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Satiety in rats following blueberry extract consumption induced by appetite-suppressing mechanisms unrelated to *in vitro* or *in vivo* antioxidant capacity

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Abstract

Water extracts of two blueberry cultivars ('Centurion' and 'Maru') were tested for their ability to modify appetite in a rat model. The fruits of 'Centurion' had higher *in vitro* antioxidant capacity (as measured using the ferric reducing antioxidant power (FRAP) assay) and higher total phenolic content (TPC, as measured by the Folin–Ciocalteu method) than fruits of 'Maru'. When rats were gavaged with water-soluble blueberry extract (BBE; 1 ml/day) of both cultivars for 6 days, serum FRAP increased significantly when compared to water-gavaged controls, indicating that BBE may have the ability to elevate circulating antioxidant potentials *in vivo*. Both cultivars had a satiating influence on experimental rats, as evidenced by their ability to decrease food intake by 8.6% ('Maru') and 6.2% ('Centurion'), although a statistically significant decrease over the control rats was achieved only for the 'Maru' treatments. In addition, body weight gain of rats gavaged with extracts from 'Maru' and 'Centurion' cultivars decreased by 9.2% and 5.3% relative to the rats in the control group, respectively. The reduction in food intake over a 4 h period compared to a control treatment preloaded with the same volume of water suggests that the decrease in food intake was mainly a consequence of a satiating effect, rather than a stomach distension effect. The observed results suggest that the reduction in food intake and decrease in body weight in experimental animals is not merely a consequence of antioxidant mechanisms. BBE may provide a good satiety inducer and weight management modulator.

Keywords: Blueberry; Cultivars; Satiety; Rats; Antioxidant; FRAP

1. Introduction

Obesity is increasingly being recognised as a significant health challenge facing all ages of the population (Peters, Barendregt, Mackenbach, Mamun, & Bonneux, 2003). Obesity is a risk factor for several chronic disorders such as diabetes, cardiovascular disease, hypertension, sleep apnea, osteoarthritis of weight-bearing joints, reduced fertility, asthma and some cancers (Rippe, 1998) and hence, effective treatment of obesity can lead to a reduction of risk factors for these diseases and may result in decreased morbidity and mortality. The etiology of obesity is multifactorial. Genetic, environmental, metabolic and behavioural issues may all contribute to the development of obesity (Rippe, 1998). The culture of over-eating and a sedentary lifestyle is compounding the effects of a dietary profile that contains a large percentage of energy-dense processed and conventional foods (WHO, 1998).

In the United States, where the epidemic is particularly evident, the number of approved drug treatments available to treat the disease has been reduced, rather than increased, over the last 5 years. Two commonly prescribed drugs, dexfenfluramine and fenfluramine, were recently linked to valvular heart disease (Connolly et al., 1997) which led to their withdrawal. Although there are functional foods indicated for proactive control of some chronic disease conditions such as cardiovascular disease and cancer, there

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is no such food indicated for control of obesity (St-Onge, 2005) and hence efficient, effective and satisfying dietary interventions are required.

Blueberries (*Vaccinium* spp.) are rich sources of bioactive compounds, such as phenolics and organic acids, which have antimicrobial activities against human pathogens (Puupponen-Pimiä et al., 2001, 2005). Several components in blueberry fruit, including anthocyanins (Cao, Booth, Sadowski, & Prior, 1998), have been shown to have antioxidant activity. Recent research conducted by Lau, Shukitt-Hale, and Joseph (2005) has shown that nutritional antioxidants, such as the polyphenols found in blueberries, can reverse age-related declines in neuronal signal transduction as well as cognitive and motor deficits. Other studies have demonstrated for the first time that polyphenolic compounds are able to cross the blood–brain barrier and localise in various brain regions important for learning and memory (Andres-Lacueva et al., 2005). Kraft et al. (2005) reported that lowbush blueberries contain a range of compounds such as phytosterols, phenolic acids, flavan-3-ols, anthocyanins and oligomeric proanthocyanidins that protect against the initiation, promotion and progression stages of carcinogenesis and that different compounds are effective against each of these stages.

