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Original article

Korean turmeric is effective for dyslipidemia in human intervention study

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ABSTRACT

Background: Turmeric is a plant that belongs to the ginger family, Zingiberaceae, and is one of the main ingredients in curry powder. Turmeric is often called the golden spice and has been used for medicinal purposes for thousands of years. Curcumin, which gives turmeric its yellow color, has been confirmed to have antioxidant, anti-inflammatory, and anti-infectious effects. It is also known to prevent dementia and promote liver health. For these reasons, turmeric is regarded as a therapeutic food additive with many health benefits beyond its nutritional value. In this paper, the health benefits of turmeric were examined through human intervention studies.

Methods: Thirty chosen individuals (28 men and 2 women) with slightly elevated alanine aminotransaminase levels consumed 1 g of turmeric powder (TP) as two capsules after each meal, three times a day, during the test period of 12 weeks. Changes in the lipid profile and in the levels of serum glucose, malondialdehyde, and metabolites in the sera were measured prior to and after TP consumption.

Results: The participants exhibited dyslipidemia, fasting hyperglycemia, and oxidative stress prior to TP treatment, and these symptoms were alleviated after treatment. On metabolomics analysis of sera, levels of branch-chained amino acids (valine and leucine/isoleucine) were decreased, whereas those of aromatic amino acids (tryptophan and phenylalanine) were increased. Pronounced changes were also noted in the levels of total lysophosphatidylcholine (lysoPC) and acylcarnitine: the levels of total lysoPC were decreased whereas those of acylcarnitine were increased. Serum levels of xanthine and hypoxanthine, which are intermediates of purine degradation, were increased in the participants, although that of the final product, uric acid, was decreased.

Conclusion: Oral consumption of TP alleviated dyslipidemia and changed metabolites patterns by accelerating metabolic activities with less oxidative stress in participants with mild liver dysfunction.

1. Introduction

Turmeric (*Curcuma longa* L.; Fig. 1) is a perennial herbaceous plant species belonging to the family Zingiberaceae, known to have originated in India. Currently, it is grown in Korea, Japan, Taiwan, and Indonesia, along with other countries. The oldest written record of turmeric dates back to B.C. and indicates that it was used as a food additive (spice), food preservative, and food coloring.

In India, turmeric is called haldi [1] and has been traditionally used as a dyeing agent for clothes due to its yellow color [2–3]. Turmeric has also been used in important ceremonies and rituals, such as weddings, by applying turmeric paste on the body or by tossing rice mixed with turmeric power towards the bride and groom [4–6]. Leprosy, chicken pox, and skin ulcers have been treated with ointments made from turmeric and by bathing in water with turmeric [7,8]. In the *Ayurveda*, the first Indian publication on medicine, turmeric is described as a common anti-inflammatory agent [9]. Additionally, turmeric has been used in

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Fig. 1. Roots of turmeric and their powder.

traditional medicines to treat various conditions such as swelling from muscle sprain or injury, bilious attack, loss of appetite, rhinitis, colds, diabetic wounds, liver disease, rheumatism, and sinus infection [1,10].

Turmeric powder has been shown to play an important role in increasing nutritional value, prolonging freshness, and preserving the quality, preferability, and integrity of shape [11,12]. Because of these characteristics, turmeric became one of the major ingredients in curry, a staple and famous dish in Indian cuisine.

China and many Southeast Asian countries have used turmeric to dye clothes, color food, and brew alcoholic drinks [11]. In Japan, turmeric (or Ukon in Japanese) has been widely enjoyed as a tea and medicinal herb [3,13]. A written record of the cultivation and therapeutic use of turmeric in Korea is found in the *Sallimkyungje* (山林經濟), a comprehensive book on households published during the late reign of the Chosun Dynasty (Fig. 2) [14]: Turmeric can only be grown in certain areas. Fine turmeric has a shape that resembles the stomach of a cicada. Its fragrance is mild and the qi (氣) is light and fast (揚) … (在處有之 形如蟬肚者佳 此物不甚香 但其氣輕揚 能致達酒氣於高遠 以降神也). This record indicates a long history of farming and medicinal use of turmeric in Korea. As to where turmeric was grown, the Sejongsillokjiriji (世宗實錄地理志) [15] mentioned that turmeric was cultivated in the towns of Gurye, Nakan, and Suncheon in the Jeonla-do province, whereas the Sinjeung-dongguk-yeoji-seungram (新增東國輿地勝覽) (Fig. 3) [16] stated that turmeric was a native product grown in Dongbok (Hwasun), Gwangyang, Goksung, Imsil, Sunchang, and Jeonju, and also in Jeonla-do. Currently, the town of Jindo in the Jeonlanam-do province is responsible for the mass cultivation of turmeric, using a system of geographical indications, and this continues to expand [17,18].

