

Effect of purple sweet potato leaf consumption on the modulation of the antioxidative status in basketball players during training

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The aim of this study was to evaluate the effect of purple sweet potato leaves (PSPLs) consumption on antioxidative status and its modulation of that status in basketball players during training period. Fifteen elite basketball players were enrolled in this study. The seven-week study consisted of a run-in (week 1), PSPLs diet (daily consumption of 200 g PSPLs) (weeks 2, 3), washout (weeks 4, 5), and control diet (low polyphenol, with the amount of carotenoids adjusted to the same level as that of PSPLs) (weeks 6, 7). Blood and urine samples were taken for biochemical analysis. Compared with the control group, the results showed that PSPLs consumption led to a significant increase of plasma polyphenol concentration and vitamin E and C levels. Low density lipoprotein (LDL) lag time was significantly longer in the PSPLs group. A significant decrease of urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) was noted; however, there was no significant change in plasma glutathione (GSH), total antioxidant status (TAS) and malondialdehyde + 4-hydroxy-2(E)-nonenal level after consuming the PSPLs diet. In conclusion, consumption of PSPLs diet for 2 weeks may reduce lipid and DNA oxidation that can modulate the antioxidative status of basketball players during training period.

Key Words: purple sweet potato leaves, polyphenols, basketball, training, antioxidative status

INTRODUCTION

High consumption of vegetables and fruits has been linked epidemiologically to a decreased risk of cancer and cardiovascular disease.¹ Their beneficial effects have been attributed partly to the presence of numerous polyphenolic compounds, which display antioxidant and free radical scavenging properties.² Flavonoids are polyphenolic compounds found in rich abundance in fruits and vegetables. A variety of *in vitro* studies have shown that flavonoids are antioxidants,³ immunomodulators,⁴ and exhibit anti-genotoxic effects.⁵

Many health benefits of exercise are well known. Exercise is recommended for the prevention and management of many chronic diseases and for the maintenance of optimal health. Strenuous physical activity can increase oxygen consumption by up to 10- to 20-fold over resting levels to meet energy demands, and oxygen uptake in active skeletal muscle increase 100- to 200-fold.⁶⁻⁸ Increased oxygen uptake during exercise is accompanied by an elevation in reactive oxygen species (ROS), which might cause lipid peroxidation of polyunsaturated fatty acids in membranes, DNA damage, and decreases antioxidants levels in target tissues and blood.⁹⁻¹⁰ Oxidative stress can be defined as imbalance between oxidative reactions and antioxidant capacity that results directly or indirectly in cellular damage.¹¹⁻¹² Therefore, antioxidant supplements are marketed

and used by athletes as a means to counteract the oxidative stress of exercise. Many studies have indicated that polyphenols with their antioxidative capacity can help to prevent such oxidative damage and thereby support appropriate physiological function.¹³⁻¹⁵

Purple sweet potato [*Ipomoea batatas* (L.) Lam] leaves (PSPLs), which are easily grown in tropical areas such as Taiwan, have the highest polyphenolic content (33.4 ± 0.5 mg gallic acid/g dry weight) of all the commonly grown vegetables in this country, and exhibit free radical scavenging ability.¹⁶ Limited information is available on the human experimental studies about the physiologic and biochemical effects of dietary PSPLs.

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We have recently shown that PSPLs had higher bioavailability of polyphenol using stir-fried method, and could modulate the antioxidative status of healthy adults.¹⁷ However, the effects of PSPLs on exercise-induced oxidative stress are unknown. Therefore, the purpose of this study was to evaluate whether PSPLs consumption providing a variety of polyphenols at a physiological dose range affects a variety of biomarkers of antioxidative status in basketball players during training period.

