J Nutr Health. 2022 Aug;55(4):450-461
https://doi.org/10.4163/jnh.2022.55.4.450
eISSN 2288-3959

JNH
Journal of Nutrition and Health

Research Article

Check for updates

A plant-based multivitamin, multimineral, and phytonutrient supplementation enhances the DNA repair response to metabolic challenges

Eunji Yeo [1,*], **Jina Hong** [2,*], **Seunghee Kang** [3], **Wonyoung Lee** [1], **Oran Kwon** [3,4], **and Eunmi Park** [1]

[1]Department of Food and Nutrition, Hannam University, Daejeon 34054, Korea
[2]Access Business Group International, LLC, Buena Park, CA 90621, USA
[3]Department of Nutritional Science and Food Management, Ewha Womans University, Seoul 03760, Korea
[4]Department of Nutritional Science and Food Management, Graduate Program in System Health Science and Engineering, Ewha Womans University, Seoul 03760, Korea

OPEN ACCESS

Received: May 31, 2022
Revised: Jul 28, 2022
Accepted: Aug 8, 2022
Published online: Aug 24, 2022

Correspondence to
Oran Kwon
Department of Nutritional Science and Food Management, Ewha Womans University, 52 Ewhayeodae-gil, Seodaemun-gu, Seoul 03760, Korea.
Tel: +82-2-3277-6860
Email: orank@ewha.ac.kr

Eunmi Park
Department of Food and Nutrition, School of Life Science and Nano-Technology, Hannam University, 1646 Yuseong-daero, Yuseong-gu, Daejeon 34054, Korea.
Tel: +82-42-672-8793
Email: eunmi_park@hnu.kr

*These authors contributed equally to this work.

ABSTRACT

Purpose: DNA damage and repair responses are induced by metabolic diseases and environmental stress. The balance of DNA repair response and the antioxidant system play a role in modulating the entire body's health. This study uses a high-fat and high-calorie (HFC) drink to examine the new roles of a plant-based multivitamin/mineral supplement with phytonutrients (PMP) for regulating the antioxidant system and cellular DNA repair signaling in the body resulting from metabolic stress.
Methods: In a double-blind, randomized, parallel-arm, and placebo-controlled trial, healthy adults received a capsule containing either a PMP supplement (n = 12) or a placebo control (n = 12) for 8 weeks. Fasting blood samples were collected at 0, 1, and 3 hours after consuming a HFC drink (900 kcal). The blood samples were analyzed for the following oxidative stress makers: areas under the curve reactive oxygen species (ROS) levels, plasma malondialdehyde (MDA), erythrocytes MDA, urinary MDA, oxidized low-density lipoprotein, and the glutathione:oxidized glutathione ratio at the time points. We further examined the related protein levels of DNA repair signaling (pCHK1 (Serine 345), p-P53 (Serine 15), and γH2AX expression) in the plasma of subjects to evaluate the time-dependent effects of a HFC drink.
Results: In a previous study, we showed that PMP supplementation for eight weeks reduces the ROS and endogenous DNA damage in human blood plasma. Results of the current study further show that PMP supplementation is significantly correlated with antioxidant defense. Compared to the placebo samples, the blood plasma obtained after PMP supplementation showed enhanced DNA damage response genes such as pCHK1(Serine 345) (a transducer of DNA response) and γH2AX (a hallmark of DNA damage) during the 8 weeks trial on metabolic challenges.
Conclusion: Our results indicate that PMP supplementation for 8 weeks enhances the anti-oxidant system against oxidative stress and prevents DNA damage signaling in humans.

Keywords: vitamins; phytochemicals; antioxidants; DNA repair; blood

ORCID iDs
Eunji Yeo
https://orcid.org/0000-0002-4295-9223
Jina Hong
https://orcid.org/0000-0002-8867-3431
Seunghee Kang
https://orcid.org/0000-0001-8408-2637
Wonyoung Lee
https://orcid.org/0000-0002-9433-7362
Oran Kwon
https://orcid.org/0000-0002-2031-7238
Eunmi Park
https://orcid.org/0000-0002-1911-4652

Funding
This study was supported by Amway Korea, Ltd. (Seoul, Korea).

Conflict of Interest
There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Our body is consistently exposure to oxidative stress caused by environmental stresses such as metabolic stress, air pollutants, ultraviolet-B radiation, pathogen infection [1,2]. Furthermore, excessive reactive oxygen species (ROS) production and oxidative stress are associated with DNA damage and repair response, cell-cycle arrest, and related cell signaling pathways with chronic disease [3]. The DNA repair response system repairs DNA damage by ROS, maintains genomic stability, and DNA integrity in cellular levels [4]. Though multivitamins/minerals with phytonutrients intake as a source of antioxidants is believed to be involved in DNA repair, little information is available on how multivitamin/mineral with phytonutrients regulate DNA damage under oxidative stress in human blood.

Previously we reported multivitamin/mineral with phytonutrients reduced ROS in plasma, improving ROS scavenging activity, and decreased for DNA tail length in peripheral blood mononuclear cells (PBMC) for 8 weeks [5]. In qPCR array of RNA analysis on PBMCs, superoxide dismutase 2 (SOD2), NAD(P)H oxidase (NOX), NOX activator 1 (NOXA1) gene expression related to ROS such as superoxide metabolic pathway were upregulated in plant-based multivitamin/mineral with phytonutrients (PMP) supplementation group. Although the effects of multivitamin/mineral with phytonutrients on DNA damage and repair signaling have been examined, relatively little is known of these relationships in a time dependent of metabolic challenges on the background of whole body. The present study was undertaken to investigate the role of multivitamin/mineral with phytonutrients intake in DNA repair signaling under metabolic challenge from human in a high-fat and high-calorie (HFC) drink induced model [6].

