Original Article

Effect of purple sweet potato leaves consumption on the modulation of the immune response in basketball players during the training period

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The aim of this study was to evaluate the effect of the consumption of purple sweet potato leaves (PSPLs) on the immune response and the modulation of that response in 15 basketball players during a training period. They completed the 7-week study consisted of a run-in period (week 1), a PSPLs diet (200 g PSPLs/d; weeks 2, 3), a washout period (weeks 4, 5), and a control diet (low polyphenols content and carotenoid content adjusted to the same level as that of PSPLs diet; weeks 6 and 7). Blood, urine, and saliva samples were collected for biochemical analysis. The results showed that the plasma polyphenols concentration increased significantly in the PSPLs period. Compared with the control period, the PSPLs consumption produced a significant increase in the proliferation responsiveness of peripheral blood mononuclear cells (PBMC), cytotoxic activity of nature killer (NK) cells, and secretion of interferon (IFN)-γ. However, no significant increase in the secretion of salivary immunoglobulin A (sIgA), interleukin (IL)-2, or interleukin-4 was observed after PSPLs consumption. In conclusion, consumption of a PSPLs diet for 2 weeks can modulate the immune response of basketball players during a training period.

Key Words: purple sweet potato leaves, polyphenols, basketball, training, immune response

INTRODUCTION

A high consumption of vegetables and fruit 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, a class of secondary plant metabolites, which display antioxidant and free radical scavenging properties.[2] A variety of studies have shown that polyphenols, such as flavonoids, are antioxidants,[3] are immunomodulators,[4] and exhibit antigenotoxic effects.[5]

Regular physical exercise and training at moderate levels are important factors for the prevention and management of many chronic diseases and for the maintenance of optimal health. However, strenuous exercise 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 increases 100- to 200-fold, which induces oxidative stress and generates excess reactive oxygen species (ROS).[6-8] Generation of ROS and antioxidant status may be linked to alterations in the immune response after exercise, including cell adhesion, inflammation, and lymphocyte proliferation.[9,10] Epidemiologic data suggest that endurance athletes are at increased risk of upper respiratory tract infection (URTI) during periods of heavy training and for up to 2 weeks after competition.[11] Therefore, knowledge about how antioxidant nutrients are handled during exercise may help to establish what the benefits of physical exercise are and support the possible need for the dietary intake of antioxidant nutrients for the prevention of exercise-induced oxidative stress and immune changes.

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 of the commonly grown vegetables in this country, and they have free radical scavenging ability.[12] We recently showed that the bioavailability of polyphenols from PSPLs was higher when the stir-fried method was used, and this higher bioavailability could

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modulate the antioxidative status of healthy adults and basketball players.[13,14] Furthermore, based on a low-carotenoid diet, it was shown that 2 weeks of PSPLs supplementation produced a significant increase in lymphocyte proliferation and interleukin (IL)-2 and IL-4 secretion of peripheral blood mononuclear cells (PBMCs) in healthy adults.[15] However, the effects of PSPLs on exercise-induced immune responses are unknown. Therefore, the purpose of this study was to evaluate whether the consumption of PSPLs, which provided a variety of polyphenols at physiologic doses, would affect a variety of biomarkers of immune response in basketball players during a training period.

MATERIALS AND METHODS

Subjects

Fifteen basketball players (six men and nine women aged 20-24 years) were recruited for the study. The participants were nonsmokers and did not take any medications or vitamin supplements routinely during the study period. Training history, weight, height, and body mass index (BMI) were recorded (Table 1). The percentage of total body fat was assessed with the use of a body fat impedance analyser (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 hr/day for 3 days/week. The training protocols consisted of a general warm-up and stretching (approximately 20 min); technical-tactical training (approximately 30 min); heavy training, including training for counterattacks and simulated full- or half-count basketball games (approximately 60 min); and a cool-down phase (approximately 20 min). The study was approved by the Medical Ethical Committee of the Taipei Medical University, and all participants gave written informed consent.