MATERIALS AND METHODS

Subjects

Fifteen elite basketball players (six men and nine women, aged 20-24 years) were recruited for the study. The participants were non-smokers and not taking any routine medications and vitamin supplements during the study period. Training history, weight, height and body mass index (BMI) were recorded (Table 1). Total body fat percentage was assessed using a body fat impedance analyzer (Inbody 3.0; Biospace, Seoul, Korea). For the intervention session, a training program was designed by a nationally certified senior coach for each player, and the training proceeded for 2-3 h/day for 3 d/week. The training protocols consisted of a general warm up and stretching (approx. 20 min), technical-tactical training (approx. 30 min), heavy training including training of counterattacks and simulated full- or half-count basketball games (approx. 60 min) and finally a cool-down phase (approx. 20 min). The study was approved by Medical Ethical Committee of the Taipei Medical University, and all participants gave informed written consent.

PSPLs prepared

The purple sweet potato leaves (PSPLs) were obtained from plants that had grown and tended at the Taoyuan District Agriculture Improvement Station, Taipei Branch, Taiwan. They were transported to the laboratory and stored at 4°C until cooking. For cooking process, soy cooking oil was preheated to 200 °C, fresh PSPLs was added to the oil and stir-fried for 3 and 5 min with repeated stirring. After cooking, the PSPLs were taken out and placed in the carton to service.

PSPLs are rich in carotenoids. In order to calibrate the antioxidant effect of carotenoids, the amount of carotenoids consumed by the study and control diets were similarly adjusted. We have previously demonstrated that the level of polyphenol in PSPLs and carrots were 4.15 and 1.87 mg gallic acid equivalent (GAE)/g wet weight and the level of β -carotene was 117.10 and 268.76 μ g/g wet weight.¹⁷ Control subjects ingested the same amount of β -carotene from 40-45 g carrots each diet.

Experimental design

The diet-control study was conducted during September to November 2004. The study was divided into 2 periods each lasting 2 weeks, for a total study period about 7 weeks. Subjects were provided the PSPL and control diet for lunch and dinner by the Department of dietetics of Taipei Medical University Hospital. During the study period, the subjects were instructed to exclude polyphenol-rich foods from their diet. A list of the food products that the subjects were not allowed to eat was provided (ex-

onions, green vegetable, tea etc.). The study consisted of a run-in period (week 1), consumption of PSPLs diet (200 g/day) (weeks 2 and 3), a washout period (weeks 4 and 5), and consumption of control diet that low in polyphenol but containing an equivalent amount of carotenoids (weeks 6 and 7). Therefore, PSPL-related daily dietary intake was 902 mg GAE of total polyphenol and 23.42 mg of β -carotene. Controls subjects ingested the same amount of β -carotene and the content of polyphenol was 149.6-168.3 mg GAE each day.

Collection and preparation of blood samples

At the beginning of the study and at the end of each experimental period, blood samples from fasting subjects was collected in the morning between 07:00 to 09:00 am. Blood was drawn from an antecubital vein into tubes containing 1.6 g/L of EDTA or Li-heparin, and then these were immediately placed on ice in the dark. Plasma collected by centrifugation at 1500 \times g for 10 min at 4°C was stored at -80°C until analysis. For vitamin C analysis, freshly plasma (500 μ L) was mixed with an equal volume of 10% metaphosphoric acid and centrifuged to remove the precipitated portion. The supernatant was frozen at -80°C until analysis.

Collection and preparation of urine samples

All subjects were asked to collect 24-h urine sample. During the collection period, urine was stored at 4°C and kept in dark bottles. Exact urine volumes were determined, and each sample was stored at -20°C until analysis.

Sample analysis

Measurement of blood and biochemical parameters. Routine complete blood counts (CBC) and biochemical parameters were taken using an automatic haematology analyser (HITACHI 7170; Hitachi Co., Tokyo, Japan).

Measurement of total polyphenol in plasma and urine. Total polyphenol in plasma and urine were measured using the Folin-Ciocalteu-method.¹⁸ The absorption at 750 nm was measured spectrophotometrically. The total polyphenol content was expressed as gallic acid equivalents (GAE).