METHODS

Subjects and study design

The subject criteria, experimental protocol, and execution of the intervention study have previously been described in detail [5]. Briefly, healthy adults (39.9 ± 1.2 years of age) with habitually low fruit and vegetable intake who had Recommended Food Score (RFS) of 19.3 ± 1.0 and body fat percentage of 30.9 ± 0.7% were assigned to take 6 tablets, twice/day of PMP (n = 48) or placebo (n = 48) for 8 weeks in a randomized, double-blind and parallel-arm trial. On the evening prior to each examination day (baseline and week 8), subjects ate an ordinary meal (approximately 640 kcal; carbohydrate:protein:lipid = 65:23:12) consisted of steamed rice (340 kcal in 170 g) and Bulgogi (300 kcal in 170 g; roasted beef marinated with soybean sauce) and fasted overnight for at least 12 hours. On after collection of fasting blood samples (0 hour), subjects (PMP = 12, placebo = 12) consumed a HFC drink with their assigned supplement (a unit dose) within 10 min in secondary analysis (**Figs. 1** and **2**). The high fat challenge test formula composed of a HFC drink: 60 g palm oil (Lotte Foods Co., Ltd., Seoul, Korea), 83.5 g dextrose (Daesang Corporation, Seoul, Korea) and 20 g protein powder (Protifar; Nutricia, Hoofddorp, The Netherlands) filled up with water to 500 mL [6]. The total energy content of this challenge was about 900 kcal. Blood samples were collected at regular time points (0 hour, 1 hour, 3 hours) up to 6 hours. No other foods or beverages were allowed during the day, except water. The plasma samples were separated by centrifuge at 4°C (1,500 × g for 10 minutes) from whole blood. All the samples were stored at −80°C until analyzed. The study protocol was approved by the Institutional Review Boards of Ewha Womans University (IRB No. 119-16) and registered in the International Clinical Trials Registry Platform of the WHO (KCT0002055).

https://doi.org/10.4163/jnh.2022.55.4.450

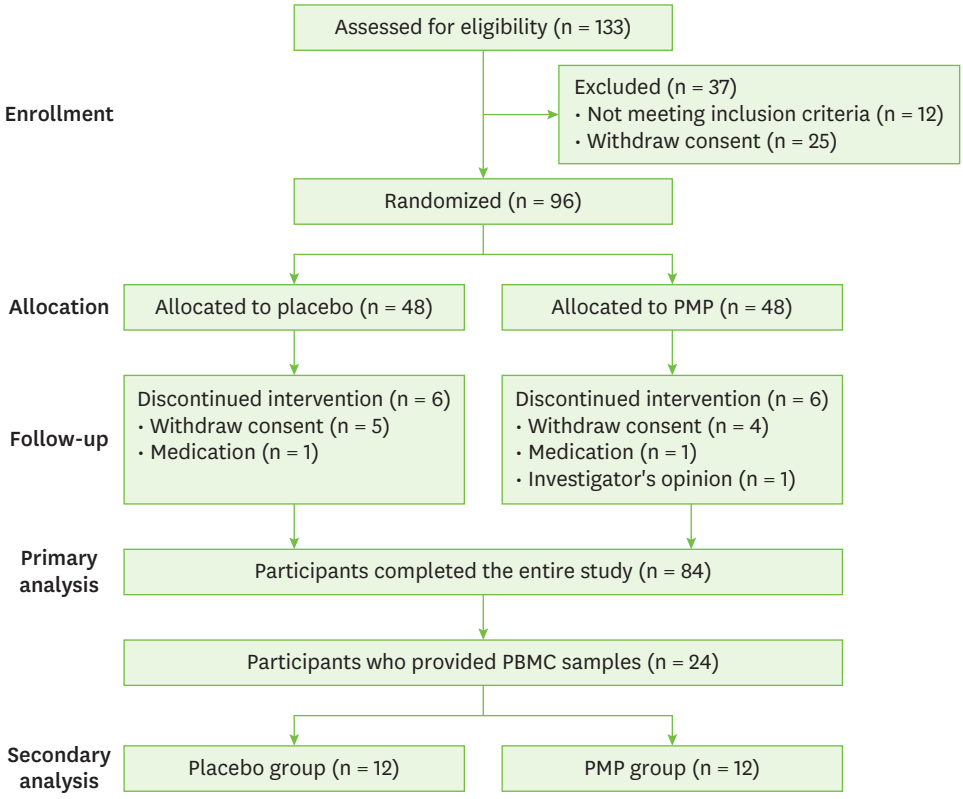

Fig. 1. CONSORT flow diagram of the secondary analysis study.
Diagram presents from the subject enrolment through to data analysis.
PBMC, peripheral blood mononuclear cells.

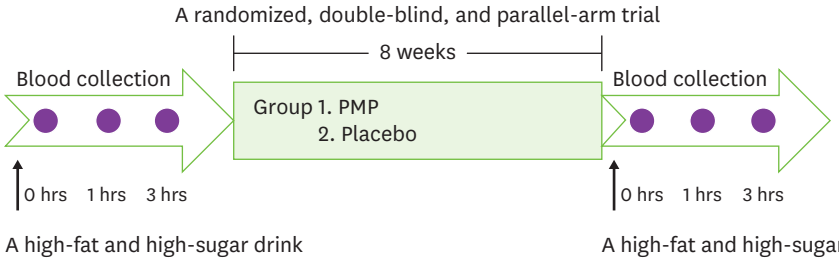

Fig. 2. The diagram of study design.
96 subjects of recruited 133 subjects were enrolled in the randomized, double-blind, parallel-arm and placebo-control trial, excluding for the eligible criteria [5] of 12 subjects and withdrawing the consent of 25 subjects. In pre-study, PMP group (n = 48) and placebo group (n = 48) collected the fasting blood at 0, 1, and 3 hours in a HFC drink challenge. The metabolic challenge test formula composed of a HFC drink was 60 g palm oil, 83.5 g dextrose and 20 g protein powder filled up with water to 500 mL in total energy of 900 kcal [6]. After 8 weeks of supplementation of multivitamin and mineral with phytonutrients, PMP group (n = 12) and placebo group (n = 12) collected the fasting blood at the same time point in the metabolic challenges.

Oxidative stress-related biochemical analysis

Analysis details of biochemical parameters were reported in our previous publication [5]. Briefly, luminol amplified chemiluminescence (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma-Aldrich, St. Louis, MO, USA) was used for plasma ROS level. The incubation temperature was at 37°C in a chemiluminescence Fluoroskan Ascent FL system

https://doi.org/10.4163/jnh.2022.55.4.450

(Thermo-Labsystems, Helsinki, Finland). High-performance liquid chromatography-fluorescence detection system (HPLC-FLD; Shiseido Co, Ltd., Tokyo, Japan) was used for plasma, erythrocytes and urine of malondialdehyde (MDA) levels. A standard curve using 1,1,3,3-Tetraethoxypropane (Sigma-Aldrich) was used for the MDA levels. A sandwich enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden) was used for plasma oxidized low-density lipoprotein (Ox-LDL). The level of reduced glutathione (GSH) using commercial kit (Cayman; Ann Arbor, MI, USA) was measured in spectrometer. Also, the level of oxidized glutathione (GSSG), and antioxidant enzyme activities (SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase) were deliberated spectrophotometrically according to the manufacturer's instructions using commercially available kits (Cayman).