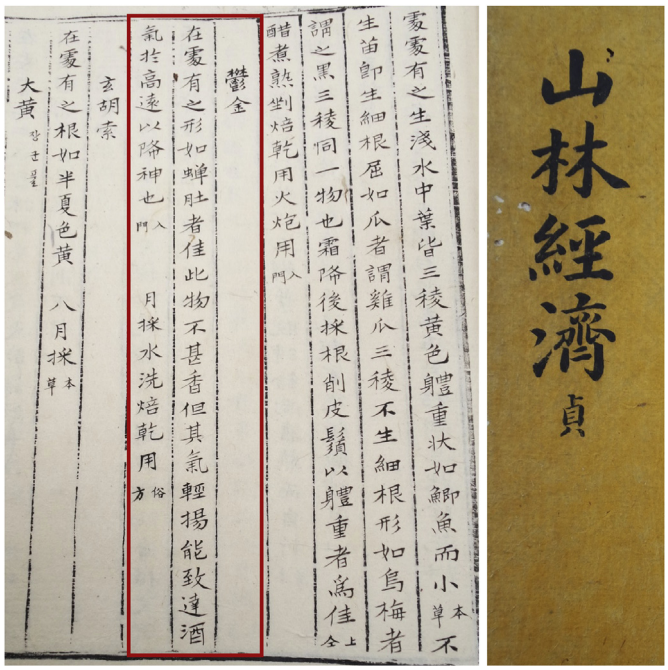

Fig. 2. Turmeric was recorded in Sallimkyungje (山林經濟) written by the Hong Man-Seon (洪萬選) in the late 17th century. The ancient documents are provided by the Jangseogak Archives at the Academy of Korean Studies.

According to the Korean medical encyclopedia called *Donguibogam* (東醫寶鑑) (Fig. 4), translated literally as *Principles and Practice of Eastern Medicine* and first published in 1610, during the reign of the Chosun Dynasty, turmeric is described as being both cold (or soothing) and hot (or causing body temperature increase) while being nontoxic; *C. longa* L. was used to treat a mass caused by stagnant blood, decrease qi (氣) flow, stop a nosebleed, and eliminate blood stasis [19]. The medical book claims that turmeric can help relieve blood extravasation, as well as alleviate qi (overflow of energy), both of which are thought to be harmful. It also recommends the rhizome as antidotes for hematuria and anxiety. This indicates that turmeric has been traditionally used for improving health in Korea.

1.1. Effects of curcumin

Turmeric is rich in phenolic curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin [20,21]. It is known to have antioxidant, anti-inflammatory, and anti-infectious activities [7,20,21]. Animal and human studies with turmeric have revealed the versatility of the rhizome both as a prophylactic agent and a cure for many ailments as well as a hepatoprotective agent for the alleviation of obesity [20,21]. Turmeric has been shown to be an effective therapeutic agent against arthritis, ischemic stroke, dyslipidemia, nonalcoholic steatohepatitis, and atherosclerosis in many experimental animal studies [22]. In addition, curcuminoids have been demonstrated to improve insulin sensitivity by suppressing adipogenesis, reducing elevated blood pressure, and mitigating inflammation and oxidative stress. Curcumin, one of the major curcuminoids in turmeric, is an inhibitor of cyclooxygenase-2 expression, which is involved in inflammation [23]. The efficacy of turmeric has been evaluated in limited randomized controlled clinical trials. Panahi et al [24] reported that curcuminoids plus piperine effectively reduce serum levels of low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), and lipoprotein A, and elevate high-density lipoprotein cholesterol (HDL-C) levels in patients with metabolic syndrome. As dyslipidemia results in the deposition of fatty streaks in the blood vessels when pro-oxidants damage the vessels, dyslipidemia is associated with arthrosclerosis [25]. Turmeric can prevent arthrosclerosis by improving dyslipidemia and antioxidant capacity. In addition, fermentation changes the form of bioactive components that generally increase the activity by removing glycosides of the components [26].

The health benefits of turmeric cited by many sources were garnered through a human intervention study using turmeric produced from Jindo, a southern region in Korea. During the 12-week test period, improved dyslipidemia was observed in participants after consuming turmeric.

