **P* < 0.05.

Mean values were significantly different from those of the control group (least squares difference test): †*P* < 0.05.

‡ There were no significant differences for baseline measures (*t* 0) between treatment groups (paired *t* test): *P* > 0.05

§ For details of subjects, test meals and supplements, see Tables 1–3 pp. 390–391.

|| Serum lipids and glucose analyses were conducted at an Ontario Ministry of Health Licensing and Inspection Branch licensed laboratory (Guelph General Hospital Core Laboratory Services, Guelph, Ont., Canada).

¶ LDL-cholesterol was calculated using the Friedwald equation (Friedwald *et al.* 1972).

has been proposed as a preventative means to reduce the development of these diseases (Kaplan & Aviram, 1999; Vendemiale *et al.* 1999; Willett, 2001). The aim of the present trial was to examine the effects of wild-blueberry supplementation on serum antioxidant status in healthy human volunteers. Previous studies have demonstrated the *in vitro* antioxidant properties of wild blueberries to be higher than that of most fruits and vegetables, as well as that of cultivated highbush blueberries (Satué-Gracia *et al.* 1997; Prior *et al.* 1998). The present study indicates the antioxidant potential for the blueberry or a blueberry extract as a functional food under physiological conditions.

Supplementation

The decision to feed the subjects a high-fat meal was based on the evidence that the consumption of a high-fat meal is associated with postprandial lipaemia and increased oxidative stress (Papas, 1996; Hennig *et al.* 2001). We therefore aimed to create a dietary stress similar to that encountered when eating a typical 'fast-food' meal. The decision to supplement the subjects with 100.0 g dried blueberry powder was based on achieving a maximal level of anthocyanins without exceeding a dietary acceptable

level of fibre (Howarth *et al.* 2001). The 100.0 g blueberry powder supplement contained 17.8 g fibre. It is important to note that 100.0 g dried blueberry powder is the equivalent of approximately 500–650 g whole blueberries, which exceeds that of a typical serving size. The design of the present study does not allow for the determination of the minimal dose required to achieve a significant response; however, now that an effect has been observed, subsequent studies are needed to determine if a statistically significant response could be observed with lower doses of blueberries. Furthermore, if supplementation is considered beneficial, further refinements could decrease the quantity of powder needed considerably, as well as producing a blueberry extract with high concentrations of berry phytochemicals.

Possible effect of other dietary components on serum antioxidant status

The blueberry-supplemented group as studied herein was provided with 17.8 g fibre more than the control group, but this did not result in the attenuation of postprandial serum glucose or triacylglycerol absorption (Table 4). Previous studies have shown that certain fibres can attenuate postprandial increases in serum glucose (Ou *et al.* 2001).

