PANMINERVA MED 2014;56(Suppl. 3 to No. 2):1-7

Delphinol® standardized maqui berry extract reduces postprandial blood glucose increase in individuals with impaired glucose regulation by novel mechanism of sodium glucose cotransporter inhibition

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***Aim*. The impetus of our study was to investigate the effects of a nutritional supplement Delphinol®, an extract of maqui berries (Aristotelia chilensis) standardised to ≥25% delphinidins and ≥35% total anthocyanins, on postprandial blood glucose and insulin levels and identify the physiologic mechanism involved.**

***Methods*. Postprandial blood glucose and insulin were investigated in double-blind, placebo-controlled, cross-over fashion in ten volunteers with moderate glucose intolerance. Longer term effects on blood sugar levels were investigated in streptozotocin-diabetic rats over a four months period. Effects of maqui berry delphinidins on sodium-glucose symport were examined in rodent jejenum of the small intestine.**

***Results*. Delphinol® intake prior to rice consumption statistical significantly lowered post prandial blood glucose and insulin as compared to placebo. We identified an inhibition of Na^+-dependant glucose transport by delphinidin, the principal polyphenol to which Delphinol® is standardised. In a diabetic rat model the daily oral application of Delphinol® over a period of four months significantly lowered fasting blood glucose levels and reached values indistinguishable from healthy non-diabetic rats.**

***Conclusion*. Our results suggest a potential use of Delphinol® for naturally controlling post-prandial blood glucose owed to inhibition of sodium glucose co-transporter in small intestine.**

KEY WORDS: Glucose - Dietary supplements - Postprandial period - Blood glucose.

Nutritional approaches for improving general health are gaining increasing interest, by the general public and researchers alike. Metabolic problems represent one of the major health concerns which are attractive for being addressed by nutritional interventions, as these are directly connected to dietary habits. A recent survey published in JAMA Internal Medicine describes more than half of the US adult population use dietary supplements and among the most common indications heart health is found.[1] Second to cholesterol concerns, obesity and hyperglycaemia were found to represent a major motif for consumption of supplements in order to improve cardiovascular health. Botanical extracts represent a mainstay in this health category, despite the circumstance that commonly composition and physiologic activity in humans are inadequately addressed.

Different berries have been proposed for treatment and prevention of several disorders including cardiovascular disease, neurodegenerative processes, obesity and metabolic disorders.[2-5] The biological properties of berries are attributed largely to their high levels of anthocyanins.

A standardized extract of maqui berries (*Aristotelia chilensis*) Delphinol® (trademark owned by MNL Chile) is popularly used as dietary supplement, owed to its high antioxidant properties.[6] These small deep-purple to black maqui berries are particularly rich in anthocyanin pigments, predominantly delphinidin and cyanidin derivatives of glucose and sambubi-

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ose.[7] Mapuche Indians from Chile traditionally consume them as food, but also employ them for various ailments, such as diarrhoea, infections, wound healing, hemorrhage and fever.[8] Various groups have pointed to interesting virtues of maqui berry extracts: prevention of light-induced photoreceptor apoptosis, prevention of neurodegeneration in Alzheimer models, antioxidant protection of cholesterol and endothelial cells and anti-inflammatory activity and adipogenesis inhibition.[8-11] While such *in vitro* studies help understand possible health contributions of maqui berries, these may not be fully applicable to humans as unaltered delphinidins and anthocyanins have been proposed to be of limited bioavailability. In animal and human studies, anthocyanins are poorly absorbed and therefore show low bioavailability.[12] Moreover, it has been suggested by several groups that dietary cyanidins and delphinidins may be subject to extensive biotransformation in humans, most likely involving the colonic microbiota.[13] Irrespective of metabolization and absorption considerations, maqui berry extract has recently been demonstrated in a type II diabetes rodent models to significantly lower post-prandial blood glucose and insulin levels.[14]

We describe here for the first time that the standardised extract of maqui berry (Delphinol®), bearing ≥25% delphinidins, significantly lowers post prandial blood glucose in volunteers presenting with moderate glucose intolerance. Mechanistic investigations suggest that Delphinol® affects the sodium-glucose co-transporter (SGLT) activity. Botanical extracts utilised for sugar control commonly inhibit a-glucosidase and a-amylase, enzymes which hydrolyse complex sugar to glucose and other monosaccharides. SLGT inhibition delays the absorption of glucose irrespective from its origin, such as cane sugar and also extends to pure glucose consumed.