2. Materials and methods

2.1. Ethics approval and study design

This study was approved by the Functional Foods Institutional Review Board of Chonbuk National University Hospital (CUH IRB 2010-02-17), Jeonju, South Korea. Between November 2010 and

Fig. 3. Turmeric was a local food product in Korea, which was reported in the Sinjeung-dongguk-yeoji-seungram (新增東國輿地勝覽), written by Lee Haeng (李荇) et al in 1530. The ancient documents are provided by the Kyujanggak Institute For Korean Studies.

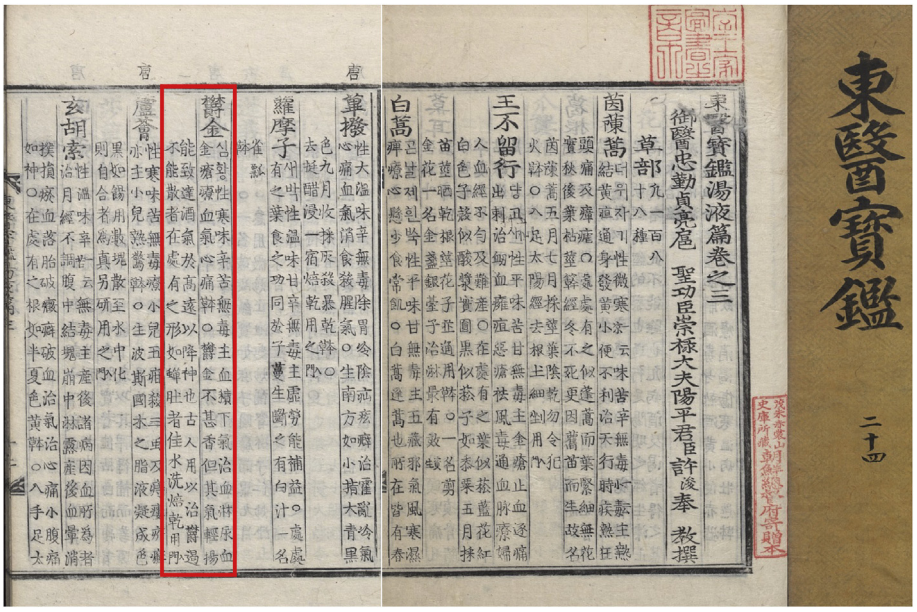

Fig. 4. The efficacy of turmeric was described in Donguibogam (東醫寶鑑), written by Heo Jun (許浚) in 1610. The ancient documents were provided from the Jangseogak Archives at the Academy of Korean Studies.

April 2012, 30 participants, all aged > 20 years, were recruited to evaluate the metabolic alterations in test individuals who consumed TP. All participants lived in the city of Jeonju, Cholla Province, South Korea. As part of the initial workup, the participants who signed up for the test underwent hepatic ultrasonography and typical liver function tests [e.g., tests to measure levels of enzymes such as alanine aminotransaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase]. The other biochemical indices determined were TC, TG, HDL-C, and LDL-C levels. Thirty participants with ALT levels between 40 IU/L and 200 IU/L, which were slightly above the normal range of 10–40 IU/L, were chosen as the test participants. The exclusion criteria were as follows: positive serology results for hepatitis B virus and hepatitis C virus, abnormal transferrin saturation, decompensated cirrhosis, and pregnancy. After none of the patients were excluded, all participants were asked to provide written consent prior to the start of the study. The participants were asked to take 3.0 g/d of powdered turmeric for 12 weeks, i.e., six capsules of turmeric per day, which corresponds to two capsules after each meal. As this study was a part of a randomized double-blinded clinical trial, the participants were not informed about what they were consuming. The participants were asked to maintain their everyday lifestyle, such as eating habits, exercise, and alcohol consumption [27].

2.2. TP capsule preparation

The dried turmeric powder was capsulated with 500 mg in each capsule. The TP capsules were kindly provided by Korea INS Pharmaceuticals, Inc. (Hwasoon, Jeonnam, South Korea). The curcumin

content of the TP was tested using high-performance liquid chromatography at the Korea Health Supplement Institute (Jeonju, Jeollabuk-do, South Korea) and was found to be approximately 0.79 mg/g [27].

2.3. Biochemical analysis

All serum samples were kept frozen at −70°C until they were assayed. Serum glucose levels were measured using an enzymatic colorimetric method with a commercial enzymatic assay kit (Asan Pharmaceutical Co., Seoul, Korea). The TC, TG, HDL-C, and LDL-C levels in serum were measured according to the manufacturer's instructions (Asan Pharmaceutical Co., Seoul, Korea). All analyses were measured using a UV spectrometer (JASCO, Tokyo, Japan). Serum malondialdehyde (MDA) levels were determined using the thiobarbituric acid reactive substances method with commercial kits (Cell Biolabs, San Diego, CA, USA). Glutathione levels in serum were measured using the Glutathione Assay Kit (Cayman, Ann Arbor, MI, USA) according to the manufacturer's instructions.

