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High-Antioxidant Potatoes: Acute in Vivo Antioxidant Source and Hypotensive Agent in Humans after Supplementation to Hypertensive Subjects

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ABSTRACT: Potatoes have the highest daily per capita consumption of all vegetables in the U.S. diet. Pigmented potatoes contain high concentrations of antioxidants, including phenolic acids, anthocyanins, and carotenoids. In a single-dose study six to eight microwaved potatoes with skins or a comparable amount of refined starch as cooked biscuits was given to eight normal fasting subjects; repeated samples of blood were taken over an 8 h period. Plasma antioxidant capacity was measured by ferric reducing antioxidant power (FRAP). A 24 h urine was taken before and after each regimen. Urine antioxidant capacity due to polyphenol was measured by Folin reagent after correction for nonphenolic interferences with a solid phase (Polyclar) procedure. Potato caused an increase in plasma and urine antioxidant capacity, whereas refined potato starch caused a decrease in both; that is, it acted as a pro-oxidant. In a crossover study 18 hypertensive subjects with an average BMI of 29 were given either six to eight small microwaved purple potatoes twice daily or no potatoes for 4 weeks and then given the other regimen for another 4 weeks. There was no significant effect of potato on fasting plasma glucose, lipids, or HbA1c. There was no significant body weight increase. Diastolic blood pressure significantly decreased 4.3%, a 4 mm reduction. Systolic blood pressure decreased 3.5%, a 5 mm reduction. This blood pressure drop occurred despite the fact that 14 of 18 subjects were taking antihypertensive drugs. This is the first study to investigate the effect of potatoes on blood pressure. Thus, purple potatoes are an effective hypotensive agent and lower the risk of heart disease and stroke in hypertensive subjects without weight gain.

KEYWORDS: *purple potato, anthocyanins, chlorogenic acids, phenolic acids, plasma antioxidant capacity, urine polyphenols, hypertension, blood pressure*

INTRODUCTION

The potato (*Solanum tuberosum*) is a herbaceous annual that grows up to 100 cm tall and produces a tuber, also called a potato. This tuber is rich in starch and ranks as the world's fourth most important food crop, after maize, wheat, and rice. Potatoes are the leading vegetable crop in the United States, and the United States is fourth in production behind China, Russia, and India.[1] Only about one-third of U.S. potatoes are consumed fresh. The bulk of potatoes, 60%, is processed into frozen products (fries and wedges), crisps, and dehydrated potato, whereas 6% is reused as seed potato. The average American eats >54 kg of potatoes per year (158 g/day or ~1 medium potato) and potatoes are the number four source of polyphenol antioxidants in the U.S. diet.[2]

The potato is a good source of energy and some micronutrients, and its protein content is very high in comparison with other roots and fibers. Potatoes are rich in vitamin C, and a single medium potato eaten with its skin provides nearly half of the daily adult requirement of 100 mg of ascorbate (USDA, National Nutrient Database). Potato is high in fiber (2 g/medium potato) and low in fat (<0.5 g), and boiling potatoes in their skins prevents loss of nutrients. The health aspects of potatoes have been reviewed, first by Friedman[3] and most recently by Camire and coauthors.[4] However, recent publications have emphasized the negative aspects of potatoes. Potatoes boiled, baked, or mashed all had high glycemic indices, >70, except baked white potato with skin, which had a glycemic index of 70.[5] Frying for a short time in hot oil (140−180 °C) causes a high absorption of fat in the potato and thus increases energy input and significantly reduces mineral and ascorbic acid content. The recent diatribe was an epidemiology study on weight gain in 121 000 Americans followed for a period of 10 years and their lifestyle factors and diet measured.[6] On the basis of increased daily servings of individual dietary components, 4-year weight change was most strongly associated with the intake of potato chips (0.76 kg) and potatoes (2.82 kg) per serving. These values were taken from the abstract and reported by the press. However, the value for potatoes includes French fries and when the weight gain on nonfried (baked, boiled, roasted, or microwaved) was calculated in the text of the paper, weight gain was only 0.3 kg over 4 years versus a 1.5 kg gain for French fries.