Materials and methods

Reagents

Delphinol® maqui berry extract, is standardised to contain ≥35% w/w total anthocyanins and ≥25% w/w total delphinidins, as detailed in accompanying specification sheet, and was provided by the study sponsor MNL Santiago, Chile. The product was registered and authorised as an investigational new compound at the Institute of Public Health of Chile (#002698). Delphinidin (>98% purity) was purchased from Extrasynthese (Lyon, France). Streptozotocin (STZ) was purchased from Calbiochem, Darmstadt, Germany.

Preclinical investigation

Animal diabetes model experiments

Ten Sprague Dawley rats for investigation of diabetes were provided by the Catholic University Pontificia, Chile, at the age of 8 to 9 weeks, weighing 250 to 300 g. All animals were fed a standard rodent pelleted diet and water *ad libitum*.

STZ-Diabetic rats (N.=5) was induced by single tail injection of streptozotocin dissolved in citrate buffer (pH 4.5) at a dose of 55 mg/kg. Control rats (N.=10) were injected the citrate buffer vehicle only.

Delphinol was administered to rats by gavage. Delphinol was dissolved in distilled water and applied to animals for an approximate dosage of 20 mg/kg body weight. For controls tap water was applied to rats by gavage.

Fasting blood glucose of rats from both groups was investigated at trial start and again after four months of ad libitum access to standard rodent pelleted food chow. Glucose (2.0 $g \cdot kg^{-1}$ body weight) was administered by gavage after twelve hours fasting. Blood glucose was measured by standard photoenzymatic method.

Electrophysiological study of intestinal glucose transport

C57Bl/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The mice were maintained at the Specific Pathogen Free mouse facility of the Centro de Estudios Científicos (CECS), Valdivia, Chile and prior to the experiments they had free access to water and food. Animals were killed after anaesthesia by cervical dislocation and jejunum was isolated and opened longitudinally along the mesenteric border and rinsed with phosphate buffered saline (PBS).

Two sections of jejunum per animal were mounted in Ussing Chambers and maintained in Ussing buffer (120 mM NaCl, 25 mM $NaHCO_3$, KH_2PO_4 3.3 mM, 0.8 mM K_2HPO_4, 1.2 mM $MgCl_2$ and 1.2 mM $CaCl_2$) supplemented with 10 mM D-glucose in the serosal

side. The temperature was maintained at 37 °C and the solution was continuously gassed with carbogen 5% CO_2. Once the preparation reached a stable record of electrical parameters, 10 mM D-glucose was added to the mucosal side of the preparation in order to stimulate the transport of D-glucose coupled to Na^+. The effect of 50 μM delphinidin on sodium-coupled transport of glucose was determined by application in the mucosal side of the preparation. The transepithelial electrical potential difference (VM), defined as the potential side compared to serous mucus preparation was recorded continuously under current clamp configuration using a VCC MC2 amplifier (Physiological Instruments).

The values of short circuit current (I_{sc}) were calculated from experimental data using Ohm's law. Results were expressed as the intensity of the I_{sc} (μA/cm^2).

Clinical investigation

Participants

Ten adult volunteers of both sexes were recruited at the Hospitals San Juan de Dios and Jose Joaquin Aguirre (Santiago, Chile). Inclusion criteria for participation were fasting glucose levels <100 mg/dL and an altered glucose tolerance defined as ≥100 mg/dL and <125 mg/dL 120 minutes after intake of 75 grams of boiled rice.

Exclusion criteria were a chronic pharmacological therapy, body mass exceeding 30 kg/m^2, lactating or pregnant women, cigarette smokers, or individuals with a history of drug or alcohol abuse. Medications affecting hepatic activity 28 days prior to the study or known allergies to medications, renal insufficiency and all food allergies were exclusion criteria.

Volunteers were instructed to refrain from taking supplements including vitamins, not to change dietary or exercise habits, beginning from seven days prior to participation in the trial. Acute non-prescription medications were permitted, but were required to be reported.