Antioxidant status. Both plasma vitamin C and vitamin E were analyzed using reverse-phase high-performance liquid chromatography (HPLC) with a UV detector at 254 nm and 292 nm. A 4 \times 250 mm and 4 \times 125mm Lichrosphere 100RP-18 column (Merck, Darmstadt, Germany), containing 5-mm particles protected by a guard column, were respectively used for vitamin C and E analysis. The mobile phase for vitamin C contained 0.5 mM PICB (1-Pentane sulfuric acid sodium salt) adjusted to pH 3.1 with glacial acetic acid. Pure methanol was used as the mobile phase for vitamin E analysis. Red blood cell portions of whole-blood samples were separated out for subsequent glutathione (GSH) analyses using commercial kits (Calbiochem Co., San Diego, CA, USA). 50 μ L of plasma was mixed with 200 μ L of freshly prepared 6% methaphosphoric acid. After centrifugation (3,000 \times g for 10 min at 4°C), the supernatant of 100 μ L was mixed with buffer no. 3 (200 mM potassium phosphate [pH 7.8]

containing 0.2 mM diethylene triamine pentaacetic acid) up to a volume of 0.9 ml. 50 μ L of 12 mM solution of chromogenic reagent in 0.2 N HCl and 50 μ L of 30% NaOH were added, mixed thoroughly, and incubated for 10 min in the dark. The absorbance was measured at 400 nm.

The measurement of total antioxidant status (TAS) was based on the method of Miller *et al.*¹⁹ using commercial kits (Randox Laboratories Ltd., Crumlin, Antrum, UK). In this assay, metmyoglobin reacts with hydrogen peroxide to form ferrylmyoglobin free radical species. Ferrylmyoglobin was incubated with the substrate (2, 2'-amino-di-[3-ethylbenzthiazole sulfonate]) and measured spectrophotometrically at 600 nm. Antioxidants in the sample suppress production of blue-green color in proportion to their concentration.

LDL was isolated using a rapid two-step ultracentrifugation and the kinetics of LDL oxidation was measured by monitoring the formation of conjugated dienes at 234 nm with a Hitachi U 3000 spectrophotometer at 2 min intervals at 37 °C after *in vitro* Cu²⁺-catalyzed oxidation. The lag time of lipid peroxidation is defined as the time interval between the initiation and the intercept of the two tangents drawn to the lag and propagation phase of the absorbance curve at 234 nm, and it was expressed in min.

The lipidperoxides [Malondialdehyde (MDA) and 4-hydroxy-2(E)-nonenal (4-HNE)] in plasma were using commercial kits (Merck; Calbiochem Co., San Diego, CA, USA) and measured by mixing 200 μ L of plasma with 650 μ L of N-methyl-2-phenylindole and 150 μ L of methansulfonic acid, incubate for 60 min at 45°C and the

absorbance at 586nm.

Urinary 8-OHdG was measured by an ELISA. The absorbance was measured at 450 nm. Urine samples were centrifuged to remove any particulate material. Fifty μ L of sample and 50 μ L of reconstituted primary antibody were added. The plate was covered, incubated at 37°C, and mixed continuously for 1 h. The antibodies bound to the 8-OHdG in the sample were washed with 0.05% Tween 20/phosphate buffered saline buffer. An enzyme-labeled secondary antibody was added to the plate, which was then incubated at 37°C and mixed continuously for 30 min, and the unbound enzyme-labeled secondary antibody was washed away. The amount of antibody bound to the plate was determined by the color developed from the addition of a chromatic substrate and read at 450 nm.

Statistical analysis

All values are expressed as mean \pm SD. Data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Statistical significance between mean values was determined using the *t*-test and one-way ANOVA with repeated measures. *p* < 0.05 was considered statistically significant.

RESULTS

All participants tolerated the intervention well and completed the study. Physical characteristics, blood and biochemical parameters of subjects are summarized in Table 1-3. There were no significant changes in body weight, BMI, percentage of body fat, or any of the blood and biochemical parameters during the study.