This study was conducted to determine whether water-soluble blueberry extracts (BBE), given shortly before the onset of a meal, would influence total daily food intake or body weight gain. *In vitro* antioxidant capacity of the berry fruits and *in vivo* antioxidant capacity in serum, after berry extract ingestion, were also monitored.

2. Materials and methods

2.1. Chemicals and standards

2,4,6-Tripyridyl-*s*-triazine (TPTZ), sodium acetate, ferric chloride and gallic acid, Folin–Ciocalteu's phenol reagent and ferrous sulfate were purchased from Sigma (Sigma–Aldrich Pty. Ltd., Castle Hill, NSW 1765, Australia).

2.2. Preparation of extracts

Crude aqueous extracts from two rabbiteye blueberry (*Vaccinium asheii*) cultivars ('Maru' and 'Centurion') were prepared by weighing fresh fruits (100 g), mixed with 100 ml of distilled water and then milled using a commercial mini-processor (Braun Miniprimer MR300, Germany). The crushed berries were put in centrifuge tubes. Tubes were centrifuged (3000*g*, 15 min) and the clear supernatant fluid was collected and used either within 1 h of collection or stored at −80 °C for further work.

2.3. Estimation of total phenolic content

The total phenolic content (TPC) was quantified according to the method of Yuan, Bone, and Carrington (2005). Briefly, aliquots of water-soluble extracts (100 μL) were mixed with 2.0 ml of 2% Na_2CO_3 and were allowed to sit at room temperature for 2 min. At this time, 100 μL of 50% Folin–Ciocalteau's phenol reagent was added and the reaction tubes were vortexed and allowed to sit at room temperature for a further 30 min prior to reading the absorbance at 720 nm. Calibration was achieved with an aqueous gallic acid solution (100–1000 μg/ml). Total phenol values are expressed as gallic acid equivalents (GAE) based on the calibration curve.

2.4. Evaluation of antioxidant activity

The antioxidant capacity of blueberry extracts was determined using the FRAP assay, a colorimetric assay that measures the ability of the tested sample to reduce the intense blue ferric tripyri-dyltriazine complex to its ferrous form, thereby changing its absorbance (Benzie & Strain, 1996). The working FRAP reagent was prepared by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 1 volume of 10 mmol/L TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mmol/L hydrochloric acid and 1 volume of 20 mmol/L ferric chloride. Freshly prepared FRAP reagent (1.5 mL) was warmed to 37 °C and then 50 μL of extract and 150 μL of deionised water was added to the FRAP reagent. Absorbance readings were taken after 30 min at 593 nm (Wicklund et al., 2005). Aqueous solutions of the $FeSO_4 \cdot 7H_2O$ concentrations, in the range of 100–2000 μmol/L, were used for calibration. The results were corrected for dilution and expressed in μmol Fe(II)/L. All solutions were used on the day of preparation and all determinations were performed in duplicate and the mean of the results of two experiments are presented.

2.5. Animals and housing

Thirty female Sprague Dawley rats aged ten weeks that had been weaned onto a balanced semi-synthetic diet were housed individually, in hanging wire-mesh stainless steel cages, in a room with a temperature of 22 ± 1 °C and a 12-h light: dark cycle (light on at midnight) and they had free access to water throughout the study. The animal protocol was approved (Protocol # 05/129) and followed the procedures set by the Animal Ethics Committee of the Massey University (Palmerston North, New Zealand).