The study protocol was approved by the Research Ethics Committee of the Occidental Metropolitan Health Service, Ministry of Health, Chile, resolution #2872011, including an insurance policy for the participants. A written and signed informed consent was obtained from all participants at the beginning of the study. Participants were informed that they may discontinue participation in the study at any time without being required to provide reasons for their departure. The investigation was carried out at the centre of pharmacology and toxicology (IFT), medical faculty of the University Santiago, Chile.

The study was designed in double-blind, placebo-controlled, crossover fashion. Computerised randomisation by code assignment was employed for groups A and B with six participants for each group. Two envelopes contained each individual's group assignment, one set was kept with the Pharmacology and Toxicology Research Center (Valdivia, Chile) for emergency care and the other was kept with the principal investigator. The two sets of envelopes remained sealed until data analysis. Participants and treating physicians and staff were unaware of the group assignment. The study physicians did not share findings during the trial period and were not involved in the handling of study products. Groups A and B crossed-over with a seven day wash-out period in between the two sets of experiments as illustrated in Figure 1. The physicians completed a file of adverse events for each participant, to record and classify (low, moderate, severe) all observations during the investigation. In case of severe adverse events, a notification to the Chilean National Center of Information of Medicines and Pharmaceutical Vigilance would be mandatory.

For the day of experiments participants were required to appear at the clinic following a fasting period of twelve hours. Subjects took a single portion of the investigative product dissolved in in 250 ml fresh tap water. In the case of Delphinol® 250 ml of water bearing 200 mg were consumed. Because the solution of Delphinol® presents with characteristic dark purple colour, the placebo control product was

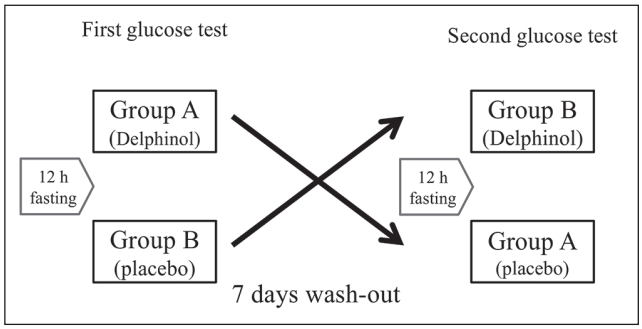

Figure 1.—Groups A and B crossed-over with a seven day wash-out period in between the two sets of experiments are illustrated.

likewise coloured by using a commercially available powdered (instant) berry juice beverage from the company Livean, Santiago Chile (www.livean.cl). This beverage bears artificial colouring and is visibly indistinguishable from an aqueous Delphinol® solution, yet being void of anthocyanidins and also sugar free.

Study participants were cannulized for blood sample collection in intervals. A first sample was taken 10 minutes prior to administration of Delphinol® or placebo-solution. Then drinks were swiftly consumed and this moment defined as time "zero". Fifteen minutes after consumption of the investigative products another blood sample was collected. Another 15 minutes later (t=30 min) 75 g of boiled rice were quickly consumed by all participants. The white rice (grade 1) portion was prepared by a nutritionist.

Blood sample analyses

Specimens were collected during each session. A first sample 10 minutes prior to the consumption of investigative product or control. Fifteen minutes after intake of products another blood sample was collected. Thirty minutes after intake of products rice was consumed and at this time also blood samples were collected. Thereafter, consecutive samples were taken at 60, 90, 120 and 180 minutes after intake of Delphinol or placebo. Glucose was measured in plasma by photo-enzymatic method (GOD PAP from DiaSys Germany) and insulin measured by immune assay using the AxSYM kit from Abbott Laboratories (IL, USA).

Statistical analysis

To identify statistical significance between parameters of groups, volunteers and animals, the multifactorial variance test (ANOVA) was employed. Values are given as mean ± standard deviation. The level of significance was set at $P<0.05$.

