Delphinidin-rich maqui berry extract (Delphinol®) lowers fasting- and post prandial glucose and insulin in dose-effect size study using oral glucose tolerance test

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Delphinidin-rich maqui berry extract (Delphinol®) lowers fasting- and post prandial glucose and insulin in dose-effect size study using oral glucose tolerance test

RUNNING TITLE:

Delphinol® effects on glucose metabolism

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ABSTRACT

Delphinidin anthocyanins have previously been described to protract carbohydrate absorption by means of glucose transport inhibition. We investigated effects of standardized (≥25% delphinidin glycosides) maqui berry (*Aristotelia chilensis*) extract Delphinol® on glucose and insulin plasma levels in fasting conditions and during an oral glucose tolerance test (OGTT).

We here describe for the first time that acute application of Delphinol, dose-dependently (60, 120 and 180 mg), and significantly, at all applied dosages, lowers fasting glucose and -insulin within one hour, as compared to untreated control ($p < 0.05$).

Overnight fasted 36 pre-diabetic study participants underwent four consecutive OGTT with minimum one week wash-out period in between experiments. Acute Delphinol supplementation dose-dependently retarded 30 min post prandial glucose and insulin rise following glucose challenge, presenting with higher values thereafter until completion of the 2 hour postprandial monitored period. Statistical significant reduction of 30 min post-prandial glucose versus untreated control was identified for 120 mg Delphinol ($p = 0.048$). At later time points, 60 and 90 min post OGTT, glucose and insulin were elevated with 60 and 120 mg compared to untreated control, with borderline significance for 60 mg at both 60 min ($p= 0.056$) and 90 min ($p= 0.056$) post OGTT.

WORDS n = 200

INTRODUCTION

Frequent excessive post-prandial glucose and -insulin excursions represent a risk factor for developing diabetes, associated with impaired glucose- and insulin-tolerance, inflammation, dyslipidemia, β-cell dysfunction and endothelial dysfunction [Ludwig, 2008]. The maintenance of healthy blood sugar levels and controlled carbohydrate metabolism is a rapidly growing concern in most developed countries and increasingly also in developing countries, owed to the increased awareness of the hyperglycemia risk resulting from unhealthy diets and sedentary lifestyle [Kharroubi et al, 2015]. Further to dietary self-limitation and physical activity efforts, consumption of plant secondary metabolites may substantially contribute to improve carbohydrate metabolism [Williamson, 2013].

Long term epidemiologic studies, foremost the Nurses's Health studies (NHS), have pointed to dietary factors affecting the risk for developing diabetes, using validated food questionnaires. Investigation of data from the NHS have resulted in interesting findings related to elevated regular consumption of different flavonoid classes and disease risk reduction [Wedick et al., 2012]. Higher consumption of anthocyanins was associated with lower risk for type II diabetes in US adults, based on the follow-up of 70359 women in the NHS (1984-2008), and 89201 women in NHSII (1991-2007) and also 41334 men in the Health Professionals Follow-Up Study (1986-2006), who were free from diabetes and cardiovascular disease at baseline. Interestingly, this study found no significant correlation between other flavonoid subclasses and even total flavonoid consumption related to risk-reduction for type II diabetes. A follow-up of the

NHS II (93600 women) suggested that anthocyanin intake, in form of blueberries and strawberries would correlate with decreased myocardial infarction risk [Cassidy et al., 2013]. A recent epidemiologic study suggests that regular higher intake of flavonoid species anthocyanins, flavones, and flavanones is associated with greater likelihood for good health and wellbeing in individuals surviving to older ages [Samieri et al., 2014].

Polyphenols are well described to exhibit inhibitory effects on α-glucosidase and α-amylase enzyme activities, thus delaying absorption of complex food carbohydrates [Hanhineva et al., 2010]. Especially the oligomeric proanthocyanidins potently delay hydrolysis of starchy foods to glucose, some of which appear to be more effective than acarbose medication [Schäfer & Högger, 2006]. Consumption of a crowberry-fortified blackcurrant juice, bearing all six anthocyanidin species as anthocyanins, sugared with 50 g glucose, was described to attenuate, though statistical significantly only at time point 90 min past ingestion, the post-prandial blood glucose and -insulin peak, as compared to consumption of the same sugared beverage void of crowberry fortification [Törrönen et al., 2012].

Delphinidin anthocyanins extracted from maqui berries (*Aristotelia chilensis*), indigenous to Chile, have recently been ascribed to inhibit SGLT1 in rat duodenum, and significantly inhibit absorption of glucose from rice in human volunteers with impaired glucose tolerance. The extract Delphinol® (MNL, Chile) standardized to 25% w/w delphinidin glycosides and 35% total anthocyanins, was found to significantly inhibit post-prandial glucose at single dosage of 200 mg at time point 60- and 90 min after rice intake [Hidalgo et al., 2014]. We here describe Delphinol® dose-effect size investigations on blood glucose and insulin in fasting conditions and post-prandial

effects applying a standard OGTT in study participants with impaired glucose tolerance.