Table 1. Physical characteristics of subjects¹

	Before		After	
	Male (n=6)	Female (n=9)	Male (n=6)	Female (n=9)
Training history (years)	7.83 \pm 3.25	4.11 \pm 3.59	7.83 \pm 3.25	4.11 \pm 3.59
Height (cm)	181 \pm 6	162 \pm 5	181 \pm 6	162 \pm 5
Weight (kg)	71.8 \pm 5.81	56.9 \pm 5.61	72.4 \pm 5.34	56.8 \pm 6.33
BMI (kg/m ²)	22.0 \pm 1.32	21.5 \pm 2.36	22.3 \pm 0.93	21.5 \pm 2.33
Body fat (%)	12.9 \pm 2.05	25.8 \pm 5.29	13.4 \pm 1.85	25.5 \pm 5.44

¹ Values are Mean \pm SD (n=15)

Table 2. The blood parameters of subjects^{1, 2}

	Before	After
WBC ($\times 10^3/\mu$ L)	5.78 \pm 0.87	5.91 \pm 0.72
RBC ($\times 10^3/\mu$ L)	4.49 \pm 0.34	4.53 \pm 0.34
HGB (g/dL)	13.6 \pm 1.28	13.7 \pm 1.28
HCT (%)	40.5 \pm 3.49	41.0 \pm 3.52
MCV (fL)	90.0 \pm 4.36	90.5 \pm 5.18
MCH (pg)	30.2 \pm 1.67	30.1 \pm 1.93
MCHC (g/dL)	33.5 \pm 0.46	33.3 \pm 0.54
PLT ($\times 10^3/\mu$ L)	250 \pm 32.4	272 \pm 42.4
NEUT (%)	50.3 \pm 8.16	50.4 \pm 7.96
LYMPH (%)	38.8 \pm 7.48	39.6 \pm 7.06
MONO (%)	7.73 \pm 1.28	7.04 \pm 1.78
EOS (%)	2.67 \pm 1.80	2.56 \pm 1.57
BASO (%)	0.48 \pm 0.16	0.47 \pm 0.19

¹ Values are Mean \pm SD (n=15). ² WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet count; NEUT: neutrophils; LYMPH: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophils

Table 3. The biochemical parameters of subjects ^{1,2}

	Before	After
TC (mg/dL)	170 ± 25.0	171 ± 32.6
TG (mg/dL)	51.9 ± 14.0	57.5 ± 25.3
HDL-C (mg/dL)	56.4 ± 10.7	55.7 ± 11.3
LDL-C (mg/dL)	100 ± 19.9	101 ± 26.0
GOT (U/L)	21.7 ± 5.62	23.1 ± 5.22
GPT (U/L)	15.3 ± 4.61	14.5 ± 3.02

¹Values are Mean ± SD (n=15). ² TC: Cholesterol; TG: triglyceride; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvate transaminase

The concentrations of plasma and urine polyphenol measured throughout the study are summarized in Table 4. Plasma polyphenol levels significantly increased after the intervention in the PSPL group (32.22 ± 1.49 to 40.17 ± 3.63 mg GAE/dL), and were also significantly higher than those of the control group. However, urinary polyphenol levels showed no significant change in each period.

Table 5 presents the changes in antioxidative status during the study. Antioxidants as determined by the vitamin C and E and glutathione, lipid peroxidation as determined by the “lag time” during LDL oxidation and the end products MDA+4-HNE, antioxidant power as measured by the TAS, and DNA oxidation as measured by the 8-OHdG. Compared with the control group, the PSPL group had significantly higher plasma levels of vitamin C (11.89 ± 3.20 vs. 8.19 ± 3.14) and vitamin E (9.57 ± 2.55 vs. 5.04 ± 1.83), but there was no significant difference in plasma GSH. LDL lag time, as well as urinary excretion of 8-OHdG showed significant differences between PSPL and control groups. Plasma TAS and lipidperoxides (MDA+ 4-HNE) concentrations did not change signifi-

cantly between two groups.