Results

Preclinical investigations

Healthy rats and diabetic (type I model, streptozotocin treated) rats were housed for four months with ad libitum access to food and treated once a day with Delphinol® by gavage. Rats were investigated for their fasting blood sugar levels at trial start and completion. While Delphinol® application did not alter fasting glucose in normal rats after four months, a significant decrease of fasting glucose was discovered in diabetic rats (Figure 2). The fasting glucose of Delphinol® -treated diabetic rats was indistinguishable from corresponding values of healthy rats after four months. No significant change in body weight was observed in during the four months period in diabetic rats. The blood triglyceride levels in diabetic rats treated with Delphinol® remained unchanged and where of same dimension as in untreated diabetic rats (data not shown).

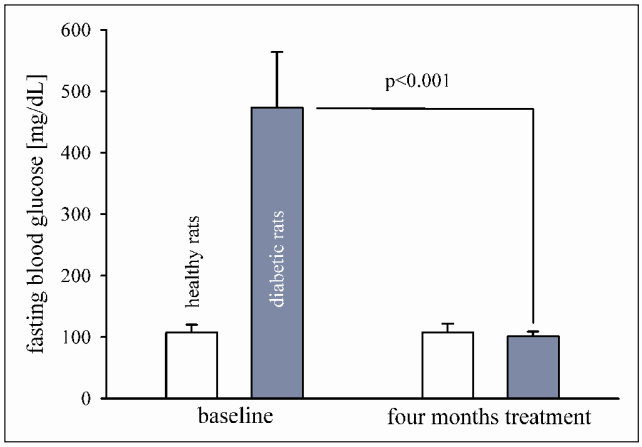

Figure 2.—Delphinol® reduces hyperglycemia in STZ-diabetic rats. The blood fasting glucose of two groups or rats is presented, healthy- and diabetic rats, and baseline and again after four months unlimited access to food and daily supplementation with Delphinol (20 mg/kg body weight daily) by gavage. Whereas no change to the fasting glucose was found in the group of healthy rats, a significant decrease of fasting glucose was evident in the group of diabetic rats, which matched the healthy glucose level of non-diabetic rats after four months. The results are the means±error standard, N.=5.

We investigated mouse jejunal mucosa samples using an Ussing chamber to explore the possibility that Delphinol® constituents may impact the activity of glucose transporters. The uptake of glucose by the Na^+-glucose co-transporter incurs an increase in short-circuit current (ΔIsc) resulting from the electrogenic transport of glucose coupled to Na^+ reaching the serosal side of the jejunum. Mouse mucosa was challenged by addition of glucose to the chamber for reaching a concentration of 20 mM glucose. When delphinidin had been added to the chamber at final concentration of 50 μM prior to glucose challenge,

the corresponding ΔIsc was significantly decreased from ΔIsc 7.6±1.1 μAcm^{-2} to 2.6±0.6 μAcm^{-2}, corresponding to a reduction 63% as compared to glucose-challenge in absence of delphinidin (Figure 3).

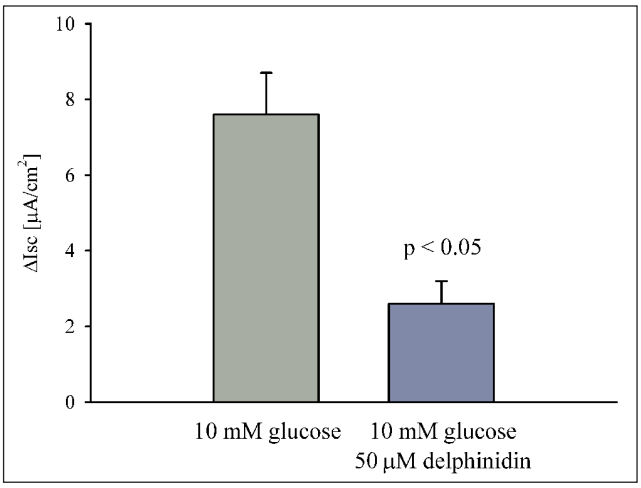

Figure 3.—Effects of delphinidin on glucose-induced changes in short circuit current (ΔIsc) studied in mouse jejunal mucosa in Ussing chamber. On the left (light bar), the short circuit change resulting from challenge with 10 mM glucose is presented. On the right the corresponding readout with 50 μM delphinidin concentration installed three minutes prior to challenge with 10 mM glucose is presented (dark bar). A 60% inhibition of glucose-induced Isc was found with delphinidin. N.=4 (different animals).