Preparation of PSPLs

The 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 cooked. The cooking process was as follows: soy cooking oil was preheated to 200 °C, fresh PSPLs were added to the oil, and the PSPLs were stir-fried for 3–5 min with repeated stirring. After being cooked, the PSPLs were taken out and placed in cartons to be served.

PSPLs are rich in carotenoids. To calibrate the antioxidant effect of carotenoids, the amount of carotenoids consumed in the PSPLs and the control diets were similar. We previously demonstrated that the polyphenols concentrations in PSPLs and carrots were 4.15 and 1.87 mg of gallic acid equivalent (GAE)/g wet weight, respectively, and the β-carotene concentrations were 117.10 and 268.76 μg/g wet weight, respectively.[13] Control subjects ingested the same amount of β-carotene by consuming 40-45 g of carrots with each diet.

Experimental design

The diet-control study was conducted from September to November 2004. The study was divided into 2 periods, each of which lasted 2 weeks, for a total study period of about 7 weeks. The subjects were provided the PSPLs and control diets for lunch and dinner by the Department of Dietetics of Taipei Medical University Hospital. During the study period, the subjects were instructed to exclude polyphenols-rich foods from their diets. A list of the food products that the subjects were not allowed to eat was provided (e.g., onions, green vegetables, tea etc.). All subjects completed the study consisted of a run-in period (week 1), a PSPLs diet (200 g PSPLs/day; weeks 2 and 3), a washout period (weeks 4 and 5), and a control diet (low polyphenols content and carotenoid content adjusted to the same level as that of the PSPLs diet; weeks 6 and 7).

Therefore, the daily PSPL-related dietary intake was 902 mg GAE of total polyphenols and 23.42 mg of β-carotene. The control period ingested the same amount of β-carotene as did the PSPLs period, and the polyphenols intake was 149.6-168.3 mg GAE/d.

Isolation of PBMCs and preparation of blood samples

Before and at the end of the intervention period, blood samples from fasting subjects were collected in the morning between 0700 and 0900 h. Blood samples were drawn from an antecubital vein into tubes containing EDTA or lithium-heparin, which were immediately placed on ice in the dark. Plasma was collected after centrifugation at 1500 × *g* for 10 min at 4 °C was stored at −80 °C until analysed. PBMCs were isolated by density gradient centrifugation with the use of a Ficoll-Paque (Amersham Biosciences, Buckinghamshire, United Kingdom) and were resuspended in complete RPMI-1640 culture medium (Invitrogen, Carlsbad, CA) containing 5% (v/v) heat-inactivated fetal bovine serum (FBS; Invitrogen), 2 mmol L-glutamine/L, 100,000 U penicillin/L, and 100 mg streptomycin/L.

Collection and preparation of urine samples

All subjects were asked to collect 24-h urine samples. During the collection period, urine was stored at 4 °C and

Table 1. Physical characteristics of subjects[1]

	Before		After	
	Male (n=6)	Female (n=9)	Male (n=6)	Female (n=9)
Training history (years)	7.83 ± 3.25	4.11 ± 3.59	7.83 ± 3.25	4.11 ± 3.59
Height (cm)	180 ± 6.25	162 ± 4.92	180 ± 6.25	162 ± 4.92
Weight (kg)	71.8 ± 5.81	56.9 ± 5.61	72.4 ± 5.34	56.8 ± 6.33
BMI (kg/m2)	22.0 ± 1.32	21.5 ± 2.36	22.3 ± 0.93	21.5 ± 2.33
Body fat (%)	12.9 ± 2.05	25.8 ± 5.29	13.4 ± 1.85	25.5 ± 5.44

[1] Values are Mean ± SD (n=15)

kept in dark bottles. Exact urine volumes were determined, and each sample was stored at −20 °C until analysed.

Sample analysis

Measurement of blood and biochemical variables. Routine complete blood counts (CBC) were made, and biochemical variables were measured with the use of an automatic haematology analyser (HITACHI 7170; Hitachi Co., Tokyo, Japan)

Measurement of total polyphenols in plasma and urine. Total polyphenols concentrations in plasma and urine were measured by using the Folin-Ciocalteau method.[16] The absorption at 750 nm was measured spectrophotometrically. The total polyphenols content was expressed as GAEs.