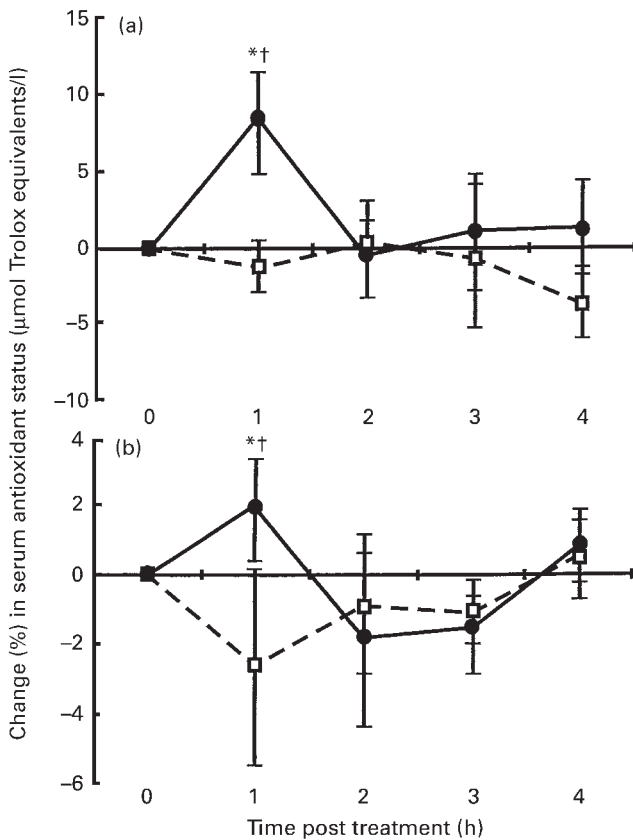


Fig. 1. Change (%) in serum antioxidant status over 4h following the consumption of a high-fat meal with a control supplement (□) or 100.0g freeze-dried wild blueberry powder (*Vaccinium angustifolium*) (●). Serum antioxidant status measured as: (a) oxygen radical absorbance capacity (ORAC)_{perchloric acid} (PCA) (water-soluble fraction) (Genox Laboratories, Baltimore, MD, USA); (b) total antioxidant status (TAS) (Randox-TEAC assay; Randox Laboratories, Mississauga, Ont., Canada). Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. For details of test meal, supplements and procedures, see Tables 2 and 3 and pp. 390 and 391. Values are means for eight subjects with standard errors shown by vertical bars. Mean values were significantly different from those of the control group at a given time point (least squared difference test); * $P < 0.05$. Mean values were significantly different from those at baseline (t_0) (paired t test); † $P < 0.05$.

The blueberry treatment was associated with a significant time and treatment effect for the appearance of serum glucose. This glucose effect was significantly ($P < 0.05$) different from the control group at the 3 and 4h sample points. We attribute the glucose effect to differences in monosaccharide content between the control and the blueberry treatment. Blueberries contain a high concentration of fructose (up to 50.0% digestible carbohydrate; Payne, 2000); however, the control supplement contained only glucose (dextrose). Previous research has determined that fructose accompanied by a similar or equivalent concentration of glucose may enhance glucose and fructose absorption (Perman, 1996) and delay glucose utilization (Shi *et al.* 1997). The conversion of fructose to glucose by the liver and subsequent release of serum glucose into the systemic circulation results in a further delayed appearance of glucose following a postprandial fructose load (Chandramouli *et al.* 1993). Furthermore, glucose is an

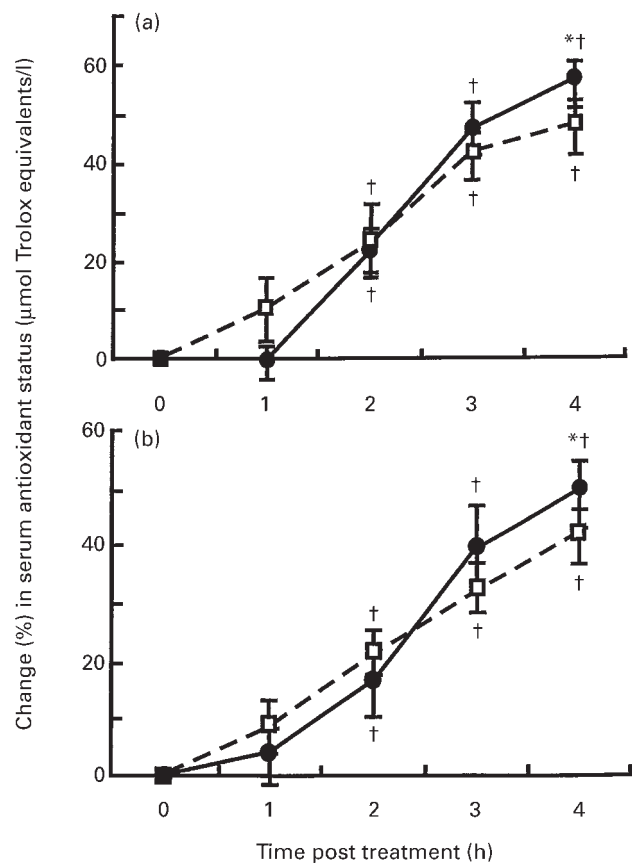


Fig. 2. Change (%) in serum antioxidant status over 4h following the consumption of a high-fat meal with a control supplement (□) or 100.0g freeze-dried wild blueberry powder (*Vaccinium angustifolium*) (●). Serum antioxidant status measured as: (a) oxygen radical absorbance capacity (ORAC)_{total} (total fraction) (Genox Laboratories, Baltimore, MD, USA); (b) ORAC_{acetone} (lipid-soluble and water-soluble fraction) (Genox Laboratories). Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. For details of test meal, supplements and procedures, see Tables 2 and 3 and pp. 390 and 391. Values are means for eight subjects with standard errors shown by vertical bars. Mean values were significantly different from those of the control group at a given time point (least squared difference test); * $P < 0.05$. Mean values were significantly different from those at baseline (t_0) (paired t test); † $P < 0.05$.