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Coffee is high in chlorogenic acids (CGA), and consumption in epidemiological studies indicates a reduced risk of hypertension.[7] In a hypertensive rat model CGA alone lowered the elevated blood pressure.[8] Potatoes are known to be high in CGA among vegetables. The higher antioxidants and especially CGA found in pigmented potatoes led us to the hypothesis that colored potatoes might be useful to lower the blood pressure in hypertensive humans.

■ MATERIALS AND METHODS

Polyphenol Analysis. One hundred milligrams of freeze-dried potato powder was extracted in a 2 mL screw-cap tube with 0.9 mL of extraction buffer (50% methanol, 2.5% metaphosphoric acid, 1 mM EDTA) and 500 mg of 1.0 mm glass beads, shaken in a BeadBeater (Biospec, Bartelsville, OK) for 15 min, and then centrifuged for 5 min at 4 °C; the supernatant was transferred to a clean tube. The remaining pellet was re-extracted with 0.6 mL of extraction buffer and centrifuged. The supernatants were combined, centrifuged, and concentrated in a Speed Vac (Thermo Savant, Waltham, MA) prior to HPLC analysis.

Analysis was conducted using an Agilent 1100 HPLC system with a quaternary pump, refrigerated autosampler, column heater, and DAD and MS detectors. A 100 × 4.6 mm, Onyx monolithic C-18 (Phenomenex) column was used at 35 °C and a flow rate of 1 mL/min with a gradient elution of 0−1 min, 100% A; 1−9 min, 0−30% B; 9−10.5 min, 30% B; 10.5−14 min, 35−65% B; 14−16 min, at 65−100% B; 16−16.5 min, 100% B (buffer A, 10 mM formic acid, pH 3.5, with NH_4OH; buffer B, 100% methanol with 5 mM ammonium formate). MS analysis was with an Agilent 1100 LC/MSD SL ion trap using an ESI source in both positive and negative ion modes. The source was operated using 350 °C drying gas (N_2) at 12 L/min, 55 psi nebulizer gas (N_2), and the source voltage with a scan range of m/z 100−1300. The external standard method of calibration was used. Neochlorogenic and cryptochlorogenic acids were quantitated as chlorogenic acid equivalents and flavonols as rutin equivalents.

Potassium Analysis. Freeze-dried potato powder was ashed at 500 °C for 3 h and then analyzed after dissolution in aqueous lanthanum nitrate by conventional flame emission spectroscopy.

Single-Dose Design. After approval from The University of Scranton IRB board had been obtained, informed consent from eight participants, a medical history, and height and weight data were collected for each. Participants were required, for 3 days prior to the study, to consume a low-polyphenol diet and refrain from consuming alcoholic beverages, coffee, tea, cola, chocolate, fruits, vegetables, and fruit juices. Meat, milk products, and pasta were allowed. The subjects then fasted for 10 h before arriving at Pennant Laboratories for fasting blood work. A 24 h urine was collected on the day prior to the appointment. Six to eight small microwaved potatoes (∼138 g total) with skins or an equivalent amount of potato starch in the form of a cooked biscuit along and 240 mL of water were consumed by each participant. The potatoes (Purple Majesty, PM) were obtained in a single shipment from the USDA (Duroy Navarre). Potatoes were microwaved for 1.5 min. A trained phlebotomist took blood samples at 0, 0.5, 1, 2, 4, and 8 h after consumption. Yogurt and water were consumed for lunch after the 4 h blood sample was taken. Participants followed the prohibitions above during the study day and collected a 24 h urine the day of the study, which includes the following morning's waking sample. After 2 weeks, the fasting and sampling were repeated with the other product. The same 3 day dietary restriction was in place prior to this sampling.

The urine and plasma samples were collected, the volumes of the urines measured with a graduate cylinder, and the samples stored at −20 °C until analysis. Plasma antioxidant capacity was measured by ferric reducing antioxidant power (FRAP). Urine polyphenols were measured by a single-step colorimetric Folin assay using catechin as the standard. Urine non-polyphenol interferences were measured after removal of polyphenols by Polyclar VT (ISP Technologies, Wayne, NJ) and the Folin assay had been conducted on the eluate. This value was subtracted from the sample Folin to determine urine polyphenols.