Lymphocyte proliferation. PBMCs (2×10^6 cells/mL) were cultured in medium containing 5% FBS and were stimulated by the T cell mitogen concanavalin A (Con A; 10 mg/L, Sigma) or B cell mitogen lipopolysaccharide (LPS; 20 mg/L, Sigma) for 48 h at 37 °C. Proliferation was measured by using the MTT assay (CellTiter96® Non-Radioactive Cell Proliferation Assay; Promega Corporation, Madison, WI). Briefly, cells were incubated for 5 h in the presence of 20 μL MTT solution (5 mg/mL in phosphate-buffered saline; Sigma). Cells were then lysed by adding 100 μL of sodium dodecyl sulfate solution (10% in 0.01 mol HCl/L; Merck, Darmstadt, Germany). After the solution was incubated overnight, absorption was measured at 490 nm.

Cytotoxicity assay. The specific cytotoxic activities of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells were tested by using a Promega CytoTox 96 kit. This colorimetric assay quantitatively measures the release of lactate dehydrogenase (LDH), a stable cytosolic enzyme, on cell lysis.[17] Released LDH in culture supernatant fluid is measured with a 30-min coupled enzymatic assay, which results in the conversion of a tetrazolium salt into a red formazan product. Briefly, PBMCs and K562 target cells resuspended in phenol red free RPMI-1640 culture medium containing 5% FBS were incubated in 96-well round bottom plates with effector cells for 4 h at 37 °C. The ratios of effector cells to target cells used were 30:1, 20:1, 15:1, 10:1, and 7.5:1. Spontaneous release of effector or target cells was controlled by the separate incubation of the respective population. At the end of incubation, the cells were lysed and centrifuged; 100-μL aliquots of each well were transferred into another 96-well flat-bottom plate, and 100 μL of fresh "LDH substrate solution" was added to each well. The plates were incubated in the dark at room temperature for 30 min, and the reaction was stopped by adding 1.0 M acetic acid. The absorbance (490 nm) was measured in a microplate reader. The percentage of cells exhibiting cytotoxic activity was calculated.

Quantification of cytokine secretion. PBMCs were stimulated with Con A as described above for 48 h at 37 °C to measure IL-2, IL-4, and interferon (IFN)-γ secretion. Cell-free supernatant fluid samples were collected and stored at −80 °C until analyzed. IL-2, IL-4, and IFN-γ were measured with enzyme-linked immunosorbent assay (ELISA) kits (Biosource, Camarillo, CA) according to the protocol of the manufacturer.

Salivary IgA secretion. Unstimulated saliva was collected by expectoration into 15-mL plastic tubes for 4 min. All samples were centrifuged at 3,000 rpm in a microliter tube. The supernatant fluid was stored at −20 °C until analyzed. Salivary IgA (sIgA) was quantitatively measured with a salivary enzyme immunoassay (EIA) kits (Salimetrics, State College, PA) according to the protocol of the manufacturer.

Statistical analysis

All values are expressed as means ± SD. The data were analyzed by using SAS software (SAS Institute Inc., Cary, NC). Statistical significance between mean values was determined by using the paired *t*-test and one-way ANOVA with repeated measures. A p value < 0.05 was considered statistically significant.

RESULTS

All participants tolerated the intervention well and completed the study. The blood and biochemical variables of the subjects are summarized in Tables 2 and 3,

Table 2. The blood variables of subjects [1, 2]