2.4. Serum analysis by ultraperformance liquid chromatography-quadrupole-time of flight-mass spectrometry

We collected 150 μL of sera from the 30 participants at the beginning and the end of the study, to which 300 μL of cold acetonitrile was added to extract soluble metabolites. After shaking for 30 minutes at 4°C, the samples were centrifuged at 5,590 *g* for 10 minutes, at 4°C. The supernatants were freeze-dried and stored at −70°C, and were subsequently dissolved in 20% methanol just prior to the ultraperformance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS) analysis [28]. Serum extracts were analyzed on a UPLC-Q-TOF-MS instrument (Waters, Milford, MA, USA), as described in our previous report [28]. The Q-TOF-MS was operated in positive electrospray ionization (ESI) mode, with a scan range of m/z 50–1,000. The cone voltage was 30 V, the capillary voltage was 3 kV, and the scan time was 0.2 second, with an interscan delay of 0.02 second. The source temperature was set at 110°C, and the desolvation flow rate was set at 700 L/h. The desolvation gas temperature was 300°C. The MS was calibrated using sodium formate, and leucine enkephalin was used as lock mass. The concentration of leucine enkephalin was 200pM, and the flow rate was set at 5 μL/min. As quality control, a mixture of five standard compounds (4-acetoaminophenol, caffeine, sulfadimethoxine, terfenadine, and reserpine) was injected after running every eight samples.

In the MS–MS experiments, argon was used as collision gas, with the collision energy alternating between 10 eV and 30 eV. The MassLynx software version 4.1 (Waters Inc.) was used to control the instrument and calculate accurate masses. Peaks were collected using a peak width at 5% height, 1 second, a noise elimination of 6, and an intensity threshold of 70. Data were aligned with a mass tolerance of 0.04 Da and a retention time window of 0.2 minute. All spectra were aligned and normalized to an external standard. Assignment of metabolites contributing to the observed variance was performed using the ChemSpider [29] and Human Metabolome Database [30].

2.5. Data processing for statistical analyses

Statistical analyses were performed on the data using the SIMCA-P+ software (ver. 12.0.1; Umetrics Inc., Umeå, Sweden). Partial least-squares discriminant analysis (PLS-DA) was used to visualize discrimination among samples, and an internal sevenfold cross-validation was carried out to estimate the performance of the PLS-DA models. Goodness of fit was quantified by R^2X and R^2Y, and the predictive ability was indicated by Q^2Y. In addition to cross-validation, model validation was also performed by a 200-times permutation test. Analysis of variance was performed to determine the statistical significance using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$ [28].

3. Results and discussion

3.1. Lipid profiles of participants

The liver plays an important role in lipid metabolism. Liver damage is caused by various factors such as alcohol, virus infection, and toxic compounds that increase inflammation and oxidative stress by damaging the hepatocytes. The damage possibly results in dyslipidemia and the accumulation of lipids, leading to nonalcoholic fatty liver disease. Thus, the alleviation of liver damage by herbs with anti-inflammation, antioxidant, and antiviral activities can also improve dyslipidemia.

The mean age of the eligible individuals was 39.0 ± 8.5 years, and the mean body mass index was 26.8 ± 3.3 kg/m^2. The mean body mass index of the participants remained virtually unchanged until the end of the study.

Prior to consuming TP, the participants had dyslipidemia; the serum TG, TC, and HDL-C levels were 198.4 ± 88.1 mg/dL, 258.4 ±25.2 mg/dL, and 27.4 ± 6.3 mg/dL, respectively (Table 1). TP consumption for 12 weeks significantly lowered serum TG, TC, and glucose levels, and increased serum HDL-C levels in comparison to the levels prior to consumption. In addition, fasting serum glucose levels decreased from 130.8 ± 20.5 to 111.0 ±15.0 mg/dL after TP treatment (Table 1). TP treatment alleviated dyslipidemia and hyperglycemia in humans with abnormal liver function.

In the present study, LDL-C oxidation was not determined, but serum MDA levels, an indicator of oxidative stress, decreased from 1.82 ± 1.99 μM to 0.80 ± 0.69μM after TP treatment for 12 weeks. Thus, TP reduced oxidative stress.