Western blot in blood plasma

As described in previous published journal [5,7], plasma samples were collected in 8 weeks of PMP supplementation on a time dependent manner of metabolic challenges. We have tested these plasma samples from Amway.

Protein samples from plasma were prepared in buffer (1% Triton X-100, 0.5% Na-deoxycholate, 0.1% SDS 50 mmol/L Tris, pH 7.3, 150 mmol/L NaCl, and 1 mmol/L EDTA) with protease inhibitor, $NaVO_4$, and NaF. Two hundred micrograms for pCHK1 (Serine 345) protein expression and 300 μg for phospho-p53 (Serine 15) and γH2AX protein expression in prepared protein samples from plasma were resolved in 10% SDS-PAGE and then transferred to PVDF membranes.

Equal loading and transfer of proteins was verified by Ponceau red staining of the membranes and by analyzing specific protein expression. Quantitative data was measured by chemiluminesence detection (Pierce ECL Western Blot Substrate, Thermo, Chicago, IL, USA) and analyzed by Fusion image system of Evolution Capt Solo 6 17.03 (Vilber, Collégien, France). Western blotting was performed using following specific antibodies; anti-pCHK1 (Ser345, 1:500 dilution; Cell Signaling, Danvers, MA, USA), anti-p-p53 (Serine 15, 1:500 dilution; Cell Singling), anit-γH2AX (1:500 dilution; Cell Signaling), and anti-β-actin (1:5,000 dilution; Cell Signaling). Samples in each group were calculated in the change fold by the target gene/β-actin (as a loading control) percentage on quality control process.

Statistical analysis

Twelve subjects who provided samples from each group were randomly selected and used for analysis. Potential outliers were identified as observations exceeding 1.5 times the interquartile range (IQR): Q1 − (1.5 × IQR) and Q3 + (1.5 × IQR), according to the Tukey's method [8]. All analyses were assessed without potential outliers. Baseline characteristics were assessed using Student's t-test for continuous variables and Fisher's exact test for categorical variables. Differences in the means of outcomes after a high fat challenge test were analyzed using repeated measure ANOVA within each group. After adjusting for fasting levels, the incremental area under the curve (iAUC) of biochemical parameter values was calculated by the trapezoidal rule. We applied a linear mixed-effect model with a random effect (subjects), random error (within-subjects), and fixed effects (group, period, and interactions of group and period) for comparisons of iAUCs between the 2 groups. All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA) and p-value < 0.05 was considered significant.

https://doi.org/10.4163/jnh.2022.55.4.450

RESULTS

Baseline characteristics

The Consolidated Standards of Reporting Trials (CONSORT) flow diagram in **Fig. 1** shows the flow diagram from the subject enrollment to the secondary data analysis. General characteristics were described in previous study [5]. We screened in eligible 96 subjects with low fruit and vegetable intake habit and body fat of twenty percentages below. 25–69 years of old of healthy male and female adults participated in 8 weeks of supplementation. 12 subjects withdrew due to withdrawal of consent, taking medications, or the investigator's judgment, and 84 subjects completed the 8-week intervention study.

Twenty-four subjects among total 84 subjects were selected for secondary analysis on subjects with PBMC samples. The baseline characteristics are not different between placebo and PMP groups (**Table 1**). The average age was 36.0 years in placebo and 39.8 years old in PMP; the percent of body fat was 30.2% in placebo and 30.0% in PMP; and the RFS score was 18.5 in placebo and 23.4 in PMP, respectively, which met the selection and exclusion criteria of study. Also, there was no difference of the RFS scores between 2 groups ($p = 0.177$) and the scores indicate low consumption of fruits and vegetables.

Change in biochemical parameters related to oxidative stress after a HFC drink

The oxidative stress indexes were analyzed before and after a HFC drink within a group (**Table 2**). We observed the value of ΔROS AUC was not significantly different in placebo group, whereas the PMP group decreased the value of ΔROS AUC after 1 hour and 3 hours ($\beta = -644.4$ at 1 hour in PMP, $p = 0.030$; $\beta = -604$ at 3 hours in PMP, $p = 0.042$; the value of β estimation was calculated from between at baseline and endpoint). Plasma MDA was also decreased in the PMP group at 3 hours in 8 weeks compared to baseline ($\beta = -1.03$ at 3 hours in PMP, $p = 0.037$).

Table 1. Baseline characteristics

Variables	Placebo group (n = 12)	PMP group (n = 12)	p-value[1)]
Age (yrs)	36.0 ± 2.9	39.8 ± 2.9	0.370
Sex (male/female)	2/10	4/8	0.640
Body weight (kg)	61.6 ± 3	64.7 ± 4.8	0.599
BMI (kg/m^2)	22.8 ± 0.7	24.4 ± 1.3	0.303
Percent of body fat (%)	30.2 ± 1.7	30 ± 2.0	0.940
SBP (mmHg)	111.3 ± 3.4	119.3 ± 4.7	0.183
DBP (mmHg)	74.4 ± 2.9	83.3 ± 3.4	0.060
Smoker	1 (8.3)	1 (8.3)	1.000
Alcohol drinker	7 (58.3)	8 (66.7)	0.478
Alcohol amount (g/wks)	19.1 ± 9.9	35.0 ± 13.2	0.346
Physical activity (MET-min/wks)	1,775.6 ± 466.3	1,072.1 ± 249.3	0.201
Sleeping time (hrs/wks)	50.1 ± 1.9	48.8 ± 1.6	0.599
RFS (0–47)	18.5 ± 2.6	23.4 ± 2.4	0.177
Dietary intake			
Energy (kcal/day)	1,830.9 ± 164.7	1,558.8 ± 142.1	0.224
Carbohydrate (g/day)	282.3 ± 29.1	232.8 ± 18.4	0.164
Protein (g/day)	64.6 ± 5.2	62.4 ± 7.5	0.814
Fat (g/day)	48.1 ± 6.1	41.4 ± 6.0	0.442
Sodium (mg/day)	3,819.8 ± 363.3	3,489.4 ± 445.9	0.571

Values are presented as the means ± SE or number (%).
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalent task; RFS, recommended food score.
[1)]Student's t-test for continuous variables and Fisher's exact test for categorical variables were used to compare differences between the groups.