METHODS

Study population

Potential subjects presenting with either, a family history of type 2 diabetes, hypertension or body mass index greater than 23 kg/m^2, dyslipidemia, age between 18 and 50 years of both genders, were invited to the clinic for screening to identify suitability to meet inclusion criteria.

Inclusion criteria comprised: 1) Age between 18 and 50 years old; 2) Abnormal response to OGTT, as the result of altered glucose values (basal glucose ≥ 100 mg/dL; any intermediate glucose value at the 30, 60 or 90 minutes ≥ 160 mg/dL; glucose at 120 minutes ≥ 140 mg/dL) or insulin (basal insulin ≥ 15 µIU/mL; any intermediate insulin value at the 30, 60 or 90 minutes ≥ 100 µIU/mL; insulin at 120 minutes ≥ 60 µIU/mL).

Exclusion criteria comprised fasting blood glucose levels ≥ 180 mg/dL, pregnancy, hormonal therapy with sexual steroids, cardiovascular disease requiring medication, hypo-glycemic medication use, allergies, and the inability to follow instructions.

Prior to enrolment all participants were introduced in detail to the purpose and rationale of the study, the product they would be taking as well as the investigational

procedures they would be exposed to. All participants provided their written informed consent for their participation in the research project. The present study was conducted according to the guidelines of the Declaration of Helsinki, the study protocol was approved by the ethical committee of Mutual de Seguridad, Santiago de Chile. Written consent was obtained from all participants. Subjects were permitted to discontinue participation at any time without providing reasons. Pregnancy testing in women with childbearing potential was only performed at the first visit. During the first health check-up specimen were collected from all enrolled participants for blood rheology, standard blood chemistry, lipid profile and complete urine analysis.

Study design and protocol

This investigation was an open exploratory study initiated for identifying acute dose-effects of a standardized maqui berry extract Delphinol® on post-prandial blood glucose. Delphinol comprises of polyphenols, standardized to bear ≥ 25% delphinidin glycosides and no less than 35% total anthocyanins, with many additional different flavonoid species present [Watson et al., 2015]. Delphinol® has been shown to be safe in acute and chronic toxicity assessments at dosage of 1g/kg body weight. Delphinol® is commercialized as dietary supplement in most parts of the developed world, predominantly as an antioxidant and for blood glucose management. Capsules bearing 60, 120, and 180 mg Delphinol® (batch 13156, MNL Chile) were manufactured by Barnafi Krause Farmacéutica S.A., Santiago de Chile.

Subjects

According to the inclusion criteria, a total number of 36 pre-diabetic subjects were initially enrolled, 20 women and 16 men aged 19 to 50 years. Additional seven participants were recruited at later time for power of statics. This group comprised of 4 women and 3 men aged 19 to 50 years, all of them pre-diabetic. At the first visit, the health status of the subjects was checked by an interview on their clinical history and a complete physical examination. Routine analysis (complete blood count, biochemical and lipid profiles) and a diagnostic OGTT were also performed. At the end of each visit participating volunteers were interviewed for adverse effects.

Oral glucose tolerance test and insulin measurements

Study participants were screened for eligibility by means of a glucose tolerance test applying Trutol (75 glucose as 296 mL aqueous solution, Thermoscientific, Pittsburgh, PA, USA), with investigation of basal (fasting), 30, 60, 90, and 120 min glucose and insulin levels, with blood -glucose and or -insulin exceeding values as afore mentioned for inclusion criteria.

For the study of dose effects, after an overnight (10-12 hours) fasting period, subjects were cannulated after arrival at the clinic, lying in a hospital bed over the entire investigational period. Subjects were given a single dose of Delphinol® (nil, 60, 120 or 180 mg, on different days with minimum one week intermission), and an hour thereafter, a 4 mL blood sample was collected in Becton Dickinson (BD) Vacutainer tubes, (with EDTA and sodium fluoride), stored at 4°C for glucose measurements. For insulin measurements, a 3.5 ml sample was collected using Vacutainer SST Tubes. All samples were centrifuged at 4.000 rpm on an ALC PK 120 centrifuge for 5 minutes. Glucose measurements were carried out using colorimetric essay GOD-PAD. Insulin

measurements were conducted by chemiluminescence in Vacutainer tubes, (with EDTA and sodium fluoride), stored at 4°C for glucose measurements. For insulin measurements, a 3.5 ml sample was collected in Vacutainer tubes. All samples were centrifuged at 4.000 rpm on an ALC PK 120 centrifuge for 5 minutes. Measurements were performed using colorimetric essay GOD-PAD. Insulin measurements were conducted by chemiluminescence. Immediately afterwards participants were subjected to an OGTT, swiftly ingesting 75 g glucose solution (Trutol). For the OGTT in total five blood samples were taken in intervals of 30 min. The first blood sample was collected one hour after Delphinol® intake, timed before glucose ingestion, as illustrated in Figure 1. The subsequent four samples were collected in 30 minutes intervals until two hours post OGTT.