	Before	After
WBC (×103/μL)	5.78 ± 0.87	5.91 ± 0.72
RBC (×103/μL)	4.49 ± 0.34	4.53 ± 0.34
HGB (g/dL)	13.6 ± 1.28	13.7 ± 1.28
HCT (%)	40.5 ± 3.49	41.0 ± 3.52
MCV (fL)	90.0 ± 4.36	90.5 ± 5.18
MCH (pg)	30.2 ± 1.67	30.1 ± 1.93
MCHC (g/dL)	33.5 ± 0.46	33.3 ± 0.54
PLT (×103/μL)	250 ± 32.4	272 ± 42.4
NEUT (%)	50.3 ± 8.16	50.4 ± 7.96
LYMPH (%)	38.8 ± 7.48	39.6 ± 7.06
MONO (%)	7.73 ±1.28	7.04 ± 1.78
EOS (%)	2.67 ± 1.80	2.56 ± 1.57
BASO (%)	0.48 ± 0.16	0.47 ± 0.19

[1] Values are Mean ± 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 biochemcal variables 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	102 ± 26.0
GOT (U/L)	21.7 ± 5.62	23.1 ± 5.22
GPT (U/L)	15.3 ± 4.61	14.5 ± 3.02

[1]Values 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

Table 4. The change of plasma and urine polyphenols levels of subjects

	Baseline	PSPLs period	Washout	Control period
Polyphenols 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]
Polyphenols in urine (mg GAE/dL,24h)	16.8 ± 5.65	18.6 ± 11.0	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 period.

respectively. No significant changes in body weight, BMI, percentage body fat, or any of the blood and biochemical variables were observed during the study.

The concentrations of plasma and urinary polyphenols measured throughout the study are summarized in Table 4. Plasma polyphenols concentrations increased significantly after the intervention in the PSPLs period (from 32.22 ± 1.49 to 40.17 ± 3.63 mg GAE/dL) and were significantly greater than those of the control period. However, urinary polyphenols concentrations did not change significantly in any period.

The 2-week dietary intervention clearly modulated various immune functions in our subjects. The lymphocyte proliferation responses of Con A and of LPS-activated PBMCs increased significantly in the PSPLs period (Fig. 1). As for lymphocyte proliferation, the cytotoxic activity of NK cells was affected by supplementation of a low-polyphenols diet with polyphenols-rich PSPLs. After 2 weeks, the PSPLs period had significantly greater cytotoxic NK cell activities than did the control period; the effector:target cell ratios were 15:1 and 7.5:1 (Table 5). Secretions of the cytokines IL-2, IL-4, and IFN-γ during the four experimental periods are shown in Figure 2. T-helper (Th) lymphocyte cytokine secretion was affected by the PSPLs diet. A statistically significant difference in IFN-γ secretion was observed between the PSPLs and control periods. No significant effect of PSPLs consumption on IL-2 and IL-4 secretion was observed. The pattern of change in sIgA concentration did not differ significantly between periods (Fig. 3).

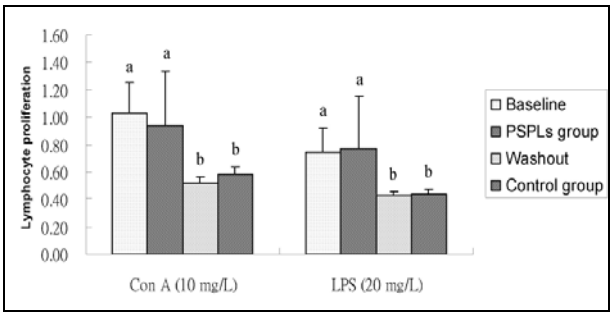

Fig 1. Proliferation response of blood lymphocytes after stimulation by Con A and LPS. Data with different superscripts ([a, b]) significantly differ from one another by one-way ANOVA with LSD test (p<0.05) within each period.