Supplementation Design. After approval had been obtained from the University of Scranton Institutional Review Board, subjects for this study were recruited via a notice sent to the University of Scranton Bulletin Board and the local Scranton newspaper, which has a regional circulation. Informed consent, a medical history, height, weight (InBody520, Biospace, Los Angeles, CA), and resting blood pressures (Prosphyg android sphygmomanometer, American Diagnostics, Hauppauge, NY) were obtained from each participant. The resting blood pressure was obtained after the participant sat quietly for 15 min in the Exercise Physiology Laboratory, and both arms were measured. Participants were then required to fast for 10 h before arriving at Pennant Laboratories for blood drawing at the beginning of the study, after 4 weeks, and at the end of the study. Participants were instructed to consume six to eight microwaved small PM potatoes with skins for lunch and dinner daily for a period of 4 weeks at either the beginning or end of the supplementation study. The potatoes were provided to the subjects, and they were allowed to microwave for a time period determined by the subject. No other potatoes were consumed during the study. Two 7-day food consumption questionnaires were filled out during the study.

This was a crossover study during which half the subjects (randomly chosen) were given potatoes at the beginning of the study and half the subjects were given no potatoes (NP) to consume. After 4 weeks, the groups were switched and given the other regimen for 4 weeks. The study ended after the second 4 week period. In addition to height, weight, and blood pressure, plasma glucose, red blood cell HbA1c, plasma cholesterol, high-density lipoprotein (HDL), and triglycerides were measured at Pennant Laboratories, Dunmore, PA.

Subjects. *Single-Dose Study.* There were seven males and one female in this study. The average age was 23 ± 9 years. The average height was 178 ± 10 cm, the average weight was 78 ± 14 kg. The average BMI was 24.7 ± 3.2. There was one subject considered to "obese" (BMI ≥ 30) and two subjects considered to be "overweight" (BMI ≥ 25 but ≤30) on the BMI scale.

Supplementation Study. In this study there were 7 males and 11 females. The average age was 54 ± 10 years. The average height was 173 ± 2 cm, and the average weight was 87.5 ± 21.3 kg. The average BMI was 29.4 ± 6.4. There were 7 subjects considered to be "obese" (BMI ≥ 30) and 6 subjects considered to be "overweight" (BMI ≥ 25 but ≤30) on the BMI scale, so the majority of subjects had excess weight. The average cholesterol, HDL, low-density lipoprotein (LDL), and triglycerides, respectively, were 201 ± 34, 48 ± 13, 130 ± 28, and 118 ± 68 mg/dL. Cholesterol was elevated (≥200 mg/dL) in 10 of 18 subjects, HDL low (≤40 mg/dL in men and ≤50 mg/dL in women) in 10 of 18 subjects, and triglycerides elevated (≥150 mg/dL) in 6 of 18 subjects. Thus, there was an increased risk of heart disease in this population. In addition, 13 of 18 subjects were taking blood pressure lowering medication.

Initially, the average systolic blood pressure (SBP) was 140 ± 15 mmHg and diastolic blood pressure (DBP), 89 ± 8. The American Heart Association states that normal SBP is <120 and normal DBP, <80.[9] There were 3 of 18 subjects with normal SBP and 1 of 18 with normal DBP. Prehypertension is defined as SBP = 120−139 and DBP = 80−89. Of the subjects 8 of 18 were prehypertensive for SBP and 9 of 18 prehypertensive for DBP. Stage 1 hypertension is SBP = 140−159 and DBP = 90−99. Of the subjects 5 of 18 were classified as stage 1 hypertension for SBP and 7 of 18 as stage 1 for DBP. Stage 2 hypertension is SBP ≥ 160 and DBP ≥ 100. One subject was classified as stage 2 for both SBP and DBP. Thus, 14 of 18 subjects can be classified as hypertensive. Fasting glucose averaged 91 ± 10 mg/dL and HbA1c was 5.6 ± 0.4%, both of which are in the high-normal range.