Glucose was estimated by GOD-PAP colorimetric assay using an auto analyzer model Selectra (Vitalab, Smithfield, USA). Calibration was done daily according to the lab protocols. All samples were processed following standard procedures. Insulin was analyzed by direct chemo-luminescence using an ADVIA Centaur XP Immunoassay System (Siemens, Germany).

Statistical analyses

Statistical analyses were carried out using MINITAB 17 (Minitab Inc., State College PA, USA), Origin Pro 8 (OriginLab Corp, Northampton MA, USA) and SAS 9 (SAS Institute, Cary NC, USA). One-tailed paired comparisons were used to identify significant differences in basal glucose values at a 5% significance level ($\alpha = 0.05$) after ingestion of different Delphinol® dosages. For the comparison of different doses on the subjects,

repeated measures mixed ANOVA model was used in which subjects were treated as a random effect and dose as a repeated effect. The variance-covariance matrix was modeled using the options for the repeated statement provided in Proc MIXED (SAS) with a Toeplitz structure to allow for an autoregressive covariance model. After the general repeated measures ANOVA, least-squares-means multiple comparisons were performed among doses. For the multiple comparisons a Dunnett-HSU approach was applied using Proc MIXED (SAS Institute). One-tailed hypothesis testing was used to compare the control dose (zero) to 60, 120, and 180 mg doses. All reported mean values are least-square means due to the non-balanced mixed model to fit the overall comparisons model.

Due to practical reasons and design limitations, after recruitment of seven additional subjects, a priori (planned) comparisons between control dose (zero) and dose 120 mg were done using one tailed paired t-tests for glucose and insulin. For this comparison the complete dataset was analyzed using the same statistical model structure but comparisons were limited only to doses zero and 120 mg.

RESULTS

Study population

A total number of 36 pre-diabetic subjects 20 women and 16 men were initially enrolled. The mean age was 30.1 (SD = 9.64; range = 20—50) years for women and 32.6 (SD = 8.92; range = 19—49) years for men, BMI was 29.57 (SD = 5.19; range = 20.1—39.2) kg/m^2 for women and 31.97 (SD = 24.3; range 24.3—48.5) kg/m^2 for men,

and fasting plasma glucose at enrolment was 88.4 (SD = 10.82; range=74.0—117.0) mg/dL for women and 95.75 (SD=7.05; range = 83.0—110.0) mg/dL for men. Specifically for *a priori* statistical evaluations at 120 mg Delphinol additional seven participants were recruited. For those patients, the mean average age was 32.5 (SD = 11.12; range 37—43) years for women and 32.0 (SD = 14.73; range 39—43) years for men; BMI was 24.63 (SD = 1.46; range = 23.7—26.8) kg/m^2 for women and 24.93 (SD = 2.97; range 22.2—28.1 kg/m^2) for men, and fasting plasma glucose at enrolment was 91.0 (SD = 15.74; range=75—109) mg/dL for women and 99.0 (SD=6.55; range = 93—106) mg/dL for men

Dose effects of Delphinol® on fasting glucose and -insulin

As detailed in Table 1, the mean over-night fasting glucose and -insulin level of subjects, investigated during four separate occasions, decreased within 60 minutes after single intake of Delphinol® in dose-dependent fashion and statistical significantly for all dosages 60, 120 and 180 mg, respectively, as compared to non-Delphinol treated control.

Table 1: Statistical evaluation of acute dose effects related to Delphinol intake on fasting glucose and -insulin in 36 subjects, prior to OGTT. Each basal value was obtained at different days in the morning, with subjects fasted overnight and in the morning, 60 min after intake of respective single Delphinol doses. Dose zero was the diagnostic test in which no Delphinol was administered (baseline).

