Effect of Highbush blueberry consumption on markers of metabolic syndrome

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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 $FeSO_4$/g FB, DPPH; > 65% inhibition at 5 mg FB). Further i*n 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 *Lactobaccillus 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.

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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
$FeCl_3$	ferric chloride

$FeSO_4$	ferrous sulphate
FFA	free fatty acids
FFM	fat-free mass
FISH	fluorescent in situ hybridization
FPG	fasting plasma glucose
FPI	fasting plasma insulin
FRAP	ferric reducing antioxidant power
FW	fresh weight
G6Pase	glucose-6-phosphatase
GAE	gallic acid equivalent
GIT	gastrointestinal tract
GLUT	glucose transporter
HDL-C	high density lipoprotein cholesterol
HF	high-fat
HFD+BB	high fat diet plus blueberry
HFHS	high-fat-high-sugar
HFR	high-fructose
HFR1B	high-fructose diet containing 1% freeze-dried blueberry powder
HFR4B	high-fructose diet containing 4% freeze-dried blueberry powder
HOMA-IR	homeostasis model assessment of insulin resistance
HPLC	High Performance Liquid Chromatography
HS	high-sugar (sucrose)
iBAT	interscapular brown adipose tissue
IC_{50}	inhibitory concentration of 50%
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL-6	interleukin-6
IL-10	interleukin-10
IRS	insulin receptor substrate
ITT	insulin tolerance test
LFD	low fat diet
LPS	lipopolysaccharide
MRS	Man-Rogaso-Sharpe

MS	metabolic syndrome
Na_2CO_3	sodium carbonate
NAFLD	non-alcoholic fatty acid liver disease
NCEP-ATP III	National Cholesterol Education Program Adult Treatment Panel III
NGSP	National Glycohemoglobin Standardization Program
NHANES	National Health and Examination Survey
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
ob/ob	leptin-deficient obese mouse model
OD	optical density
OGTT	oral glucose tolerance test
ORAC	oxygen radical absorbance capacity
PAs	proanthocyanidins
PCA	principal component analysis
PEPCK	phosphoenolpyruvate carboxykinase
PI3K	phosphotidylinositol 3-kinase
PKB/Akt	protein kinase B
*p*NPG	*p*-nitrophenyl α-D-glucopyranoside
PPAR-γ	peroxisome proliferator-activated receptor gamma
QUICKI	quantitative insulin sensitivity check index
ROS	reactive oxygen species
SAPU	Small Animal Production Unit
SD	Sprague-Dawley
T2DM	type II diabetes mellitus
TG	triglycerides
TNF-α	tumour necrosis factor α
TPC	total polyphenolic content
TPTZ	2,4,6-tripyridyl-s-triazine
VHFD	very high fat diet
WAT	white adipose tissue
WC	waist circumference
WHO	World Health Organization
WHR	waist-hip ratio