For the first seven days of the study, the rats were conditioned to a meal-feeding regimen, whereby a feeder containing more than one day's expected intake of food was made available to each rat for a total of 4 h (1000 h; 2 h in light cycle, and 2 h in the dark cycle to accommodate the rat's natural behaviour of nocturnal eating). The time period in which rats had access to food was restricted to 4 h/day in an attempt to provide a more controlled and exaggerated appetite response at feeding and accentuate gastric mechanisms of intake regulation (Froetschel, Azain, Edwards, Barb, & Amos, 2001).

2.6. Premeal administration

The rats were given a premeal of 1 ml of BBE administered via gavage (at the back of the throat) using a very soft silicon rubber tube attached to a 1-ml syringe. In order to ensure that the rats were comfortable with this procedure, they were handled daily during the 7 day adaptation period and for the last 3 days of the adaptation period, a premeal of distilled water (at room temperature) was gavaged into each rat's throat before the daily meal was offered. This gavaging technique guaranteed that the rats received orally a defined amount of load.

For the remainder of the study (from day 8 to the end) the rats were divided into 3 equal groups ($n = 10$). The rats in the first group were gavaged once a day with 1 ml of distilled water for 6 consecutive days to serve as a control group. The rats in the second group were gavaged with 1 ml of extract prepared from the fruits of 'Maru' while those in the third group were gavaged with 1 ml of extract from the fruits of 'Centurion' for 6 days.

The premeal was given to each rat 20 min before the normal meal time, and the impact of the premeal was determined by monitoring changes in each rat's total food intake for that day. The diet (AIN-93 G) offered to the rats during the normal meal time was standardised across all treatments and was formulated to meet the nutrient requirements of growing rats. During the entire study daily food intake and water intake were measured and the body weight of each rat was monitored twice weekly.

The amount of premeal given to each rat was individually calculated based on body weight, at a rate of 4 ml (diluted extract)/kg body weight. This rate was selected based on the assumption that a 60 kg person might consume a 120 ml volume of full-strengh juice, which is equivalent to 2 ml/kg body weight, which can be translated to 4 ml of diluted (50%) extract per kg body weight in the rat.

2.7. Serum antioxidant capacity

Blood (~2 ml) was collected from each rat in the control group and those gavaged with blueberry extracts. Samples were allowed to clot at room temperature for 25 min. Samples were then immediately centrifuged (1000*g*) for 15 min at 4 °C to recover serum. Serum was extracted and then stored at −80 °C for further use.

In order to assess the effect of blueberry extracts on serum antioxidant status, the FRAP assay was used. Ferric reducing ability of serum was determined on 50 μL serum samples and the tripyridyltriazine complex formed with the reduced ferrous ions was measured spectrophotometrically as described above.

2.8. Data calculations and statistical analysis

All intake data were normalised by expressing intake per kg of metabolic body weight (true body weight to the power of 0.75; units $kg^{0.75}$). Metabolic body weight (MBwt) calculations allow for a comparison of intake across variable body weights.

The % reduction in food intake (%FIR) was calculated by using the following equation:

$$\%\text{FIR} = \frac{[\text{Food Intake (Control)} - \text{Food Intake (BBE Premeal)}] \times 100}{\text{Food Intake (Control)}}.$$

Results are expressed as mean ± SEM. Statistical analysis was carried out using SAS for Windows (Version 9). The significance of differences was assessed by one-way ANOVA for comparison of individual means. The level of significance was set at $P < 0.05$.

3. Results

3.1. Antioxidant activity and total phenolic content of blueberry extracts

The antioxidant activity and TPC of extracts from the fruits of 'Centurion' were significantly higher than their counterparts from 'Maru' (Table 1).

The FRAP and TPC values were strongly correlated to each other in extracts prepared from both cultivars ($R^2 = 0.8456$ for 'Maru' extract and $R^2 = 0.9291$ for 'Centurion' extract; Table 1).