Delphinol dose [mg]	Basal glucose [mg/dL) 60 min after Delphinol intake mean value and adjusted SE	Dunnett HSU Comparison		
		Dose [mg]	Difference of least squares means	Adjusted P
0	91.67 ; 1.37			
60	88.94 ; 1.37	0 vs 60	2.72	0.037
120	88.53 ; 1.37	0 vs 120	3.14	0.034
180	88.06 ; 1.37	0 vs 180	3.61	0.040

Delphinol dose [mg]	Basal insulin [µIU/mL] 60 min after Delphinol intake mean value and adjusted SE	Dunnett HSU Comparison		
		Doses [mg]	Differences of least squares means	Adjusted P
0	15.46 ; 1.15			
60	13.78 ; 1.15	0 vs 60	1.68	0.072
120	13.79 ; 1.15	0 vs 120	1.66	0.079
180	12.05 ; 1.15	0 vs 180	3.40	< 0.001

The decrease of fasting blood glucose subsequent to an acute intake of Delphinol® coincided with dose-dependent and significant decrease of fasting insulin as compared

to untreated control. One-tailed paired comparisons showed highly significant differences between basal glucose mean values and after ingestion of all three doses of Delphinol® (Table 1) at a 5% significance level (α=0.05). Regarding estimated effect sizes, mean reductions of 2.7, 3.14, and 3.61 (mg/dL) were observed for the 60, 120, and 180 mg dose, respectively.

One-tailed paired comparisons showed highly significant differences between basal insulin mean value and after ingestion of 180 mg of Delphinol® (Table 1) at a 5% significance level (α=0.05). Regarding estimated effect size, a mean reduction of 3.4 (µIU/mL) was observed for the 180 mg dose.

Dose-effects of Delphinol® on post-prandial glucose in an OGTT

As presented in Figure 2, the blood-glucose and -insulin curves present with markedly different kinetics in response to the Delphinol dose applied. Within the thirty minutes post glucose ingestion, a dose-dependent effect of Delphinol on the rapidly increasing post-prandial glucose was observed. As shown in Figures 1 and 2, both glucose and insulin rose markedly higher during the diagnostic tests in absence of Delphinol, than with prior intake of Delphinol®, the latter showing dose-dependent retardation of post prandial glucose and -insulin. Borderline statistical significance versus untreated control was identified for 120 mg ($p = 0.117$) and 180 mg ($p = 0.126$) Delphinol, respectively, 30 min past OGTT. Based on these results, we chose to increase the sample size for the 120 mg dose in order to elevate the statistical power of the test. Seven additional pre-diabetic subjects, 4 women and 3 men, aged 19 to 50 years, were recruited and investigated by same procedures as described earlier. The glucose and

insulin single *a priori* comparisons, including 7 additional recruits, testing 120 mg Delphinol versus control, are presented in Table 2.

Table 2: Statistical evaluation of glucose and insulin with seven added subjects to the cohort of 36 subjects, for an a priori comparison at 120 mg versus untreated control. Values are given as least square means with adjusted standard error.

dose [mg]	basal glucose [mg/dL]	difference of least square means	30 minutes glucose [mg/dL]	p value
0	92.45 ; 1.29		141.93 ; 4.03	
120	88.36 ; 1.29	4.08	134.63 ; 4.03	0.048

dose [mg]	basal insulin [µIU/mL]	difference of least square means	30 minutes insulin [µIU/mL]	p value
0	14.57 ; 1.06		142.64 ; 11.90	
120	13.08 ; 1.06	1.49	132.84 ; 12.60	0.190

As a result from a priori comparisons (table 2) we found that application of a single 120 mg Delphinol® dose significantly decreased 30 min post-prandial (OGTT) blood glucose ($p < 0.05$), whereas the corresponding post-prandial lowering of insulin remained statistically insignificant.

During the subsequent period from 30 min to 60 min minutes past OGTT, blood glucose and insulin curves show surprisingly diverging developments in response to the

afore applied Delphinol® dose. With untreated control the glucose level from this point in time almost linearly decreases until end of the monitored period. The highest Delphinol dose of 180 mg shows marginally higher glucose level than untreated control 60 min post OGTT, yet the curve gradually declines from this point in time as with untreated control. In contrast, at lower dosages of 60 and 120 mg Delphinol a continued rise of glucose- and insulin values persists until 60 min post OGTT. Surprisingly the lowest applied dose of 60 mg Delphinol presents with the highest glucose level at 60 min, reaching borderline statistical significance ($p = 0.056$) versus untreated control. The insulin level recorded for the 60 mg dose correspondingly presents with the highest value of all four applied Delphinol dosages at this time point (Figure 2). It is noteworthy that 60 min past OGTT the lowest glucose level was found for untreated control, whereas the lowest insulin value was presented with 180 mg Delphinol.