■ RESULTS

The purple potato (PM) polyphenol composition is shown in Table 1. In this cultivar of potato the anthocyanins predominated over the phenolic acids. The total polyphenol concentration was 10.2 mg/g dry weight or 2.0 mg/g wet weight. This does not include the four caffeoyl polyamines

Table 1. Polyphenol Analysis of Purple Majesty Potatoes

Purple Majesty immature potatoes	mg/g dry weight (av ± SD)
total anthocyanins	6.5 ± 2.0
chlorogenic acid	2.72 ± 0.38
neochlorogenic acid	0.147 ± 0.030
caffeolyputrescine	0.021 ± 0.003
cryptochlorogenic acid	0.387 ± 0.067
caffeic acid	0.401 ± 0.128

detected, which were tris(dihydrocaffeoyl)spermine, bis(dihydrocaffeoyl)spermidine, trisdihydrocaffeoyl spermidine, and trisdihydrocaffeoyl spermidine. These compounds were identified but not quantified due to lack of appropriate standards.

The concentration of potassium was 549 mg/138 g serving. This value is comparable to the value found in the USDA National Nutrient Database of 567 mg/serving.

Single-Dose Study. The pharmacokinetics of the plasma antioxidant capacity (AC) after potato starch and PM are shown in Figure 1. The initial fasting plasma AC was 301 ± 106

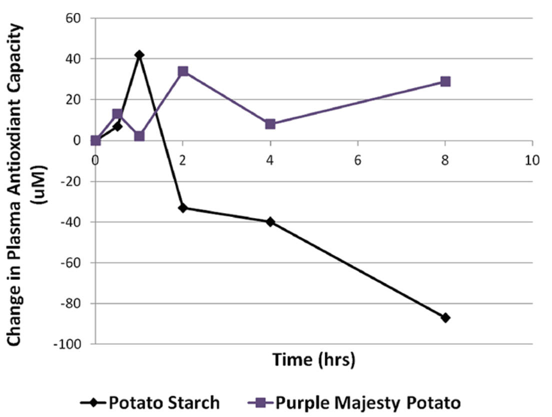

Figure 1. Change in plasma antioxidant capacity (μM FRAP) after consumption of potato starch or purple potato.

μM catechin equivalents for starch and 261 ± 100 μM for PM. As can be seen, the starch produced a small rapid increase in AC at 1 h followed by a rapid and then slow large decline, which lasted for at least 8 h, when the sampling was discontinued. The potato showed an initial biphasic curve with maxima at 30 min and 2 h with a slight rise until 8 h. The plasma area under the curve (AUC) was 150 ± 480 μM/h after the potato and −266 ± 508 μM/h after the starch. The areas were not significantly different as deteremined by a paired t test ($p = 0.11$).

Figure 2 displays the average urine polyphenols before and after consumption of the starch and PM. There was no significant difference between the levels before and after consumption for either of the groups. The change in urine polyphenols was negative for the starch and positive for the potato, but the difference was not quite significant between the two products ($p = 0.09$). PM consumption caused a 92% increase in 24 h urine polyphenols, whereas the starch produced a small net decrease (3.5%).

Supplementation Study. Of the original 20 subjects, 2 dropped out, 1 for health reasons and the other complaining of the bad taste of the potatoes. Thus, the complete data set is for 18 subjects. The percent change in the blood pressure and biochemical measurements is shown in Figure 3 for the no

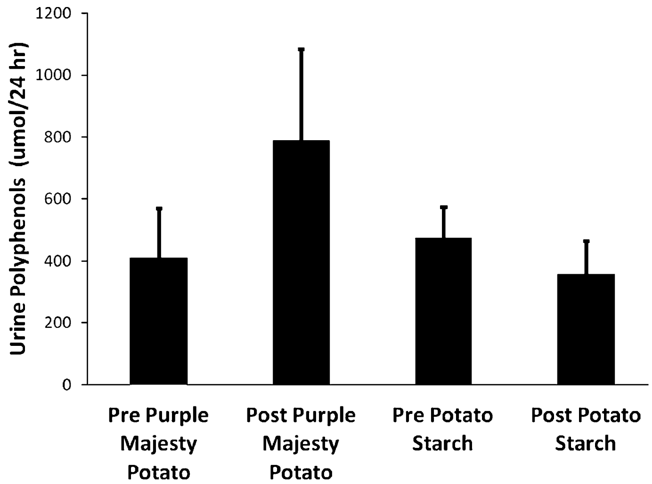

Figure 2. Urine polyphenol excretion (μmol) 24 h before and after consumption of potato starch or Purple Majesty potato.

