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Effect of Highbush blueberry consumption on markers of metabolic syndrome

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Nutritional Science

at Massey University, Palmerston North
New Zealand

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2014
Abstract

**Background**:

Metabolic syndrome (MS) is becoming a major public health challenge worldwide, and is associated with a higher risk of the development of several chronic diseases including type II diabetes. Being physically active would provide the most effective management for metabolic disorders; however, the use of dietary bioactive compounds from various plants has also been proposed as an alternative approach. A number of experimental studies indicate that Lowbush blueberries may be able to reduce symptoms of MS but the evidence for Highbush blueberries, which are commonly consumed, is scarce and their benefits remain doubtful. Therefore, the primary objective of this thesis was to investigate the effect of selected Highbush blueberries grown in New Zealand on their potential for managing metabolic-related disorders in order to provide further knowledge of the role for bioactive compounds from Highbush blueberries.

**Method**:

The selected eight Highbush blueberry cultivars were initially characterised by measuring total phenolic content using a Folin-Ciocalteu procedure; anthocyanin profiles and chlorogenic acid concentration by HPLC; and antioxidant capacity by the ferric reducing antioxidant power (FRAP) and by 2,2, diphenyl-picrylhydrazyl (DPPH) assays (Chapter 3). Further experiments were then carried out to investigate whether these Highbush blueberries possess any activity against measures of MS in vitro. The ability of Highbush blueberries to inhibit α-amylase and α-glucosidase, the enzymes involved in breaking down starch, and their abilities to enhance the growth of beneficial probiotic bacteria, another mechanism associated with improving insulin resistance, were tested in Chapter 4. Finally, the physiological effects of Highbush blueberry consumption on metabolic syndrome biomarkers were assessed in vivo using animal models of diet-induced metabolic syndrome (Chapter 5-7).

**Results**:

Our results demonstrated that selected Highbush blueberries grown in New Zealand contained considerable amounts of polyphenolics and total anthocyanins, and exhibited high antioxidant activities, with ‘Burlington’ and ‘Elliott’ cultivars exhibiting the highest total phenolic content (> 3.4 mg GAE/g frozen berries (FB)), total anthocyanins (> 2.2 mg/g FB) and antioxidant capacities (FRAP; > 3.0 mg FeSO₄/g FB, DPPH; > 65% inhibition at 5 mg FB). Further in vitro experiments
supported the ability of these blueberries to inhibit α-amylase (10-40% inhibition at 20 mg FB) and α-glucosidase (10-50% inhibition at 25 mg FB); additionally, some blueberry cultivars possessed the ability to increase the growth of the probiotic bacteria Lactobacillus acidophilus by more than 0.5 log_{10} CFU/mL. However, the extent of these benefits was not closely correlated with total phenolic content ($R^2 < 0.27$), total anthocyanins ($R^2 < 0.23$), or antioxidant capacities (FRAP; $R^2 < 0.42$, DPPH; $R^2 < 0.24$) across all genotypes, indicating that these anti-metabolic syndrome abilities were not simply due to the total bioactives or antioxidant capacities presented in the berries. ‘Burlington’ and ‘Bluecrop’, which exhibited strong enzyme inhibition as well as enhanced beneficial probiotic bacterial growth but contained different components of individual anthocyanins, were chosen for further testing in vivo. Rats fed a high-fat-high-sugar diet plus 1% freeze-dried whole blueberries (both cultivars) for 8 weeks showed signs of improvement of glucose tolerance and exhibited between 30 and 36% decrease in the degree of insulin resistance (HOMA-IR) as compared to the controls. The blueberries also showed a trend to increase the growth of beneficial bacteria, Lactobacillus spp. ($P = 0.20$) and Bifidobacterium spp. ($P = 0.15$), in the rats’ caecal content. However, no reduction in body weight or fat accumulation was observed with blueberry supplementation. There were no significant differences ($P > 0.05$) in the abilities of ‘Burlington’ and ‘Bluecrop’ to modulate any metabolic biomarkers assessed in vivo.

**Conclusion:**

Inclusion of the blueberries into the diet showed promise for management of some markers of metabolic syndrome, in particular the improvement of insulin sensitivity and glucose tolerance. The results of these studies shed some light on the beneficial effect of selected NZ Highbush blueberries against insulin resistance associated with metabolic syndrome.
Acknowledgements

To me, the human body is the best invention ever made in the world. Understanding how organs in our body work is my passion for studying nutritional science and inspires me to pursue a PhD in this area. However, undertaking a PhD is a long journey and this study would not have been possible without the support from many people along the way.

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Abbreviations

2DG 2-deoxyglucose
2-h PG 2 hours plasma glucose
A1C hemoglobin A1C or glycated hemoglobin
AACE American Association of Clinical Endocrinology
ACNs anthocyanins
ACP acepromazine
AHA/NHLBI American Heart Association/Nation Heart, Lung and Blood Institute
AMPK adenosine monophosphate-activated protein kinase
ANOVA analysis of variance
AOA antioxidant activity
ATPIII National Cholesterol Education Program Adult Treatment Panel III
AUC area under the curve
BB blueberry
BMI body mass index
BW body weight
CFU colony forming unit
Cmax maximum concentration
CONT starch-based control diet
CRP C-reactive protein
CVD cardiovascular disease
db/db mouse model of diabetes and obesity where leptin receptor is deficient
DEXA dual-energy x-ray absorptiometry
DNS 3,5-dinitrosalicylic acid
DP degree of polymerization
DPPH 2,2-diphenyl-1-picrylhydrazyl
DSF defatted soybean flour
EGCG epigallocatechin gallate
EGIR European Group for the study of Insulin Resistance
ER endoplasmic reticulum
ESR Environmental Science and Research
FB frozen berries
FeCl₃ ferric chloride
FeSO₄  
FFA  
FFM  
FISH  
FPG  
FPI  
FRAP  
FW  
G6Pase  
GAE  
GIT  
GLUT  
HDL-C  
HF  
HFD+BB  
HFHS  
HFR  
HFR1B  
HFR4B  
HOMA-IR  
HPLC  
HS  
iBAT  
IC₅₀  
IDF  
IFG  
IGT  
IL-6  
IL-10  
IRS  
ITT  
LFD  
LPS  
MRS
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<tr>
<td>MS</td>
<td>metabolic syndrome</td>
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<tr>
<td>Na₂CO₃</td>
<td>sodium carbonate</td>
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<td>NAFLD</td>
<td>non-alcoholic fatty acid liver disease</td>
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<td>NCEP-ATP III</td>
<td>National Cholesterol Education Program Adult Treatment Panel III</td>
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<td>NGSP</td>
<td>National Glycohemoglobin Standardization Program</td>
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<td>ob/ob</td>
<td>leptin-deficient obese mouse model</td>
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<td>OD</td>
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<td>oxygen radical absorbance capacity</td>
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<td>PEPCK</td>
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<td>PPAR-γ</td>
<td>peroxisome proliferator-activated receptor gamma</td>
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<td>QUICKI</td>
<td>quantitative insulin sensitivity check index</td>
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<td>Small Animal Production Unit</td>
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<td>tumour necrosis factor α</td>
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<td>TPTZ</td>
<td>2,4,6-tripyridyl-s-triazine</td>
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<td>VHFD</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WHR</td>
<td>waist-hip ratio</td>
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