DISCUSSION

The study presents the results of the effect of polyphenol-rich PSPLs diet on the changes in plasma antioxidant capacity, LDL oxidation, lipid peroxidation, and DNA oxidation in basketball players. In the study, consumption of PSPLs diet resulted in a marked increase in total polyphenols level in the plasma. To provide protection against oxidative stress *in vivo*, PSPLs polyphenols must be absorbed and retained in the body in a form that still has antioxidant properties. Studies showed when dietary polyphenols from food sources are absorbed from the gut, the circulating species are almost entirely conjugated,²⁰ and that many of these conjugated metabolites have antioxidant properties *in vitro*.²¹⁻²² We hypothesized that repeated ingestions (2 weeks) of polyphenols would result in increased plasma polyphenol concentrations, as shown for the ingestion of onions for 1 week, which results in elevated plasma quercetin concentrations.²³ The urinary polyphenol level during a PSPLs intervention was not significantly higher than control group (18.59 ± 10.95 and 14.12 ± 3.52 mg GAE/dL, respectively). Although urinary content dose not reflect absolute absorptive efficiency because absorbed polyphenols may be metabolized, stored or excreted through other routes such as the biliary tract. However, the present study suggests that urinary excretion reflects the absorption and can be used as a better marker of bioavailability than dietary intake.²⁴

The recognized dietary antioxidants are vitamin C, vitamin E, selenium, and carotenoids. However, recent studies have demonstrated that polyphenols, such as flavonoids found in fruits and vegetables may act as antioxidants.³ The flavonoids generally contain one or more aromatic hydroxyl groups that have been shown to be important for the antioxidant activity.²⁵ A recent study by van Acker and colleagues in the Netherlands suggested that flavonoids can replace vitamin E as chain-breaking

Table 4. The change of plasma and urine polyphenol levels of subjects during the study ^{1,2}

	Baseline	PSPL group	Washout	Control group
Polyphenol in plasma (mg GAE/dL)	32.2 ± 1.49^a	40.2 ± 3.63^b	34.2 ± 2.08^c	31.4 ± 2.58^a
Polyphenol in urine (mg GAE/dL,24h)	16.8 ± 5.65	18.6 ± 10.95	18.1 ± 6.59	14.1 ± 3.52

¹ Each value represents the mean ± SD (n=15). ² Data with different superscripts (^{a, b, c}) significantly differ from one another by one-way ANOVA with LSD test ($p < 0.05$) within each group.

Table 5. The levels of antioxidative status in subjects ¹⁻³

	Baseline	PSPL group	Washout	Control group
Vit C (µmol/L)	10.8 ± 4.10^{ab}	11.9 ± 3.20^a	7.56 ± 4.25^c	8.19 ± 3.14^{bc}
Vit E (µmol/L)	9.54 ± 2.77^a	9.57 ± 2.55^a	8.57 ± 2.67^a	5.04 ± 1.83^b
GSH (µmol/L)	52.9 ± 12.7	58.5 ± 18.6	59.3 ± 14.9	58.7 ± 12.5
TAS (mmol/L)	0.82 ± 0.09^a	1.14 ± 0.10^b	1.11 ± 0.10^b	1.14 ± 0.12^b
LDL lag time (min)	81.9 ± 26.3^a	74.5 ± 19.5^{ab}	60.8 ± 18.6^{bc}	54.0 ± 15.8^c
MDA+4-HNE (µmol/L)	2.16 ± 1.11	2.0 ± 1.0	2.23 ± 0.80	2.14 ± 1.09
8-OHdG (ng/mg creatinine)	20.5 ± 5.7^a	20.3 ± 7.5^a	22.6 ± 6.6^a	30.1 ± 12.3^b