https://doi.org/10.4163/jnh.2022.55.4.450

Among biochemical parameters of endogenous non-enzymatic antioxidants, GSH and GSSG were increased in PMP group (β = 40.8 at 3 hours of GSH, p = 0.010; β = –18.2 at 3 hours of GSSG, p = 0.008). Therefore, the ratio of GSH/GSSG also was increased at 3 hours in the PMP group in 8 weeks (β = 0.78 at 3 hours of ΔGSH:GSSG ratio in PMP, p = 0.021). The other hand, the value of ΔGSH:GSSG ratio was decreased in the placebo group (β = −0.99 at 3 hours of ΔGSH:GSSG ratio in placebo, p = 0.022). GSH is used to measure the oxidative stress induced by the metabolic challenge test [5,9], it can be seen that PMP product

Table 2. Change in biochemical parameters related to oxidative stress after metabolic challenge test

Variables	Placebo group				PMP group			
	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]
ΔROS AUC								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−68.0 ± 59.2	94.2 ± 68.7	162.2	0.095	571.3 ± 240.5	−73.1 ± 46.3	−644.4	0.030
3 hrs	−41.0 ± 52.3	1.7 ± 25.3	42.7	0.664	570.7 ± 219.9	−30.7 ± 43.6	−601.4	0.042
6 hrs	101.8 ± 45.9	−30.3 ± 67.8	−132.1	0.168	−161.0 ± 228.3	91.9 ± 23.3	252.9	0.377
p-value[2)]	0.129	0.656			0.427	0.649		
ΔPlasma MDA (μmol/L)								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	−0.27 ± 0.27	0.17 ± 0.15	0.44	0.262	0.43 ± 0.31	−0.15 ± 0.24	−0.58	0.233
3 hrs	−0.13 ± 0.24	−0.19 ± 0.10	−0.07	0.868	0.80 ± 0.26	−0.24 ± 0.27	−1.03	0.037
6 hrs	0.38 ± 0.29	0.02 ± 0.21	−0.36	0.356	0.05 ± 0.28	0.16 ± 0.26	0.11	0.822
p-value	0.163	0.937			0.888	0.625		
ΔErythrocyte MDA (μmol/L)								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	−0.73 ± 0.48	−0.13 ± 0.57	0.60	0.460	−0.94 ± 0.44	−0.60 ± 0.25	0.34	0.625
3 hrs	−0.43 ± 0.51	0.28 ± 0.44	0.70	0.389	−0.53 ± 0.52	−0.33 ± 0.47	0.21	0.768
6 hrs	0.64 ± 0.51	0.06 ± 0.38	−0.57	0.477	−0.61 ± 0.28	0.23 ± 0.35	0.84	0.228
p-value	0.266	0.912			0.209	0.645		
ΔUrinary MDA (μmol/g Creatinine)								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	−0.38 ± 0.21	−0.03 ± 0.22	0.35	0.427	0.10 ± 0.07	0.07 ± 0.10	−0.03	0.911
3 hrs	−0.06 ± 0.20	−0.24 ± 0.17	−0.19	0.672	0.26 ± 0.19	0.28 ± 0.14	0.03	0.899
6 hrs	0.69 ± 0.33	−0.31 ± 0.34	−1.00	0.024	0.14 ± 0.11	0.25 ± 0.20	0.11	0.644
p-value	0.029	0.309			0.425	0.149		
ΔOxLDL (U/L)								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	−0.20 ± 2.20	1.51 ± 2.00	1.70	0.639	−2.02 ± 3.90	−2.51 ± 2.53	−0.49	0.922
3 hrs	−5.70 ± 2.26	0.25 ± 2.81	5.94	0.105	−5.75 ± 2.98	−3.36 ± 1.92	2.39	0.631
6 hrs	−1.18 ± 2.05	−0.11 ± 1.09	1.07	0.777	−2.68 ± 3.09	1.86 ± 2.33	4.55	0.369
p-value	0.649	0.966			0.459	0.597		
ΔGSH (μM)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	23.8 ± 5.4	1.4 ± 7.0	−22.4	0.106	−1.5 ± 6.9	38.2 ± 11.2	39.7	0.012
3 hrs	17.0 ± 11.7	2.9 ± 9.4	−14.1	0.307	−6.0 ± 7.2	34.8 ± 10.6	40.8	0.010
6 hrs	2.0 ± 7.6	4.9 ± 6.1	3.0	0.826	10.8 ± 8.5	−8.8 ± 8.2	−19.7	0.202
p-value	0.844	0.594			0.320	0.416		
ΔGSSG (μM)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−10.4 ± 4.9	0.6 ± 3.3	11.0	0.095	2.7 ± 4.2	−5.5 ± 4.9	−8.2	0.225
3 hrs	−8.9 ± 4.0	3.2 ± 4.4	12.2	0.065	13.3 ± 3.5	−4.9 ± 5.1	−18.2	0.008
6 hrs	2.3 ± 2.9	0.3 ± 2.9	−2.0	0.764	0.2 ± 2.2	2.1 ± 2.4	1.9	0.781
p-value	0.621	0.945			0.958	0.656		
ΔGSH:GSSG ratio								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	0.99 ± 0.32	−0.25 ± 0.21	−1.24	0.004	0.00 ± 0.24	0.71 ± 0.19	0.71	0.035
3 hrs	0.61 ± 0.18	−0.38 ± 0.28	−0.99	0.022	−0.32 ± 0.17	0.47 ± 0.10	0.78	0.021
6 hrs	−0.16 ± 0.27	0.13 ± 0.18	0.29	0.494	0.08 ± 0.17	−0.30 ± 0.26	−0.38	0.259
p-value	0.599	0.661			0.752	0.197		

(continued to the next page)

Table 2. (Continued) Change in biochemical parameters related to oxidative stress after metabolic challenge test