potato supplementation (NP) and for the purple potato (PM) supplementation to the same subjects in Figure 4. The only significant change for both supplementations was the effect of the PM on DBP. The data for blood pressure for the NP diet are as follows: before DBP, 87 ± 7, and after DBP, 87 ± 6 mmHg; before SBP, 136 ± 13, and after SBP, 137 ± 15 mmHg; that is, there was no change in BP. However, for the PM diet the blood pressure data were as follows: before DBP, 89 ± 7, and after DBP, 85 ± 7, a decrease of 4 mmHg ($p < 0.01$). It is informative to show the changes in DBP, which are displayed in Figure 5. For SBP, before PM subjects averaged 139 ± 16 and after, 134 ± 12 mmHg. This decrease of 5 mm was not significant.

DISCUSSION

Potato Antioxidants. The Purple Majesty cultivar had twice as many anthocyanins as CGA. In a study of 10 varieties of purple potatoes, there were significantly more anthocyanins than CGA. Also, comparing 17 white/yellow varieties, there was 5 times more CGA and 3 times more soluble phenols in the purple varieties.[10−12] Thus, purple varieties have significantly more polyphenols, anthocyanins, and CGA than the other varieties of potato. One of the concerns regarding the polyphenols in potatoes was the cooking process. It has been reported that oven-baked potatoes contain no CGA and boiled potatoes and microwaved potatoes could retain less than half of the original CGA.[13] However, this analysis was done with potatoes peeled before cooking. A newer study using HPLC found that unpeeled potatoes retained 66.4, 52.8, and 82.5% of CGA after boiling, baking, and microwave cooking, respectively.[14] Frying was found to cause the greatest loss of quercetin derivatives and CGA in white potatoes.[15]

Single-Dose Study. PM consumption produced a greater area under the curve than the starch for seven of eight subjects, but the difference was not significant ($p = 0.11$), probably due to the large variation within each group. The overall change as a result of potato starch is a decrease in plasma antioxidant capacity. For the PM the average changes postconsumption were all positive and the largest change occurred 2 h after consumption, with a gradual rise after 8 h. Thus, overall, as expected, potato starch was a pro-oxidant as was found for its gastrointestinal hydrolysis product, glucose,[16] and PM was an in vivo antioxidant because it contains a high concentration of

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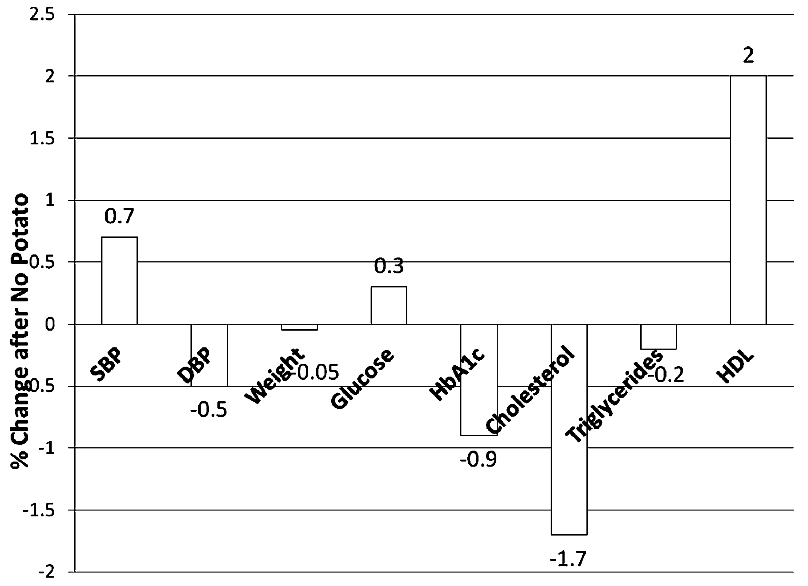

Figure 3. Percent change in biochemical parameters after 4 weeks of no-potato diet.