At 90 minutes post OGTT, blood glucose remains higher at all applied Delphinol dosages, as compared to untreated control, which rapid decline in post-prandial phase. A noticeable divergence related to dose-effects is apparent for the insulin levels 90 min past glucose challenge. Whereas higher glucose values at this time with 60 and 120 mg Delphinol are reflected by correspondingly high insulin values, the values decline in untreated control and likewise, to same extent, with 180 mg dose. Two hours past OGTT the glucose reaches lowest values with untreated control and 180 mg Delphinol.

A noteworthy observation is that with 180 mg Delphinol, the insulin level remained at lowest levels throughout all investigational time points. All subjects tolerated the treatment with Delphinol® well, no adverse reactions were reported during interviews

of participants. None of the participants departed from the study prior to completion of all experiments.

DISCUSSION

Dietary polyphenols are well described to contribute to healthier blood glucose values, based predominantly on inhibition of α-glucosidase and α-amylase activities [Hanhineva et al., 2010]. In this regard oligomeric proanthocyanidins (OPCs) show effects comparable with acarbose diabetic medication, inhibiting hydrolization of complex carbohydrates to glucose [Bahadoran et al., 2013]. While OPCs potently inhibit α-glucosidase activity and effectively lower post-prandial blood glucose following starchy meals, the absorption of monosaccharides, especially glucose itself, remains little affected by most flavonoid species. Berries of various natures, consumed as pulp or juice, have repeatedly been demonstrated to limit the rise of blood glucose and insulin in response to sucrose challenge. In all these studies inhibition of α-glucosidase was ascribed to delay hydrolysis of sucrose to glucose and fructose [Törrönen et al., 2012a, Hanhineva et al., 2010].

Some polyphenolic species such as epicatechin gallate have been ascribed to show inhibitory effects to sodium-glucose co-transporter activity [Williamson, 2013]. Probably the first identified natural SGLT1 inhibitor is phloridzin from apples, which was administered to pancreatectomized rats by subcutaneous injection, which in turn normalized insulin sensitivity [Rossetti et al., 1987]. Parenteral phloridzin was found to

lead to glucosuria resulting from inhibition of renal SGLT2, thus diminishing glucose re-absorption. Orally applied phloridzin is hydrolyzed to inactive phloretin [Rossetti et al., 1987).

Delphinidins have been demonstrated to inhibit SGLT1 activity in rat duodenum using Ussing chamber and acute intake of 200 mg delphinidin-rich maqui berry extract Delphinol® correspondingly significantly lowered post-prandial blood glucose and -insulin following 75g rice challenge in a human pilot trial [Hidalgo et al., 2014]. However, clinical evidence for inhibition of glucose absorption, such as in an oral glucose tolerance test, to date remains absent for delphinidin aglycon as well as for delphinidinic anthocyanins. Hence we conducted a study using naturally delphinidin-rich maqui berry extract Delphinol®, in order to investigate dose-effect related inhibition of glucose absorption in human volunteers using standard OGTT. We chose pre-diabetic subjects in expectation of greater post-prandial glucose-alterations following acute intake of Delphinol®. Furthermore, the Delphinol® minimum acute dose required for significant lowering of post-prandial blood glucose in OGTT, was unknown. This prompted us to test acute doses of 60, 120 and 180 mg Delphinol® versus untreated control in an OGTT in repeat investigations with the same subjects.

Unexpectedly, we discovered that acute intake of Delphinol® alone, in absence of any carbohydrate exposure, dose-dependently and significantly, at all Delphinol® dosages applied, simultaneously lowered both post-prandial fasting blood glucose and –insulin, one hour after intake. An acute simultaneous reduction of blood glucose and -insulin with dietary polyphenols at fasting conditions, to our knowledge, has not been described before. The investigation of underlying mechanisms of action related to this observation was beyond the scope of the research project. Because Delphinol® was

previously described to inhibit SGLT1 activity in small intestine, it was intriguing to speculate whether Delphinol® may potentially inhibit SGLT in kidneys, affecting renal glucose reabsorption with glucose lost to urine [Valentine et al., 2012]. Investigation of possible glucosuria was not part of the research protocol, however a post hoc investigation of only three subject volunteers participating in the trial, showed that following single intake of 180 mg Delphinol® at fasting conditions, urine collected over the subsequent hour did not bear measurable quantities of glucose (data not shown). Though the observation on absence of glucosuria is limited, this finding nonetheless suggests that delphinidin glycosides in maqui berries may not lower fasting glucose as result of glucosuria.

The effects of Delphinol® presented here shall be of particular interest for individuals with glucose intolerance and insulin resistance, as pancreatic β-cells may be less burdened by excess insulin secretion. The insulin sparing effect identified for Delphinol® is particularly appreciative as it manifests also at fasting conditions. The observation that insulin levels were lowered following Delphinol ingestion (prior to OGTT), and at single dose of 180 mg persisted lower throughout the monitored 120 min period past OGTT, as compared to untreated control as well as lower Delphinol doses, points to potential pancreatic health contributions. This discovery of Delphinol effects on insulin merits future research, which may explore fasting glucose and pancreas function related to insulin response improvement, especially following regular supplementation with Delphinol®.