Clinical investigations

The study outcome demonstrates that a single administration of 200 mg Delphinol® decreases postprandial glucose levels and insulin in subjects with moderate glucose intolerance. As shown in Figure 4A an increase of blood glucose is found beginning from 30 minutes post intake of rice in both groups. The placebo-treated group presents with the typical blood glucose peak after 60 minutes, whereas the group given Delphinol® does not show a glucose peak and the blood sugar remains steady up to 90 minutes post rice intake. The glucose concentrations are significantly lower as compared to the placebo group at the two time points 60 min and 90 min. Two hours after rice intake the values match those of the placebo group.

Correspondingly, insulin levels reached peak values 60 min after rice consumption, whereas they remain unaffected in the Delphinol®-treated group (Figure 4B). At later time points from 90 minutes and onward the insulin levels between groups align. The interviewed patients reported to have experienced no unfavourable observations or side effects.

Discussion and conclusions

Overeating and lack of physical activity represent a great challenge to the developed world, where glu-

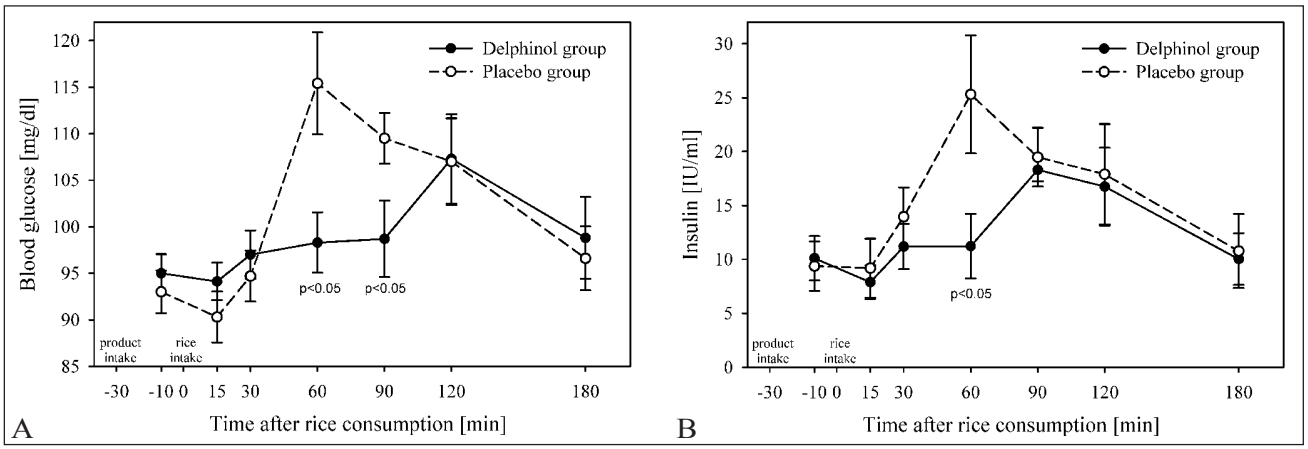

Figure 4.—A, B) Effect of Delphinol® on postprandial insulin and glucose concentrations in patients with mild glucose intolerance. The postprandial blood glucose and insulin values of ten volunteers with moderate insulin intolerance are presented. The time-line is set to zero at the moment of rice consumption. This incident was preceded by intake of Delphinol or placebo thirty minutes earlier, and measurement of fasting glucose and insulin ten minutes earlier.
Delphinol treatment significantly inhibits post-prandial glucose increase at time points 60 min and 90 min (P<0.05). Correspondingly, in the Delphinol treated group insulin levels increase in delayed fashion, rising one hour post meal, while with placebo insulin rises from 15 minutes onwards. Statistical significance between Delphinol and placebo is found 60 min after rice consumption. The results are the means±error standard, N.=10.

cose intolerance, hypercholesterolemia and eventually metabolic syndrome, obesity and type II diabetes continue to represent an epidemic. Strict control of the blood glucose level is considered essential in order to delay and/or prevent the development of diabetic complications. Vegetable and fruit represent sources of polyphenol and fibres, which naturally prolong food gut transit time and digestion and delay sugar absorption. Blueberry, cranberry, boysenberry, strawberry and other berries are considered useful for the control of glycaemia and insulin in diabetes mellitus patients and these effects have been associated to the anthocyanins in berries.[15]