Variables	Placebo group				PMP group			
	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]
ΔSOD activity (U/mL)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−0.7 ± 9.6	−5.7 ± 4.3	−5.5	0.627	−5.8 ± 6.7	0.6 ± 7.0	6.2	0.531
3 hrs	−4.2 ± 10.5	−3.8 ± 1.8	0.4	0.971	2.6 ± 6.4	−8.0 ± 5.7	−10.4	0.295
6 hrs	−10.6 ± 5.8	−1.2 ± 4.9	8.8	0.434	2.5 ± 4.4	−12.4 ± 7.5	−13.7	0.179
p-value	0.190	0.834			0.882	0.078		
ΔGPx activity (μmol/min/mL)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−2.1 ± 41.6	−88.8 ± 32.1	−86.7	0.231	13.9 ± 34.4	48.6 ± 33.8	34.7	0.570
3 hrs	65.2 ± 61.2	−75.2 ± 27.7	−140.4	0.054	24.3 ± 42.9	13.2 ± 23.4	−11.0	0.857
6 hrs	31.5 ± 39.2	11.2 ± 40.6	−20.3	0.781	9.7 ± 25.6	−29.1 ± 41.7	−38.8	0.521
p-value	0.536	0.829			0.818	0.501		
ΔCAT activity (μmol/min/mL)								
0 hrs	0.00 ± 0.00	0.00 ± 0.00			0.00 ± 0.00	0.00 ± 0.00		
1 hrs	−1.01 ± 1.85	−1.33 ± 2.67	−0.32	0.932	−4.45 ± 2.15	0.39 ± 1.95	4.85	0.122
3 hrs	−5.42 ± 2.29	−1.44 ± 1.67	3.98	0.285	0.78 ± 1.27	−1.62 ± 2.88	−2.47	0.432
6 hrs	−7.30 ± 2.97	0.90 ± 1.86	8.20	0.030	2.09 ± 1.38	0.19 ± 2.36	−1.90	0.541
p-value	0.007	0.731			0.343	0.931		

Values are presented as the means ± SE.
ROS, reactive oxygen species; AUC, area under the curve; MDA, malondialdehyde; Ox-LDL, oxidized-low-density lipoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.
[1)]The β estimates and p-values between at baseline and endpoint were obtained from repeated measure ANOVA.
[2)]The β estimates and p-values between at fasting and after 6 hours were obtained from repeated measure ANOVA.

contributed to antioxidant and reduced ROS after a HFC drink in the PMP supplement group. The data shows that the PMP product maintain endogenous antioxidants system.

However, several endogenous enzymatic antioxidants (SOD and GPx activity) did not change metabolic response to both PMP and placebo groups. CAT activity was different at 6 hours in between 0 and 8 weeks in the placebo group (p = 0.007) but there was no change in the PMP group. This data suggests that the endogenous antioxidant system was not degraded by PMP supplement, which acts as an exogenous antioxidant, as discussed in the previous study [5].

Summary value of oxidative stress-related biochemical parameters

Next, the response pattern of oxidative stress damage markers by the metabolic challenge test was statistically analyzed using a linear mixed effect model, by summarizing hourly data with iAUC (**Fig. 3**). As shown in **Fig. 3A**, the iAUC of ROS and plasma MDA were not different before and after of 8 weeks intervention in the placebo group, but significantly decreased in the PMP group (iAUC of ROS in PMP, p = 0.018; iAUC of plasma MDA in PMP, p = 0.0004). In addition, iAUC values of erythrocyte and urinary MDA were not significantly different between the 2 groups (**Fig. 3A**).

In the case of Ox-LDL, the iAUC value increased significantly in 8 weeks in the placebo group (p = 0.001), but there was no difference between 0 and 8 weeks in the PMP group.

In the clinical biomarkers related to the endogenous antioxidant system, the iAUC values of GSH, GSSG, and GSH:GSSG ratio of the PMP group compared to the placebo group were significantly improved in the group x week interaction (β = −218 of GSH, p = 0.002; β = −83.0 of GSSH, p = 0.0001; β = 5.85 of GSH:GSSG ratio, p = 0.0003) (**Fig. 3B**).

https://doi.org/10.4163/jnh.2022.55.4.450

Higher pCHK1 (Serine 345) protein expression of a HFC drink in PMP supplementation

Finally, we determined whether PMP supplementation could induce cellular DNA repair response by detecting phosphorylated checkpoint kinase 1 (pCHK1-Ser345). The single-stranded DNA breaks, in accordance with oxidative stress, activated the ATR-CHK1 pathway in physiology status [10]. Specially, C terminus of CHK1 (Serine 345) was targeted by ATR and phosphorylated for CHK1 signaling activation in human [10]. Therefore, we examined pCHK1 (Serine 345) protein expression on metabolic challenges as the cellular DNA repair signaling indicator. Interestingly, PMP group of 8 weeks intervention increased pCHK1 (Serine 345) protein levels on a time dependent manner of the HFC drink ($p = 0.007$, **Table 3** and **Supplementary Fig. 1**). The data suggests that PMP supplementation could induce DNA repair signaling effectively in blood, following metabolic challenges.

P-p53 (Serine 15) gene is one of downstream of CHK1 signal pathway. Next, we measured P-p53 (Serine 15) protein level of all samples including PMP supplement group and placebo for 8 weeks. PMP supplement group after 8 weeks tended to induce p-p53 (Serine 15) protein