The initial impetus for our study was to identify an effective acute Delphinol® dose which significantly affects absorption of dietary glucose. We found that applied Delphinol® dosages significantly lowered blood glucose 30 minutes past glucose

challenge, which may arguably be partially attributed, to the drop in fasting glucose subsequent to Delphinol® intake. Yet, our findings confirm that Delphinol® decreases absorption of dietary glucose further to previous investigations which applied starch to subjects [Hidalgo et al., 2014]. Our results are coherent with the SGLT-1 inhibition by delphinidins described by Hidalgo and co-workers.

It is a noteworthy observation, that the post-prandial blood -glucose and -insulin dynamics are altered profoundly in relation to the Delphinol® dose applied, from the time point 30 min post glucose ingestion. Surprisingly, the 60 min post-prandial blood glucose values present in inverse order to the Delphinol® dose applied to by subjects. Whereas in untreated control blood glucose dropped to average 126.8 mg/mL 60 min past glucose challenge, the average values with Delphinol® ranged higher at 129.1, 134.1 and 139.1 mg/mL, for 180, 120 and 60 mg Delphinol, respectively. This observation may appear contradictory to inhibition of SGLT1 with Delphinol. However, it is noteworthy that supplementation with Delphinol® occurred one hour prior to glucose challenge and delphinidin glycosides may potentially no longer persist at physiologically fully active concentrations in small intestine two hours past Delphinol® intake.

Other groups investigating polyphenol impact on post-prandial glucose applied anthocyanins as blackcurrant juice, fortified with additional crowberry (*Empetrum nigrum*) powder (100 g/L) and adding sucrose (50 g/L) to the juice [Törrönen et al 2012a]. Plasma glucose and -insulin were lowered with crowberry fortified sugared blackcurrant juice 30 min past consumption, tough non-significantly compared to the sweetened blackcurrant juice alone. Interestingly, a significantly higher blood glucose and -insulin was identified 90 min after consumption of sucrose-sweetened and

crowberry-fortified blackcurrant juice, as compared to sweetened blackcurrant juice without crowberry. These post-prandial glucose and insulin kinetics are in conformance with our findings, showing elevated glucose- and -insulin at 60 and 90 min, resulting from anthocyanin consumption. Törrönen and co-workers attribute the attenuation of post-prandial glucose and insulin to α-glucosidase inhibition, resulting in delayed sucrose hydrolysis and speculate on the possibility for additionally impaired intestinal glucose transport. As we applied glucose, any possible effects of Delphinol on carbohydrate hydrolysis are ruled out.

Another group investigated bilberry (*Vaccinium myrtillus*) extract effects on post-prandial blood glucose in obese subjects presenting with type II diabetes or impaired glucose tolerance [Hoggard et al., 2013]. Overnight fasted subjects were tested in an OGTT applying Polycal liquid, which bears 49% polysaccharides, 12% glucose and 0.6% maltose. Eight subjects were given a single capsule with 470 mg of bilberry extract or placebo in double-blind, placebo-controlled, cross-over fashion. Though the bilberry extract anthocyanins applied were at much higher magnitudes than dosages applied in our study, the OGTT applied predominantly polysaccharides, hence post-prandial glucose dynamics cannot be discussed in context of our findings presented here. Indeed, while blood sugar was noticeably lower from 30 min onwards, Hoggard and co-workers identified statistically significant lower post-prandial blood glucose with bilberry only at relatively late time points 120, 150 and 180 min, respectively, after challenge with Polycal. Interestingly, this study found that acute intake of bilberry extract exerted no significant effects, at any time point, on investigated incretins: gastric inhibitory polypeptide (GIP), glucagon-like-peptide-1 (GLP-1), and glucagon and amylin remained unaffected also.

In conclusion, our study points to a yet unidentified mechanism by which delphinidin glycosides, present in maqui berry, significantly acutely lower fasting glucose and -insulin simultaneously. A limitation of our study is the circumstance that we did not collect blood samples over a period longer than two hours post OGTT. It would be very interesting to learn how glucose and insulin develop in response to Delphinol® towards later time points.

The nature of the mechanisms involved shall be subject of future investigations. The glucose metabolism effects attributable to Delphinol® may be of particular interest in research on chronic health effects, such as metabolic syndrome and pre-diabetes.