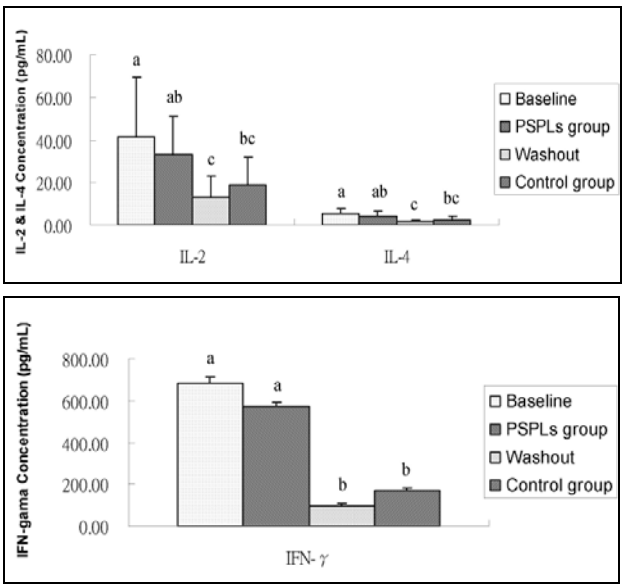

Fig 2. The secretion of cytokines (IL-2, IL-4 and IFN-γ) during the four experimental periods. Data with different superscripts ([a, b, c]) significantly differ from one another by one-way ANOVA with LSD test (p<0.05) within each period.

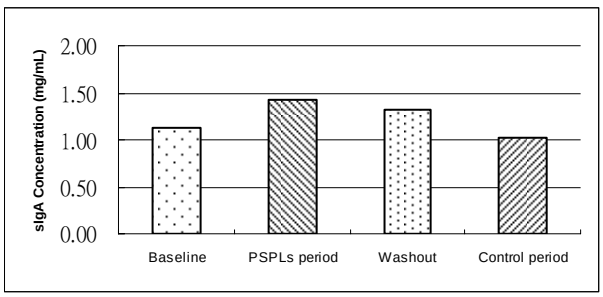

Fig 3. The concentration of sIgA in saliva during the four experimental periods

DISCUSSION

We evaluated the effects of a polyphenols-rich PSPLs diet on changes in the proliferation of lymphocytes, the cytotoxic activity of NK cells, and the secretion of IFN-γ in basketball players. Consumption of the PSPLs diet resulted in a marked increase in total polyphenols concentrations in the plasma.[14] To provide protection against oxidative stress *in vivo*, the polyphenols in PSPLs must be absorbed and retained in the body in a form that still has antioxidant properties. Studies have shown that when dietary polyphenols from food sources are absorbed from the gut, the circulating species are almost entirely conjugated[18] and that many of these conjugated metabolites have antioxidant properties *in vitro*.[19,20] We hypothesized that repeated ingestion (for 2 weeks) of polyphenols would result in increased plasma polyphenols concentrations, as was previously shown after the ingestion of onions for 1 week, which results in elevated plasma quercetin concentrations.[21] The urinary polyphenols concentration during the PSPLs intervention was not significantly greater than that during the control intervention: 18.59 ± 10.95 and 14.12 ± 3.52 mg GAE/dL, respectively. However, the urinary concentration of polyphenols does not reflect absolute absorptive efficiency because absorbed polyphenols may be metabolized, stored, or excreted through other routes, such as the biliary tract.

Table 5. Cytotoxic activity of NK cells during the four experimental periods[1,2]

	Baseline	PSPLs period	Washout	Control period
Effector/Target ratio			% cytotoxicity	
30 :1	12.4 ± 11.9	20.3 ± 43.3	14.5 ± 33.0	9.99 ± 11.0
20 :1	17.8 ± 14.4 [ab]	25.6 ± 32.7 [ab]	38.9 ± 46.1 [a]	10.6 ± 14.5 [b]
15 :1	20.5 ± 20.6 [ab]	37.6 ± 33.6 [a]	29.8 ± 41.3 [ab]	8.24 ± 9.89 [b]
10 :1	12.8 ± 11.6 [ab]	39.9 ± 40.3 [ab]	59.9 ± 99.6 [a]	8.31 ± 14.7 [b]
7.5:1	24.8 ± 46.3 [ab]	51.6 ± 40.5 [a]	36.0 ± 38.6 [ab]	10.5 ± 6.75 [b]

[1] Values are Mean ± SD (n=10); [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 period.