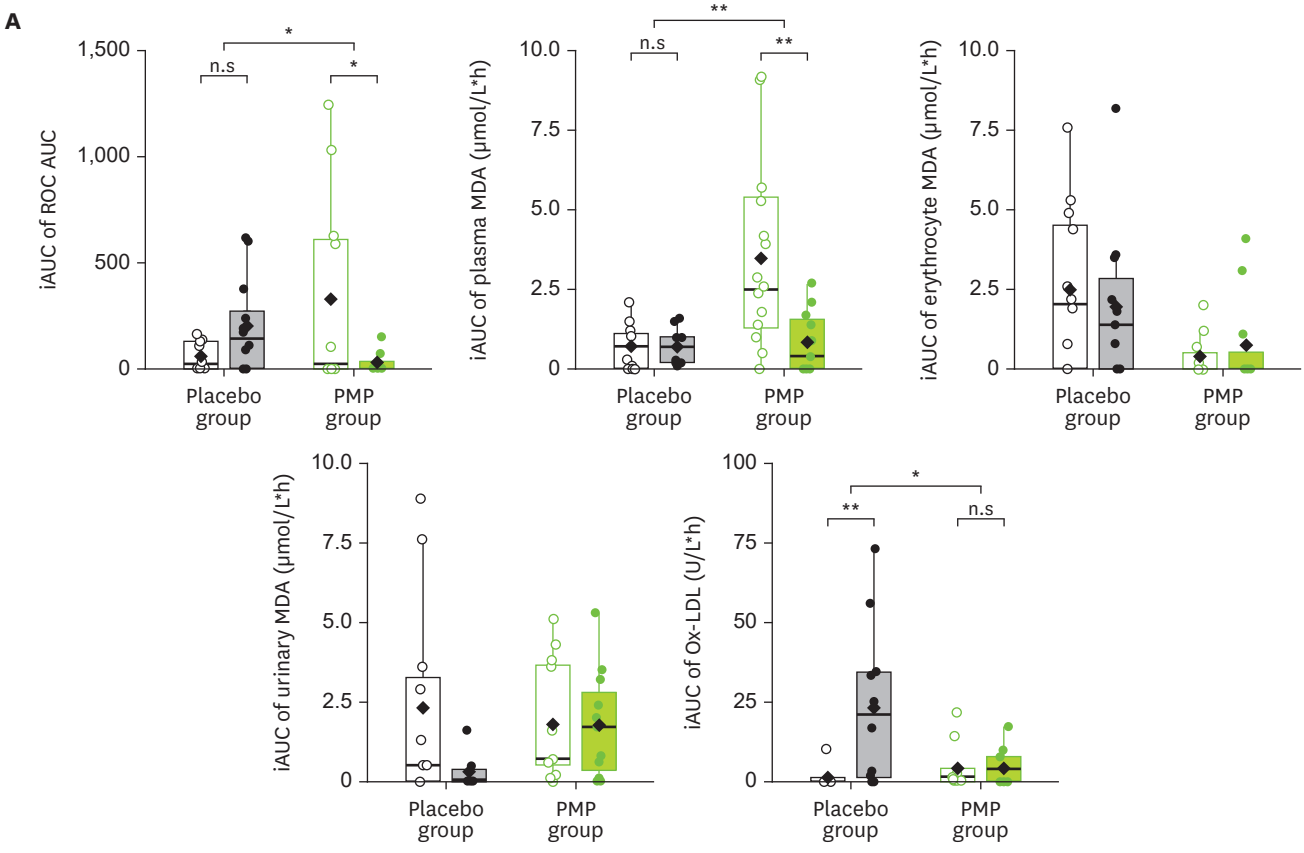

Fig. 3. Box and scatter plot of incremental AUC values of biochemical parameters after metabolic challenge test.
(A) iAUC in ROS, plasma MDA, erythrocyte MDA, urinary MDA and Ox-LDL variables. (B) iAUC of GSH, GSSG, GSH:GSSG ratio, SOD activity, GPx activity and CAT activity values. (A, B) Empty and filled dots with black are individual values of each subject in the placebo group at baseline and endpoint, respectively. Empty and filled dots with green are individual values of each subject in the treatment group at baseline and endpoint, respectively. Black diamonds indicate mean values. Linear mixed-effect model was used to compare the intra-and inter-group.
iAUC, incremental AUC; ROS, reactive oxygen species; AUC, area under the curve; MDA, malondialdehyde; Ox-LDL, oxidized-low-density lipoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.
$^{*}p < 0.05$, $^{**}p < 0.01$.

(continued to the next page)