Delphinol®, a maqui berry extract standardised to the content of anthocyanins, led to a significant reduction in fasting blood glucose concentration in STZ-diabetic rats after a four months of treatment. In the glucose tolerance test Delphinol® also decreased postprandial blood glucose levels in diabetic rats after challenge with 2.0 $g \cdot kg^{-1}$ body weight, by gavage. Furthermore, our results suggest that Delphinol® reduces basal hyperglycemia in type 2 diabetic rats. Another group recently described that maqui berry extract and delphinidin 3-sambubioside-5-glucoside, a characteristic anthocyanin limited to maqui berries, was effective in improving fasting blood glucose levels and glucose tolerance in hyperglycaemic obese C57BL/6J mice fed a high fat diet.[14] This study showed that oral glucose challenge led to significantly lower postprandial glucose levels when rodents were applied maqui berry extract prior to the glucose challenge. This observation is in agreement with our discovery described here related to sodium glucose co-transporter inhibition. Until now the mechanism involved in glycemia control of Delphinol® remained unknown. *In vitro* tests had suggested that the delphinidin 3-sambubioside-5-glucoside, an anthocyanin limited to maqui berries, may decrease gluconeogenesis.[14]

Some of the discussed effects may appear incongruous, as unaltered delphinidins and anthocyanins have been proposed to be of limited bioavailability,[12] while other groups describe that absorption mechanisms may be saturated at high doses.[15] Furthermore, in STZ-induced type 1 diabetes models pancreatic beta cell toxicity results in failure to release insulin, hence other potential targets may explain the Delphinol® effect in pre-diabetes. In the intestine, the enterocytes of the brush border membrane (BBM) are the primary site of absorption of dietary sugars and a reduction of glucose absorption may explain the decrease of blood sugar found in a type I diabetic rat model. In this regard, the co-transporter Na^+/glucose SGLT-1 transports glucose and galactose from the lumen of the intestine into the enterocytes.[16] The regulation of SGLT-1involves a postprandial mechanism, followed by a rapid adjustment of the abundance of the protein in the BBM and recruitment of glucose transporter GLUT-2, which depends on the levels of luminal sugars.[17] We observed that delphinidin inhibited the glucose transport (SGLT-1) in jejunal mucosa of healthy C57Bl/6J mice, thus we suggest glucose transport inhibition as primary mechanism of action of delphinidin, the main anthocyanin species present in Delphinol®.

Because the activity of SGLT-1 is increased in type II diabetes, Delphinol® may be useful for controlling glucose transport in individuals with impaired glucose regulation.[18,19] Moreover, cyanidin-3-glucoside suppresses the development of obesity and ameliorates hyperglycemia induced by high fat diet in mice.[20] We conducted a pilot study, to assess the possible use of Delphinol®, in pre-diabetic patients. In the current study, Delphinol® significantly reduced the postprandial glucose concentration at 60 and 90 minutes, after starch consumption in human volunteers with moderate glucose intolerance. Also, a decrease of blood insulin in the volunteers treated with Delphinol® was observed. Remarkably, none of the volunteers participating in the study showed adverse reactions to treatment, suggesting that the extract is safe in the dose administered.

Our result suggests that delphinidin, the main anthocyanin in Delphinol® reduces the glucose absorption in intestine by interaction with sodium glucose co-transporter SGLT-1. Importantly, a single dose of Delphinol® was sufficient to significantly lower postprandial blood glucose and -insulin increase after meals, and thus Delphinol® may represent a helpful dietary complement for maintaining healthy blood glucose levels.

Future research on Delphinol® may establish long term effects on blood sugar level control, such as for HbA_{1c} values and other metabolic parameters, especially also in a larger cohort.

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Funding.—We thank Maqui New Life, S.A. for the grant to Universidad Austral de Chile.

The Centro de Estudios Cientifico (CECs) is funded by the Centers of Excellence Base Financing Program of Conicyt.

Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Received on April 16, 2014.
Accepted for publication on April 17, 2014.