unlikely contributor to the ORAC values observed with the blueberry treatment. In fact the ORAC value for the treatment supplement containing mainly glucose was negligible (5 ORAC units). In addition, there was no correlation between postprandial glucose concentration and ORAC or TAS results ($P > 0.05$). We attribute the glucose effect experienced in the blueberry-treatment group to differences in fructose concentration between the blueberry treatment and the control treatment and not the difference in fibre concentrations between the two groups. In addition, the blueberry treatment was not associated with an attenuation of postprandial triacylglycerol absorption as is sometimes observed with fibre consumption (Schepach *et al.* 2001). Furthermore, previous researchers have determined that the high *in vitro* antioxidant properties of the blueberry are not a result of their fibre content (Bushway *et al.* 1983; Prior *et al.* 1998). We therefore conclude that the difference in fibre content between the treatment groups was not

substantial enough to have been responsible for the observed effects on serum glucose, lipids or antioxidant status.

An increase in ORAC_{total} and ORAC_{acetone} following consumption of the high-fat meal occurred in the control group (as well as the blueberry-treatment group) regardless of an essentially antioxidant-free control supplement. No such increase was found in the control group when using the TAS assay. This dissimilarity in findings between the two assays is likely to be a result of mechanistic differences between the assays. The TAS assay measures the inhibition of free radical action, which is similar to the basis of the ORAC assay; however, the TAS assay measures the inhibition at a fixed time (10 min) whereas the ORAC assay measures the time it takes for this reaction to reach completion (≥ 60 min). Measuring the reaction to completion allows for the contribution of all oxidizable substrates within the sample to prevent oxidation of the target molecule. Since the TAS assay has a fixed inhibition time, it has a greater ability to reflect the effect of faster-acting antioxidants in the serum (i.e. water-soluble antioxidants) and not the slower-oxidizable substrates. This is probably why there was no increase in serum antioxidant status following ingestion of the high-fat meal in the control group when using the TAS assay (Fig. 1(B)). This unexpected increase in antioxidant potential in the control group may therefore reflect the stress imposed by the high-fat meal as exhibited with the ORAC assay. We rationalize this latter effect with the following possible explanations. First, the ORAC assay (Cao *et al.* 1993) measures the area under the curve of the decay of a target molecule (β -phycoerythrin). Any potentially oxidizable substrates (i.e. amino acids, fatty acids or carbohydrates) present in the sample can theoretically retard the decay of the target (Cao & Prior, 2000). We consider that the fatty acids may have been the primary substrates causing this antioxidant effect in the control group (as well as in the blueberry-treatment group). Since the results obtained from the ORAC_{total} (containing proteins) and ORAC_{acetone} (void of proteins) assays varied by only a small margin, it is safe to assume that proteins within the serum samples were probably not acting as the primary substrates for oxidation. Second, glucose has very little antioxidant activity when analysed using ORAC, thus eliminating carbohydrate as a main substrate contributing to the antioxidant effect of the treatments. Third, the high-fat meal contained only 6.0 mg vitamin C and 2.1 mg vitamin E. Previous studies using much higher concentrations (20–90-fold) have shown no effect on serum oxidation (Priemé *et al.* 1997; van den Berg *et al.* 2001). Other possible explanations such as the up-regulation of endogenous antioxidant enzymes during the postprandial state will not be explored in the present discussion, as there are insufficient results in the literature at the present time to support this concept. Finally, the postprandial appearance of serum triacylglycerol correlated significantly ($P < 0.001$) with the ORAC_{total} and ORAC_{acetone} results. Therefore, it is logical to assume that the increase in ORAC_{total} and ORAC_{acetone}, as exhibited in the control group, is likely to be a result of the inability of the ORAC assay to differentiate between fatty acid oxidation (non-enzymatic) and the free radical-quenching capacity of serum antioxidants.

Experimental design

The design of the present study does not allow us to determine what compounds within the blueberry are responsible for the increase in serum antioxidant status observed in this trial. However, it is most likely that anthocyanins within the blueberry are the major contributing phenolic compounds responsible for this effect. Anthocyanins comprise the highest concentration of all phenolic subgroups in the blueberry (Wang & Lin, 2000). Previous research has determined that the ORAC value of the blueberry correlates more strongly with anthocyanins than with total phenolics in the berry (Mazza & Oomah, 2000). Furthermore, the antioxidant properties of anthocyanins have been validated using other systems of oxidation such as their ability to prevent LDL oxidation *in vitro* (Laplaud *et al.* 1997). Therefore, although we have not proven directly that anthocyanins are responsible for the observed antioxidant effect in the blueberry-treatment group, studies have shown that the *in vitro* antioxidant properties of wild blueberries are mainly a result of their high concentration of anthocyanins (along with other phenolics) and not their concentrations of antioxidant vitamins, minerals or fibre (Bushway *et al.* 1983; Prior *et al.* 1998; Kalt *et al.* 1999).