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Figure 1: Chronological sequence on subject treatment and specimen collection. This sequence of investigation was carried out four times, with four different Delphinol® dosages applied: 0, 60, 120 and 180 mg. At the first two investigational time points, fasting blood specimen are collected, followed immediately by Delphinol® and glucose intake, respectively.

FIGURE 1.0

Figure 2 Overnight fasted study participants were acutely supplemented at basal conditions with Delphinol® at four different dosages of nil (control), 60, 120 and 180 mg, at four different occasions with several days in between experiments. Sixty minutes after Delphinol® intake participants presented with dose-dependent lowering of basal blood glucose. At this time 75 g of glucose were consumed and resulting post-prandial glucose levels are presented. Statistical significantly lowered values compared to untreated control are indicated by an asterisk. A hash indicates statistical significantly higher values than control (n = 36 at 60 and 180 mg, n = 44 at 120 mg and control).

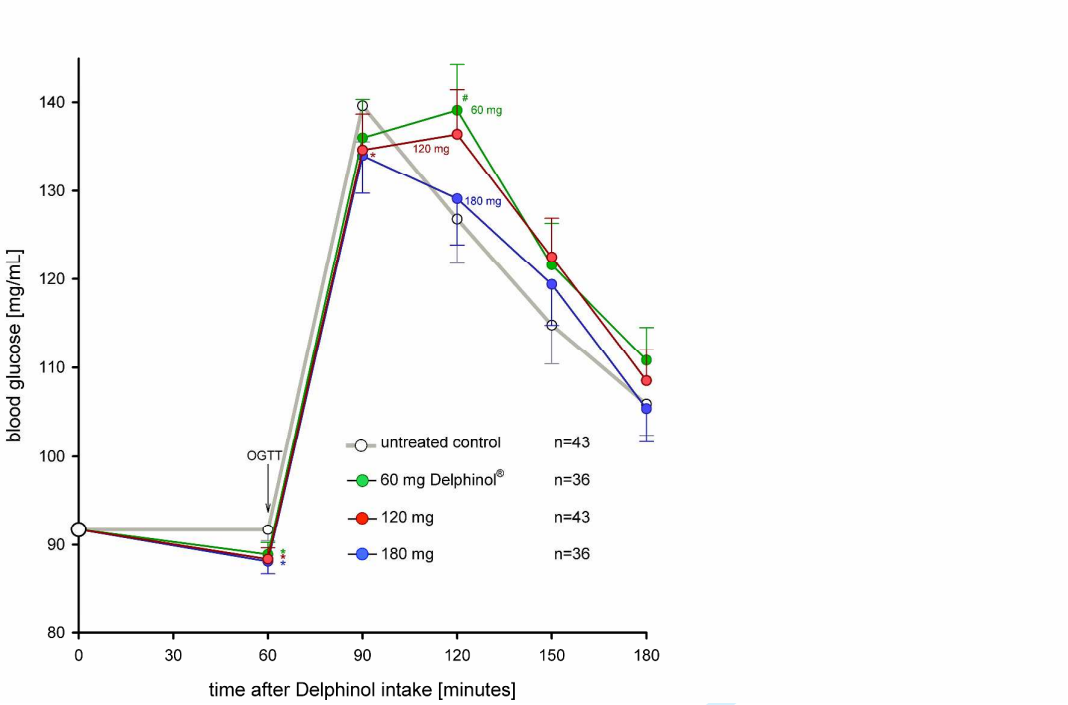
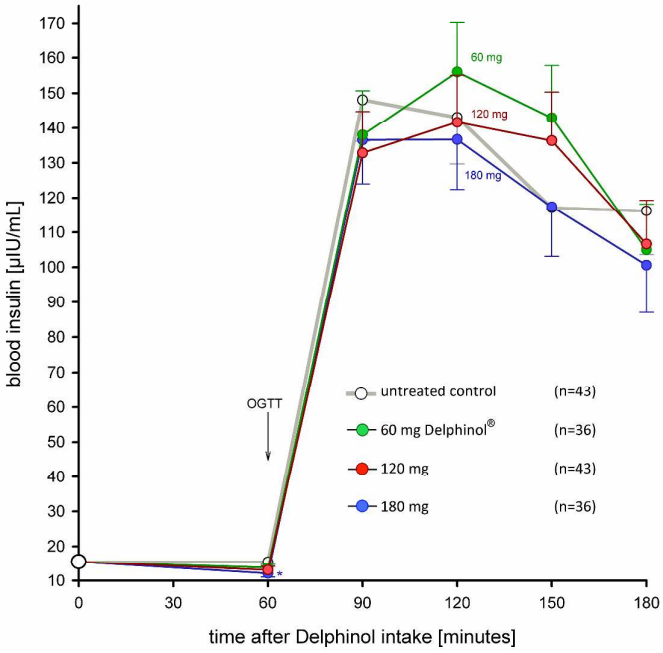
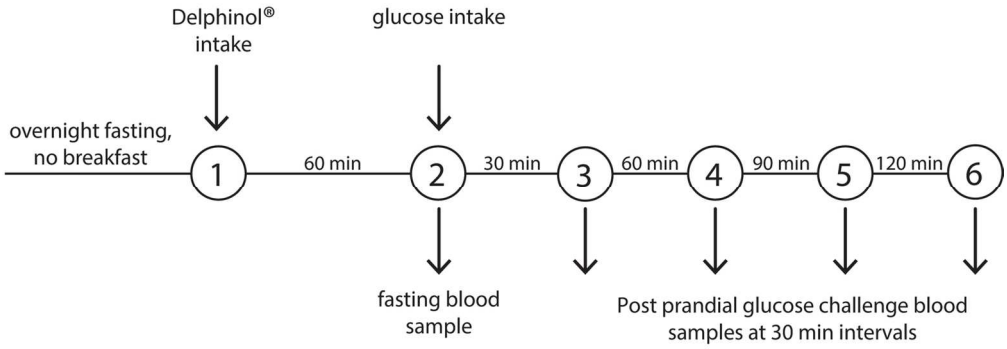