However, the present study suggests that urinary excretion reflects absorption and is a better marker of bioavailability than is dietary intake.[22]

Recent studies have shown that exercise-induced oxidative stress is related to alterations in immune functions.[10,23] Many polyphenols in fruit and vegetables have been shown to have strong antioxidant properties.[24-26] We indicated that the prevention of oxidative damage by PSPLs may be mediated by neutralizing certain free radicals or by preventing lipid peroxidation[27] because of their high polyphenols content, which thereby supports the immune system.

Lymphocyte proliferation, NK cell cytotoxic activity, and the production of the T-lymphocyte–specific cytokine IFN-γ during the PSPLs diet were significantly higher than during the control diet. These data agree with those of earlier studies, i.e., that supplementing a low-polyphenols diet with polyphenols-rich fruit juices or PSPLs resulted in a significantly increased lymphocyte response to mitogen-induced enhanced NK cell lytic activity and IL-2 and IL-4 secretions by activated PBMCs in healthy human subjects.[15,28,29] The cytotoxic activity of NK cells can be regulated by different cytokines,[30] therefore, the effects of PSPLs on NK cell lytic activity might be related to the effects of PSPLs on the production of cytokines (particularly IFN-γ) by Th1 and Th2 cells. Whereas IL-2 and IFN-γ are secreted by the Th1 lymphocytes, IL-4 is primarily produced by Th2 lymphocytes. Th1 cells are mainly involved in cell-mediated inflammatory-type immune responses, whereas Th2 cells promote antibody-mediated immune responses.[31] The dietary PSPLs intervention diet did not change IL-2 and IL-4 secretions significantly, which suggests that Th2 lymphocytes are not affected by this type of dietary intervention.

Secretory immunoglobulins found on mucosal surfaces play a protective role against microbial infection. Saliva is a mucosal secretion that contains IgA.[32] In recent years, researchers have attempted to identify nutritional countermeasures to exercise-induced changes in sIgA. In the present study, no significant effect on sIgA was observed between the PSPLs and control periods. This finding agrees with the findings of two other studies, which also reported no significant differences in sIgA concentrations between supplemented (carbohydrate or vitamin C) and placebo groups after heavy exertion[33,34]. However, sIgA secretion was greater in a PSPLs intervention period (1.43 ± 1.18 mg/mL) than in a control period (1.03 ± 0.79 mg/mL). The results indicated that a PSPLs supplement may beneficially maintain specific immunologic defenses at the mucosal surface.

In conclusion, the present results suggest that the consumption of a low-polyphenols diet with polyphenols-rich PSPLs for 2 weeks can modulate immune status in basketball players during a training period. Although it is difficult to deduce the biological significance of some physiologic changes, one can speculate that these changes contribute to a reduction in the risk of developing common diseases such as cancer and cardiovascular disease in the long term. The results obtained from this study may provide coaches and sports specialists with a foundation and reference information on which to base their designs of proper training programs for athletes.

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AUTHOR DISCLOSURES

Wen-Hsin Chang, Chiao-Ming Chen, Shene-Pin Hu, Nai-Wen Kan, Chun-Chieh Chiu and Jen-Fang Liu, no conflicts of interest.

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Original Article

Effect of purple sweet potato leaves consumption on the modulation of the immune response in basketball players during the training period

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籃球員在訓練期間紅甘藷葉的攝取對其免疫調節之影響

本研究的目的是探討籃球員在訓練期間攝取紅甘藷葉 (PSPLs)，對其體內免疫狀態之調節效應。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 200 g 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、清晨唾液及 24 小時尿液以進行評估分析。研究結果顯示，與控制組相比，攝食 PSPLs 二週後，受試者血漿中多酚類濃度顯著增加。此外，血中淋巴細胞增殖反應、自然殺手細胞毒殺力和細胞激素--IFN-γ 的分泌量也皆顯著增加；然而唾液中免疫球蛋白 A (sIgA) 的濃度及 IL-2 及 IL-4 的分泌量於二期間並無顯著差異。綜合上述，籃球員於訓練期間攝取多酚類含量高的 PSPLs 飲食 2 週後，具有調節體內免疫反應的效用。

關鍵字：紅甘藷葉、多酚類、籃球、訓練、免疫狀態。