3.2. Effect of blueberry extracts on food intake, water intake and body weight gain

The results demonstrated for the first time that rats gavaged daily for 6 days with 1 ml of extract prepared from 'Maru' fruits consumed significantly less food ($P = 0.011$) than rats in the control group (Fig. 1a); this corresponded to a 8.6% reduction in food intake (Fig. 1b). More importantly, rats gavaged with extract from 'Maru' fruits had significantly lower ($P = 0.0074$) final body weights than rats in the control group after 6 days of gavaging (Fig. 2a). Body weight gain of rats gavaged with the extract from 'Maru' fruits declined by 9.2% after 6 days gavaging (Fig. 2b) relative to the rats in the control group.

Although relative intake of food was also reduced in the 'Centurion' BBE treatment (6.2% ($P = 0.066$) reduction), the decrease was of border-line significance when compared with the control group (Fig. 1). Body weight gain of rats gavaged with the extract from 'Centurion' fruits decreased by 5.3% after 6 days gavaging (Fig. 2b) relative to the control rats.

Overall, water intake in rats preloaded with extract from both cultivars was not affected in comparison with the rats gavaged with water only (data not shown).

3.3. In vivo antioxidant status

Fig. 3 shows the differences in serum FRAP between rats gavaged with BBE and control rats gavaged with water

Table 1
Antioxidant activity (FRAP-values; μmol/L) and total phenolic contents (TPC; μg galic acid equivalent/ml) of water-soluble extracts prepared from the fruits of two blueberry cultivars ('Maru' and 'Centurion')

Measurement	'Maru' extract	'Centurion' extract
Antioxidant activity (FRAP; μmol/L)	9531 ± 63.6	$11919 \pm 20.1^{***}$
Total phenolic content (TPC; μg galic acid equivalent/ml)	1350 ± 10.3	$1441 \pm 20.8^{*}$
Relationship between FRAP and TPC values (R^2 value)	$R^2 = 0.8456$	$R^2 = 0.9291$

The mean of duplicate incubations of two identical experiments and standard errors are presented. Statistical analysis based on ANOVA, $^{*} = P < 0.05$, $^{***} = P < 0.0001$. Asterisks represent the significant difference between 'Maru' and 'Centurion' extracts.

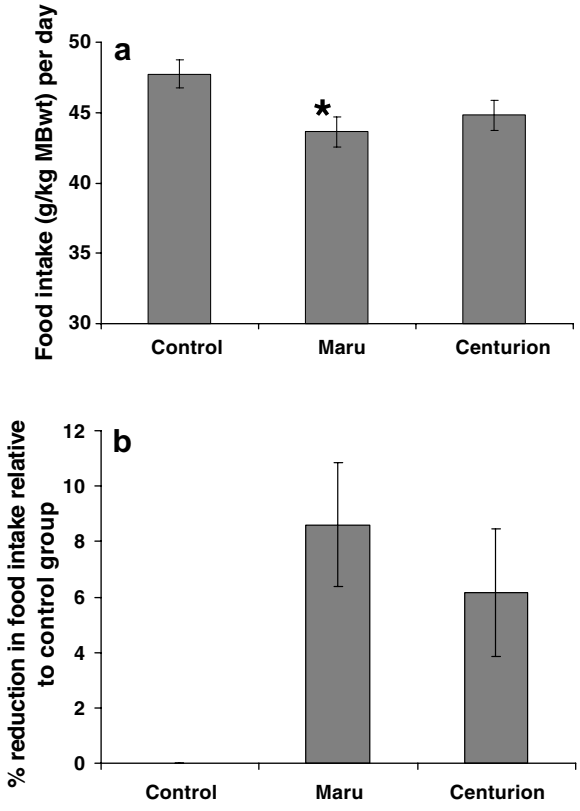

Fig. 1. Effects of 6 day oral administration of blueberry extract on food consumption (g/kg metabolic body weight (MBWT); a) and % reduction in food intake (b) in rats. Rats were divided into three groups of ten rats each. The experimental rats were gavaged with 1 ml of blueberry water-soluble extracts for 6 consecutive days. The control group was gavaged with 1 ml of distilled water for 6 days. Data are expressed as means ± SEM. Significantly different from the value in the control group: $^{*}P < 0.05$.