https://doi.org/10.4163/jnh.2022.55.4.450

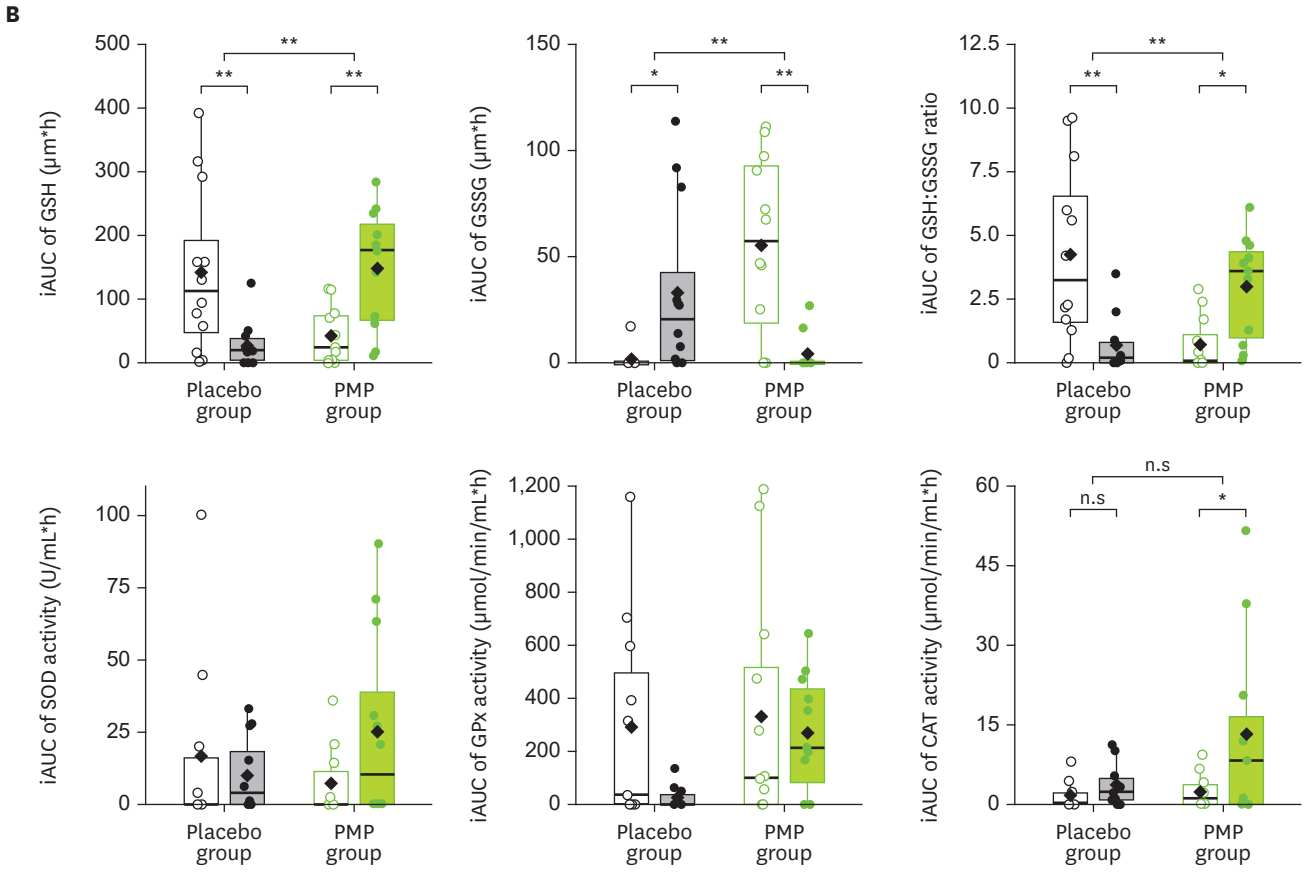

Fig. 3. (Continued) **Box and scatter plot of incremental AUC values of biochemical parameters after metabolic challenge test.**
(A) iAUC in ROS, plasma MDA, erythrocyte MDA, Urinary MDA and Ox-LDL variables. (B) iAUC in GSH, GSSG, GSH:GSSG ratio, SOD activity, GPx activity and CAT activity values. (A, B) Empty and filled dots with black are individual values of each subject in the placebo group at baseline and endpoint, respectively. Empty and filled dots with green are individual values of each subject in the treatment group at baseline and endpoint, respectively. Black diamonds indicate mean values. Linear mixed-effect model was used to compare the intra-and inter-group.
iAUC, incremental AUC; ROS, reactive oxygen species; AUC, area under the curve; MDA, malondialdehyde; Ox-LDL, oxidized-low-density lipoprotein; GSH, reduced glutathione; GSSG, oxidized glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; CAT, catalase.
$^{*}p < 0.05$, $^{**}p < 0.01$.

Table 3. Change in protein levels related to cellular DNA repair signaling after metabolic challenge test

Variables	Placebo group				PMP group			
	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]	Baseline (n = 12)	Endpoint (n = 12)	β[1)]	p-value[1)]
ΔpCHK1 (%)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−26.0 ± 7.3	2.9 ± 11.0	28.9	0.087	12.7 ± 5.9	10.6 ± 8.0	−2.1	0.924
3 hrs	−21.8 ± 11.5	−6.5 ± 12.5	15.3	0.359	11.5 ± 14.0	43.5 ± 21.9	31.9	0.143
p-value[3)]	0.068	0.578			0.450	0.007		
Δp-p53 (%)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	−37.1 ± 26.2	−41.8 ± 78.4	−4.8	0.959	−39.0 ± 19.0	−23.6 ± 23.9	15.5	0.649
3 hrs	−29.3 ± 16.3	−16.1 ± 86.6	13.2	0.888	−5.9 ± 18.8	−14.3 ± 21.1	−8.4	0.805
p-value	0.658	0.808			0.809	0.541		
ΔgH2AX (%)								
0 hrs	0.0 ± 0.0	0.0 ± 0.0			0.0 ± 0.0	0.0 ± 0.0		
1 hrs	14.0 ± 11.5	−11.1 ± 16.6	−25.1	0.311	−18.1 ± 9.7	−22.0 ± 14.7	−3.9	0.855
3 hrs	7.3 ± 9.2	27.6 ± 21.8	20.2	0.412	−5.9 ± 13.0	−19.5 ± 13.5	−13.6	0.522
p-value	0.973	0.783			0.173	0.027		

Values are presented as the means ± SE.
[1)]The β estimates and p-values between at baseline and endpoint were obtained from repeated measure ANOVA.
[2)]p-values between at fasting and after 3 hours were obtained from repeated measure ANOVA.

https://doi.org/10.4163/jnh.2022.55.4.450

level at 1 hour compared to placebo group (**Table 3**). However, p-p53 (Serine 15) protein level was increased at 1hour in PMP group compared to placebo but it was not significant (**Table 3**).

Moreover, γH2AX, as a hall biomarker of DNA damage product, protein expression was investigated in plasma samples including with 2 groups. As expected, γH2AX protein level was lower in PMP supplement group after 8 weeks intervention, at 3 hours after consuming a high fat and high calorie drink ($p = 0.027$ of PMP in endpoint, **Table 3** and **Supplementary Fig. 1**).

DISCUSSION

The metabolic challenge experiment, as a HFC drink, was provided to subjects to temporarily disturb the body's homeostasis [9,11]. When the body challenged by dietary stressors, the system is trying to restore homeostasis, usually within hours. Changes in metabolism might increase oxidative stress and induce a DNA damage in the body. This PMP supplement contains designed to work with the body's natural ability to defend against elevated, sustained oxidative stress [5]. For example, botanical extracts from rosemary, turmeric and quercetin have been shown to influence antioxidant mechanism such as ARE assay [12,13]. As previous study, we examined antioxidant activity after PMP supplementation [5]. In this study, we further investigated PMP supplementation might provide protection against metabolic changes *in vivo*, the cellular responses to antioxidant system against endogenous ROS-induced oxidative damage. Indeed, we detected the reduced oxidative stress makers of AUC ROS, plasma MDA and Ox-LDL and enhanced the GSH:GSSG ratio with PMP supplement in a HFC drink.

Chronic DNA damage response is a characteristic of human metabolic disease [1]. In the presence of body metabolic stress, human blood plasma contains ROS, and thus, damage DNA lesions [1,14,15]. It suggests crosstalk between antioxidant system and DNA repair system response might play an important role in whole body of metabolic related stress. Here, our data suggests that multivitamin and multimineral with phytonutrients induce DNA repair gene expression, the levels of pCHK1 proteins (a key transducer of DNA repair signaling), during a HFC drink. We also found that multivitamin with phytonutrients reduced the levels of γH2AX protein in HFC drink induced-human blood plasma (**Fig. 4**).

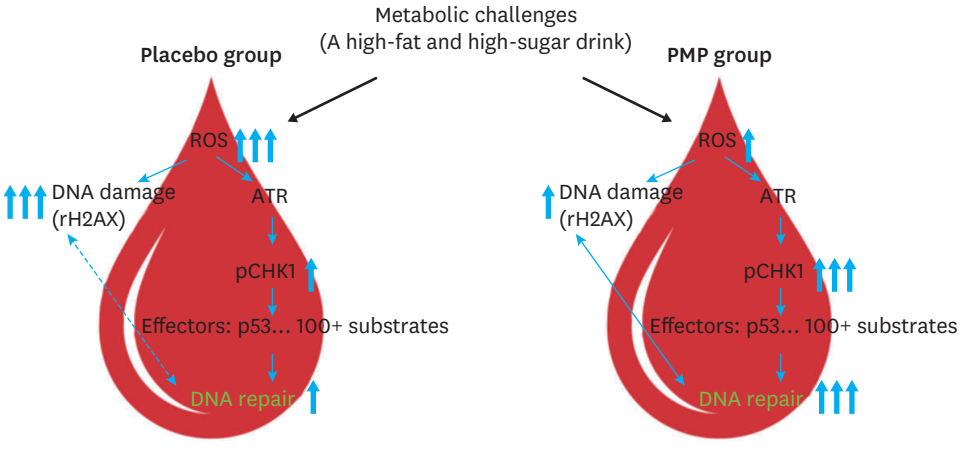

Fig. 4. The graphic summary of the study.
PMP supplementation for 8-week intervention study reduces reactive oxygen species and enhances DNA repair signaling in metabolic challenges.
ROS, reactive oxygen species.