Subsequent studies from our group (J Mazza, CD Kay and BJ Holub, unpublished results) have concluded that supplementation with 100.0 g freeze-dried wild blueberry powder results in the absorption and appearance of intact anthocyanins in the postprandial serum samples. We cannot unequivocally exclude the possibility of other absorbed phenolics contributing to the antioxidant effect as observed herein. We can, however, confidently attribute the observed effects to the consumption of the wild blueberry powder.

Although we have demonstrated that supplementation with a freeze-dried wild blueberry powder increased serum antioxidant status, the magnitude of the observed response cannot be arbitrarily translated into a decreased risk of chronic degenerative disease. Studies have indicated an increased risk of chronic disorders in individuals with low levels of fasting antioxidants (Lachance, 1998). However, at the present time there are insufficient results in the literature to determine the magnitude of chronic degenerative disease risk reduction with given increases in serum antioxidant status. There is current evidence from human subjects indicating that increased total serum antioxidant status (as determined by the TAS assay) is associated with significant reductions in breast cancer risk (Ching *et al.* 2002).

The effect of the blueberry treatment on serum antioxidant status

The blueberry treatment was associated with a significantly ($P < 0.05$) higher increase in serum antioxidant status relative to the control at 1 and 4 h post consumption on the high-fat meal. Peak serum concentration of anthocyanins as determined previously (Cao *et al.* 2001) occurred at 60–70 min after consumption. This is consistent with our present findings of a maximum water-soluble antioxidant

effect at 1 h. In our present study, ORAC_{PCA} peaked at 1 h in the blueberry-treatment group, when it was significantly ($P < 0.05$) different from that of the control group, and returned to baseline by the 2 h sample point. This difference at 1 h paralleled that of the TAS results. In contrast, the changes in ORAC_{total} and ORAC_{acetone} did not become significant ($P < 0.05$) until the 4 h sample point. We rationalize this to be a result of the water-soluble anthocyanins and other phenolics within the blueberries, which may possibly be competing with less reactive antioxidants and endogenous enzymes, therefore sparing them for later postprandial oxidation reactions. It is also possible that the anthocyanins together with other phenolic compounds within the blueberry may be regenerating oxidized lipid-soluble antioxidants in the serum, similar to the process of vitamin E regeneration by vitamin C (Niki, 1996). Although the blueberry treatment contained 10.6 mg vitamin C more than the control, we do not believe this level could be responsible for any of the observed changes in serum antioxidant status. Previous research using much higher concentrations (10–45-fold) have shown no effect on serum antioxidant status (Priemé *et al.* 1997; van den Berg *et al.* 2001). Furthermore, it has been reported that ascorbate makes very little contribution (0.8–1.5%) to the total *in vitro* antioxidant capacity of the blueberry (Prior *et al.* 1998; Kalt *et al.* 1999).

Decreased serum antioxidant status has been suggested as a risk factor in cardiovascular disease (Kaplan & Aviram, 1999), diabetes (Georgopoulos, 1999; Vendemiale *et al.* 1999), and cancer (Ames *et al.* 1995; Willett, 2001). The reduced risk of these diseases associated with increased consumption of fruits and vegetables may be attributed to the non-nutritive antioxidant phytochemicals within such foods (Loft & Poulsen, 1996; Scalber & Williamson, 2000) and not to their levels of traditional antioxidant vitamins and minerals (Steinmetz & Potter, 1996; Priemé *et al.* 1997). It is likely, however, that any such protective effects on chronic disorders occur as a result of interactions between traditional antioxidants, endogenous enzymes, and antioxidant phytochemicals.

Conclusions

In conclusion, we have demonstrated that supplementation with a freeze-dried wild blueberry powder increased serum antioxidant status following the consumption of a high-fat meal. Increasing the serum antioxidant status has been suggested as a possible method of reducing the risk of many chronic degenerative disorders (Kaplan & Aviram, 1999; Georgopoulos, 1999; Vendemiale *et al.* 1999; Willett, 2001). *In vitro* analysis of the blueberry shows it to have highly active antioxidant characteristics. The present study provides physiological evidence for the enhancement of postprandial serum antioxidant status in human volunteers consuming a blueberry-supplemented meal.

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