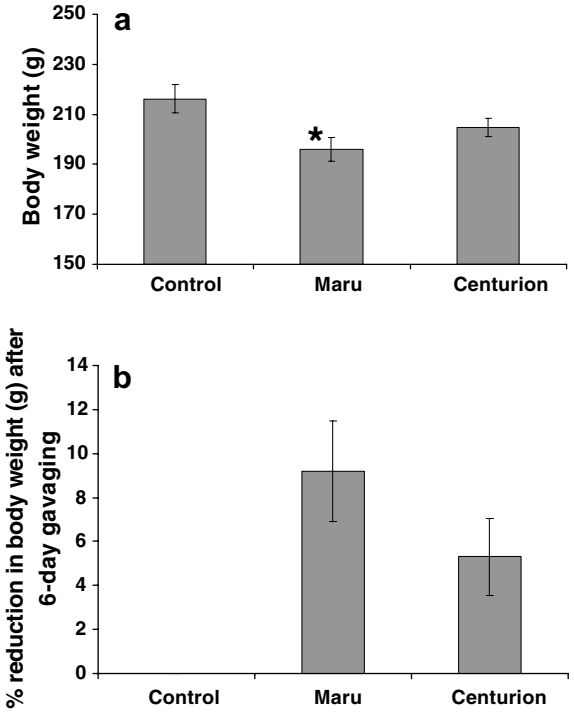

Fig. 2. Effects of 6 day oral administration of blueberry extract on body weight (a) and % reduction in body weight (b) in rats. Rats were divided into three groups of ten rats each. The experimental rats were gavaged with 1 ml of blueberry extracts for 6 consecutive days. The control group was gavaged with 1 ml of distilled water for 6 days. Data are expressed as means ± SEM. Significantly different from the value in the control group: $^{*}P < 0.05$.

only. The FRAP value, an indicator of total antioxidant defense, was significantly affected by gavaging with BBE (808.5 ± 42.7 μmol FeII/L in controls vs. 950.9 ± 39.7 μmol FeII/L in rats gavaged with 'Maru' extract; $P = 0.0404$ and 1112.5 ± 83.6 μmol FeII/L in rats gavaged with 'Centurion' extract; $P = 0.0032$).

4. Discussion

The high antioxidant activity of blueberry extracts may be related to the high levels of polyphenolic compounds as evidenced by the strong correlation between the FRAP val-

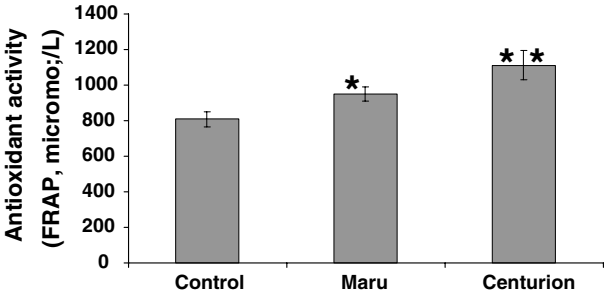

Fig. 3. Effect of oral administration of water-soluble extracts from two blueberry extracts ('Maru' and 'Centurion') on antioxidant status (FRAP values; μmol/L) in sera collected from the rats 6 days after gavaging. Data are expressed as means ± SEM. Significantly different from the value in the control rats: $^{*}P < 0.05$, $^{**}P < 0.01$.

ues and TPC values. This is consistent with previous studies which have shown that polyphenols are major contributors of the antioxidant activity (Connor, Luby, Hancock, Berkhemer, & Hanson, 2002; Prior et al., 1998; Schotsmans, Molan, & MacKay, 2007).