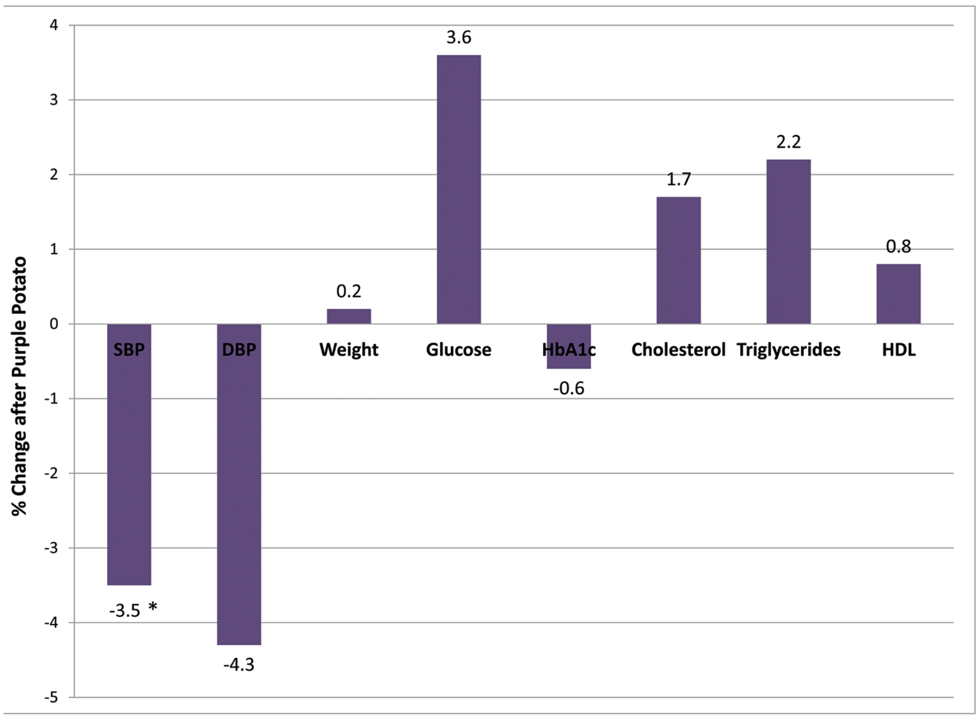

Figure 4. Percent change in biochemical parameters after 4 weeks of Purple Majesty potato diet. * indicates a significant change ($p < 0.05$).

polyphenols to counter the oxidative stress of the sugar. We have shown that other sources of polyphenols such as figs,[17] grape seed extract,[18] and cranberry juice[19] can also produce an increase in plasma antioxidant capacity even in the presence of sugars such as fructose and high-fructose corn syrup.

Urine polyphenols were very slightly decreased (4%) from consuming the potato starch, and PM caused a 92% increase. As a comparison, 230 g of tomato juice was given for 18 days to subjects, and a 202% increase in urine polyphenols (Folin) resulted.[20] Urine polyphenol analysis by Folin after solid phase extraction cleanup was pioneered by a Spanish group, who showed it to be an accurate biomarker of polyphenol intake.[21] Interestingly, urine polyphenols were inversely associated with blood pressure and positively associated with a reduction in the risk of heart disease in a large European study.[22] A group in Scotland has investigated the bioavailability and plasma antioxidant activity of the PM potato. They found a small nonsignificant increase in plasma antioxidant capacity and urinary phenolics. No evidence of intact anthocyanins was found in the urine, and phenolic acid metabolites were present (Catherine Tsang and Emad Al-Dujaili, personal communication).

Supplementation Study. The same cultivar of purple potato (PM) was also investigated in a supplementation study to healthy men, which compared white-, yellow-, and purple-fleshed potatoes in a single serving oer day for a period of 6

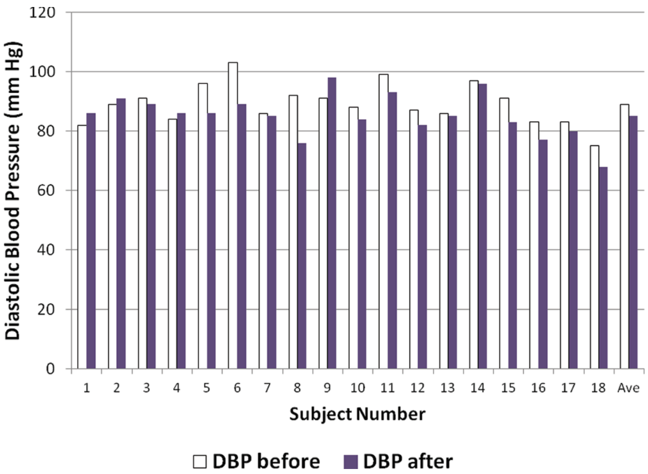

Figure 5. Individual subject diastolic blood pressure before and after 4 weeks of Purple Majesty potato diet.