¹ Each value represents the mean ± SD (n=15). ² GSH : glutathione; TAS : total antioxidant status; MDA+4-HNE : malondialdehyde + 4-hydroxy 2-(E)-nonenal; 8-OHdG : 8-hydroxy-2-deoxyguanosine. ³ Data with different superscripts (^{a, b, c}) significantly differ from one another by one-way ANOVA with LSD test ($p < 0.05$) within each group.

antioxidants in liver microsomal membranes.²⁶ It is generally accepted that antioxidants co-operate and that free electrons are transferred from one antioxidant to another. Flavonoids are excellent antioxidants and therefore it is important to know their place in the antioxidant network.²⁷⁻²⁸ In our study, PSPLs consumption resulted in significant increases in plasma vitamin C and E levels. Therefore, we suggested that polyphenols in PSPLs diet had the sparing effect on vitamin C and E.

PSPLs consumption provided significant “antioxidant capacity” as measured by the changes in LDL lag time and urinary excretion of 8-OHdG. *In vitro* studies revealed that polyphenols act as antioxidants to inhibit LDL oxidation.²⁹ Other studies have reported LDL oxidation reduced in healthy volunteers after consumption of polyphenol-rich foods or beverages.³⁰⁻³³ A reduction in lipid peroxidation in humans has been reported after blackcurrant and apple juice consumption for 7 days³⁴ and red wine consumption for 2 weeks,³¹ while others found no effect of polyphenol-rich food products on plasma thiobarbituric acid-reactive substances (TBARS).³⁵⁻³⁸ In our human intervention study, lipid peroxidation (MDA+4-HNE) did not change between consumption of the polyphenol-rich PSPLs diet and the control diet. This is in agreement with findings from other comparable studies using polyphenol-rich food products.³⁵⁻⁴¹ However, the results showed the decrease in the levels of 8-OHdG (as markers of DNA oxidation) of basketball players when they consumed PSPLs diet for 2 weeks. Therefore, we proposed that the protective antioxidant effect of PSPLs may be mediated by neutralizing certain free radicals or preventing LDL and DNA oxidation due to their high content of polyphenols.

In conclusion, this study demonstrates that consumption of a polyphenol-rich PSPLs diet for 2 weeks can modulate the antioxidative status by reducing the exercise-induced oxidative stress. Although it is difficult to deduce the biological significance of some physiological change, one may speculate that these changes contribute over a long period to a reduction in the risk of developing common disease such as cancer and cardiovascular disease. The results obtained from this study may provide coaches and sports specialists with a foundation and reference information to base their designs of proper training programs for athletes.

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Effect of purple sweet potato leaf consumption on the modulation of the antioxidative status in basketball players during training

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籃球員在訓練期間紅甘薯葉的攝取對其抗氧化力調節之影響

本研究的目的是探討籃球員在訓練期間攝取紅甘薯葉(PSPLs)，對其體內抗氧化狀態之調節效應。15 位籃球校隊的男女隊員納入本研究。7 週的研究包括調整（第一週）、PSPLs 飲食（每日攝取 200g 的 PSPLs）（第 2-3 週）、排空（第 4-5 週）及控制飲食（低多酚類且調整其類胡蘿蔔素含量與 PSPLs 一致）（第 6-7 週）。採集受試者空腹血液及 24 小時尿液以進行評估分析。研究結果顯示，與控制組相比，攝取 PSPLs 二週後，受試者血漿中多酚類濃度與維生素 C、E 量皆顯著增加。低密度膽固醇(LDL)延遲時間有顯著較長；並且尿液中 DNA 氧化傷害指標--8-氫氧 2'-去氧鳥糞核糖(8-OHdG)的含量也顯著下降。然而，血漿中麩胱甘肽(GSH)、總抗氧化物力(TAS)及丙二醛+4-hydroxy-2(E)-nonenal 含量都沒有顯著改變。綜合上述，籃球員於訓練期間攝取 PSPLs 飲食 2 週後，可以降低脂質及 DNA 的氧化作用，進而調節體內的抗氧化狀態。

關鍵字：紅甘薯葉、多酚類、籃球、訓練、抗氧化狀態。