https://doi.org/10.4163/jnh.2022.55.4.450

CHK1 is required for activation of DNA repair, apoptosis and senescence downstream in response to the presence of DNA damage [16,17]. Mostly CHK1 molecule is detected in epithelial and endothelial cells [18]. However, there are a little of studies in human blood. We observed human blood with multivitamin with phytonutrients had the high pCHK1 (Serine 345) protein levels on metabolic changes. The CHK1 also negatively regulates cell cycle progression during unperturbed cell cycles and stimulates the transcriptions of genes involved in homologous recombination related DNA repair [7]. We also speculate multivitamin with phytonutrients might regulate G1/S phase of cell cycle progression in human blood and promote HR of DNA repair.

In addition, we found PMP supplementation tended to induce p53 (Serine 15) gene expression but did not significantly change on the level after a HFC drink in blood plasma. It would more impact on p53 gene expression, as a DNA signaling mediator, in the later than 3 hours after consuming the drink. A cell-based study showed that p53 gene expression rapidly induced following irradiation and peaked at 3–6 hours later [19]. These findings suggest that PMP supplementation might involve in p53 pathway of DNA damage and repair response signaling later than 3 hours after receiving the drink.

The present study suggests PMP supplementation enhances antioxidant system against oxidative stress and DNA response of CHK1 gene, especially in human blood plasma *in vivo*, following a HFC drink-induced oxidative stress. The data shows PMP supplementation reduces the protein of γH2AX levels, a DNA damage biomarker, in a HFC drink-induced metabolic stress model. Also, PMP, as bioactive compounds, could be used in potential applications for the metabolic stress related preventive reagent in anti-chronic disease.

SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

Representative Western blot of plasma pCHK1 (Ser 345) and related DNA repair protein levels after metabolic challenge test.

Click here to view

REFERENCES

1. Shimizu I, Yoshida Y, Suda M, Minamino T. DNA damage response and metabolic disease. Cell Metab 2014; 20(6): 967-977.
PUBMED | CROSSREF
2. Barzilai A, Yamamoto K. DNA damage responses to oxidative stress. DNA Repair (Amst) 2004; 3(8-9): 1109-1115.
PUBMED | CROSSREF
3. Al-Aubaidy HA, Jelinek HF. Oxidative DNA damage and obesity in type 2 diabetes mellitus. Eur J Endocrinol 2011; 164(6): 899-904.
PUBMED | CROSSREF
4. Elledge SJ. Cell cycle checkpoints: preventing an identity crisis. Science 1996; 274(5293): 1664-1672.
PUBMED | CROSSREF
5. Kang S, Lim Y, Kim YJ, Jung ES, Suh DH, Lee CH, et al. Multivitamin and mineral supplementation containing phytonutrients scavenges reactive oxygen species in healthy subjects: a randomized, double-blinded, placebo-controlled trial. Nutrients 2019; 11(1): E101.
PUBMED | CROSSREF

6. Stroeve JH, van Wietmarschen H, Kremer BH, van Ommen B, Wopereis S. Phenotypic flexibility as a measure of health: the optimal nutritional stress response test. Genes Nutr 2015; 10(3): 13.
PUBMED | CROSSREF
7. Cho J, Park E. Ferulic acid maintains the self-renewal capacity of embryo stem cells and adipose-derived mesenchymal stem cells in high fat diet-induced obese mice. J Nutr Biochem 2020; 77: 108327.
PUBMED | CROSSREF
8. Tukey JW. Exploratory data analysis. Boston (MA): Addison-Wesley; 1977.
9. Kardinaal AF, van Erk MJ, Dutman AE, Stroeve JH, van de Steeg E, Bijlsma S, et al. Quantifying phenotypic flexibility as the response to a high-fat challenge test in different states of metabolic health. FASEB J 2015; 29(11): 4600-4613.
PUBMED | CROSSREF
10. Zhang Y, Hunter T. Roles of Chk1 in cell biology and cancer therapy. Int J Cancer 2014; 134(5): 1013-1023.
PUBMED | CROSSREF
11. Wellen KE, Thompson CB. Cellular metabolic stress: considering how cells respond to nutrient excess. Mol Cell 2010; 40(2): 323-332.
PUBMED | CROSSREF
12. Rajgopal A, Roloff SJ, Burns CR, Fast DJ, Scholten JD. The cytoprotective benefits of a turmeric, quercetin, and rosemary blend through activation of the oxidative stress pathway. Pharmacogn Mag 2019; 15(66): 449.
CROSSREF
13. Missler SR, Rajgopal A, Roloff SJ, Scholten JD, Burns CR, Patterson JA, et al. Synergistic activation of the Nrf2-ARE oxidative stress response pathway by a combination of botanical extracts. Planta Medica International Open 2016; 3(02): e27-e30.
CROSSREF
14. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013; 153(6): 1194-1217.
PUBMED | CROSSREF
15. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 2013; 123(3): 966-972.
PUBMED | CROSSREF
16. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 2003; 3(5): 421-429.
PUBMED | CROSSREF
17. Sørensen CS, Syljuåsen RG, Falck J, Schroeder T, Rönnstrand L, Khanna KK, et al. Chk1 regulates the S phase checkpoint by coupling the physiological turnover and ionizing radiation-induced accelerated proteolysis of Cdc25A. Cancer Cell 2003; 3(3): 247-258.
PUBMED | CROSSREF
18. McGowan CH. Checking in on Cds1 (Chk2): a checkpoint kinase and tumor suppressor. BioEssays 2002; 24(6): 502-511.
PUBMED | CROSSREF
19. Wittlinger M, Grabenbauer GG, Sprung CN, Sauer R, Distel LV. Time and dose-dependent activation of p53 serine 15 phosphorylation among cell lines with different radiation sensitivity. Int J Radiat Biol 2007; 83(4): 245-257.
PUBMED | CROSSREF