The results of this study demonstrated that consumption of blueberry extract could increase the antioxidant capacity of serum in rats. Significant increase in serum antioxidant level was observed in rats gavaged with extracts from both 'Maru' and 'Centurion' cultivars compared to the control rats gavaged with the same volume of water. The increased antioxidant capacity in serum (FRAP) following the oral gavage of blueberry extracts may indicate a direct absorption and/or an enhanced production of antioxidants. The antioxidants responsible for the increased antioxidant capacity following the consumption of blueberry extracts are likely to be phenolic compounds including flavonoids. Berry fruits contain wide range of bioactive compounds, such as phenolics, organic acids and anthocyanins which have antimicrobial activities against human pathogens (Puupponen-Pimiä et al., 2001, 2005) and antioxidant activity (Cao et al., 1998).

Decreased serum antioxidant status has been suggested as a risk factor in cardiovascular disease (Kaplan & Aviram, 1999) and cancer (Ames, 1995). On the other hand, increasing the serum antioxidant status has been suggested as a possible method of reducing the risk of many chronic degenerative disorders (Georgopoulos, 1999; Kaplan & Aviram, 1999; Vendemiale, Grattagliano, & Altomare, 1999).

The results demonstrated for the first time that rats gavaged daily for 6 days with 1 ml of extract prepared from 'Maru' and 'Centurion' fruits consumed less food than their counterparts preloaded with the same volume of water. The reduction in food intake, observed in rats given a preload of BBE in comparison to rats in a control group given the same preload volume of water, has led to the conclusion that the decrease in food intake was mainly the consequence of a satiating effect of the BBE rather than simply a stomach distension effect. It is also important to mention that water intake for the groups given the BBE was similar to that of rats given water only and no significant differences were observed. Moreover, the body weight gain of the rats given BBE was significantly lower than that observed in the control groups.

The time period in which rats had access to food was restricted to 4 h/day in an attempt to provide a more controlled and exaggerated appetite response at feeding (Froetschel et al., 2001). This time interval set for meal-feeding was considered to be the minimal time that would still allow the rats to consume enough food and grow comparably to those with 24-h access to food (Froetschel et al., 2001). The ability of BBE (especially from 'Maru') to reduce the food intake coupled with the decrease in body weight gain compared with their counterparts given water (control group), suggests that BBE may be a good satiety inducer and weight management modulator. Recently, St-Onge (2005) reported that the inclusion of foods or the replacement of habitual foods with others that may enhance energy expenditure (EE) or improve satiety may be a practical way to maintain a stable body weight or to assist in achieving weight loss; such foods may act as functional foods in body weight control.

Although the precise mechanisms which underlie the satiating effects of blueberry extract are not fully understood, it may trigger receptors for amino acids which have been detected in the wall of the upper intestine (Mei, 1992). The fibers from these receptors may inform certain brain centers that a source of energy and/or a specific nutrient has been ingested. Alternatively, such receptors may play a role in inducing satiety by triggering the release of hormones such as cholecystokinin (CCK), which might act directly at the central and/or peripheral levels to stimulate pancreatic juices and reduce gastric emptying (Mei, 1992).

Further studies to investigate the effect underlying mechanisms responsible for the satiating effect are needed.

5. Conclusions

The current study has demonstrated for the first time a reducing effect of blueberry extract premeals on subsequent meal intake. The underlying mechanism responsible for this response was not identified as part of this study. Extracts from 'Maru' and 'Centurion' cultivars showed satiating activity as evidenced by their ability to reduce food intake when orally gavaged into rats, compared with the food intake of control rats given a gavage of water only. The final body weight of the rats given 'Maru' and 'Centurion' extracts was lower than that observed in the control groups. In addition, the BBE administration was associated with a significant increase in serum antioxidant status above the control group.

The ability of BBE to reduce the food intake coupled with the decrease in body weight gain compared with their counterparts given water (control group), suggests that BBE may be a good satiety inducer and weight management modulator.

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