weeks.[23] The PM was higher in total antioxidants and anthocyanins than the other two varieties, and also PM was significantly the most efficacious potato in the reduction of inflammation markers and DNA oxidation. Thus, a higher polyphenol potato such as Purple Majesty results in a better reduction of risk for several chronic diseases: inflammation is linked to heart disease and diabetes, and DNA oxidation is linked to cancer. The same order of antioxidants was found in another analysis, that is, purple > yellow > white, and the purple potato was significantly more effective, suppressing proliferation and elevating apoptosis of colon cancer cells.[24] A study of Finnish vegetables showed that on a serving size basis, potatoes had a higher concentration of phenolic acids than other vegetables.[25] This study also found that the peel of the potato was very high in phenolic acids.

A serious concern of consuming more potatoes is the specter of weight gain. Two servings of medium potatoes (six to eight of our Purple Majesty potatoes) would add 1155 kJ to the diet. Our 7 day dietary record analysis indicated that the subjects consuming potato did increase their caloric intake by 707 ± 929 kJ compared to the no-potato diet ($p = 0.052$). Even with these added kilojoules, there was no weight gain with potatoes (average gain = 0.1 kg or 0.2%, not significant). In fact, purple potatoes (cv. Bora Valley) have been used as a folk remedy in Korea for weight loss, and the mechanism was investigated in a rat model. The ethanolic extract inhibited the proliferation and differentiation of 3T3-L1 adipose cells as well as reduced the cellular leptin level. Whole body fat was reduced in the animals as measured by MRI. The antiobesity effect was mediated by down-regulation of p38 mitogen-activated protein kinase (MAPK).[26] Our study is the first to investigate the effect of potatoes on weight gain. One unanswered question is whether the addition of white potatoes to the diet, with fewer polyphenols, CGAs, and anthocyanins, would or would not result in weight gain.

In this study the after-potato DBP decreased 4 mm and the SBP declined 5 mm. Of the subjects 14 of 18 experienced a decrease in DBP and 8 of 18 had a decline in SBP. This benefit occurred despite the fact that 72% were taking blood pressure medication. This result may indicate that the PM blood pressure effect occurred by a different mechanism than the medications. Potatoes are high in potassium, which is one of the regulators of blood pressure. Dietary potassium has a modest effect on blood pressure.[27] Additionally, an increased consumption of potassium is associated with a lower risk of stroke and cardiovascular disease.[28] Reduction of the blood pressure by 5 mmHg can decrease the risk of stroke by 34% and of ischemic heart disease by 21% and reduce the likelihood of dementia, heart failure, and mortality from cardiovascular disease.[29]

A recent meta-analysis of over 518 000 people indicated a much higher risk of stroke (179%) if the subject was in the upper half of the prehypertension range; that is, for DBP = 85−89 mmHg and SBP = 130−139 mmHg compared to subjects in the lower range, DBP = 80−84 mmHg and SBP = 120−129 mmHg with a lower risk of 122%.[30] The PM regimen caused six subjects to move from the higher DBP range and five from the higher SBP range to the lower range with a large reduction of risk. A large study comparing dietary advice versus no advice given to prehypertensives found DBP reduced by only an average of 1 mmHg and SBP, by 2 mmHg.[31] The PM caused a much larger decrease averaging, 4 mmHg for DBP and 5 mmHg for SBP.

The null effect of 4 weeks of PM supplementation on weight and biochemical parameters other than blood pressure is important in that it proves that an increased consumption of PM did not deleteriously affect cardiovascular and diabetes risk factors but in fact lowered the blood pressure and thus lowered the risk of cardiovascular disease.

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ABBREVIATIONS USED

PM, Purple Majesty potato; NP, no potato; FRAP, ferric ion reducing antioxidant power; CGA, chlorogenic acid and its isomers; DBP, diastolic blood pressure; SBP, systolic blood pressure.

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