Figure 3: Overnight fasted study participants were acutely supplemented with Delphinol® at four different dosages of nil (control), 60, 120 and 180 mg, at four different occasions with several days in between experiments. Sixty minutes after Delphinol® intake participants consumed 75 g of glucose. Basal and post-prandial insulin levels are presented. Statistical significantly lowered values compared to untreated control are indicated by an asterisk. (n = 36 at 60 and 180 mg, n = 44 at 120 mg and control).





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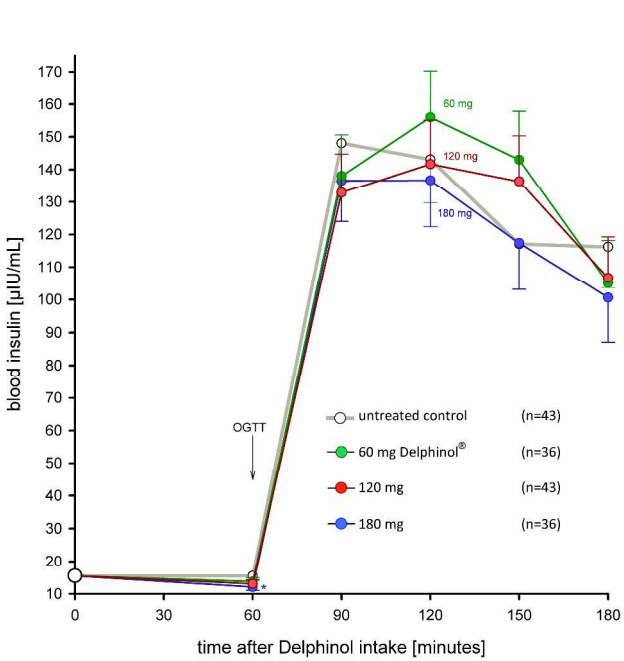

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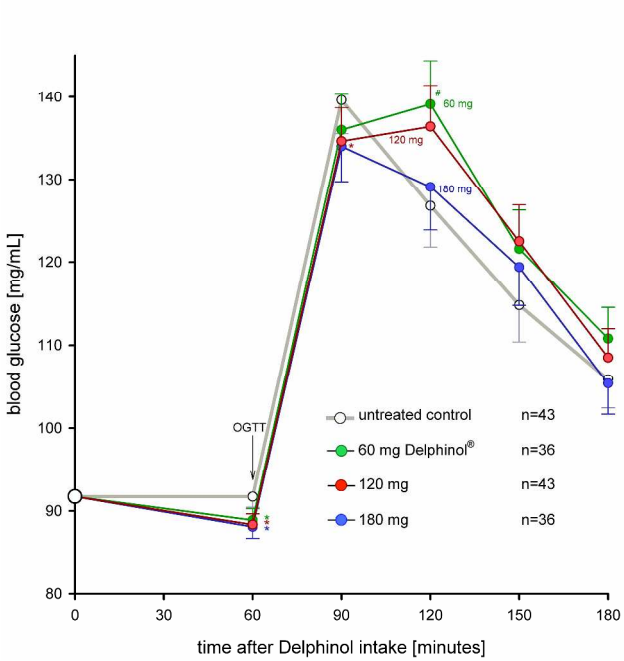
1252x827mm (150 x 150 DPI)



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1252x827mm (150 x 150 DPI)