3.2. Serum metabolic profiles

The sera from the 30 participants collected at 0 week and 12 weeks after TP were analyzed using UPLC-Q-TOF-MS. The resulting chromatograms obtained in positive ion (ESI$^+$) mode are shown in Fig. 5. The data thus obtained were used for preparing a PLS-DA score plot to elucidate the existence of class distinction between the samples (Fig. 6A). The PLS-DA score plot showed a separation between 0-week and 12-week serum samples along the axes that correspond to the first two PLS-DA components. The variation in X (R_2X) and Y (R_2Y) was 30.7% and 70.6%, respectively, with the prediction percentage of the variation in response Y (Q^2Y) for the two-component model being 34.9%. The R^2 intercept value, evaluated using the permutation test, was 0.349 and the Q^2 intercept value was 0.159. Thus, the sera of participants at 0 week and 12 weeks

Table 1
Effects of consuming TP on triglyceride, total cholesterol, HDL-cholesterol, glucose, and malondialdehyde levels of test participants.*

	0 wk	12 wk	*p*
TG (mg/dL)†	198.38 ± 88.1	162.95 ± 71.0	0.189
T-chol (mg/dL)	258.38 ± 25.2	241.52 ± 26.3	0.077
HDL-chol (mg/dL)	27.44 ± 6.3	29.98 ± 8.03	0.187
Glucose (mg/dL)	130.75 ± 20.5	110.96 ± 15.0	0.017
Malondialdehyde (μM)	1.82 ± 1.99	0.80 ± 0.69	0.019

* Mean ± standard error of the mean.
† Tested by long transformed.
BMI, body mass index; HDL-chol, high-density lipoprotein cholesterol; T-chol, total cholesterol; TCI, total calorie intake; TFE, total energy expenditure; TG, triglyceride.

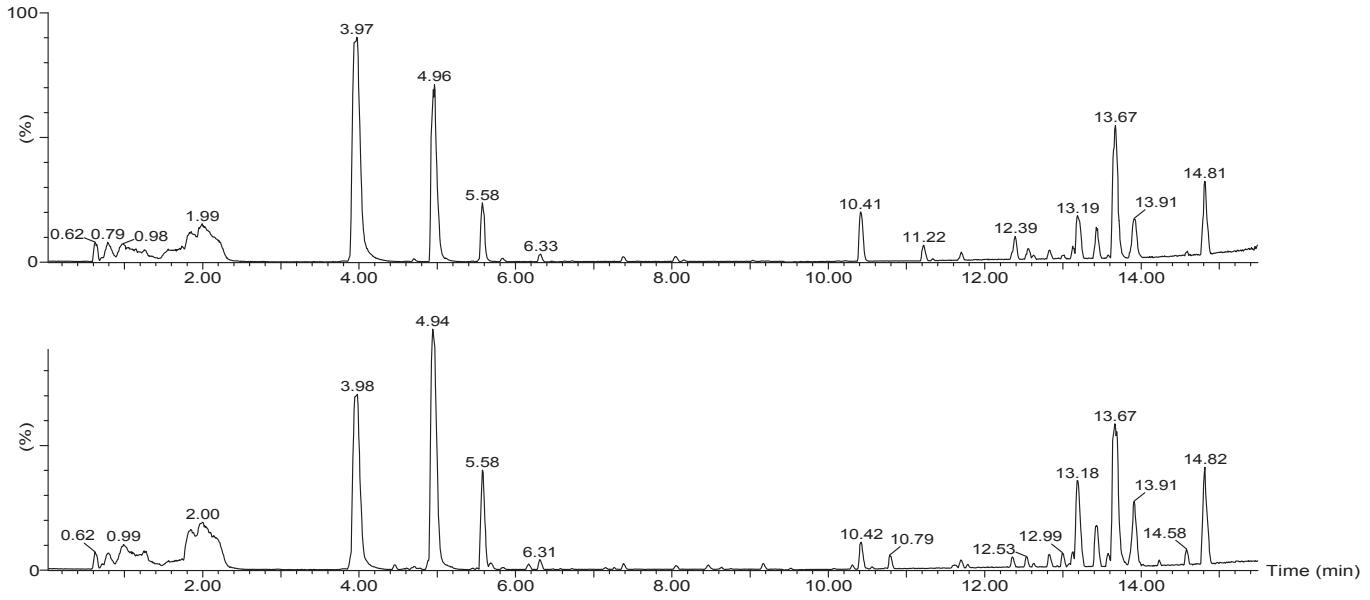

Fig. 5. High performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS) profiles of sera from human participants who were controlled by TP prior to and after 12 weeks of intervention.

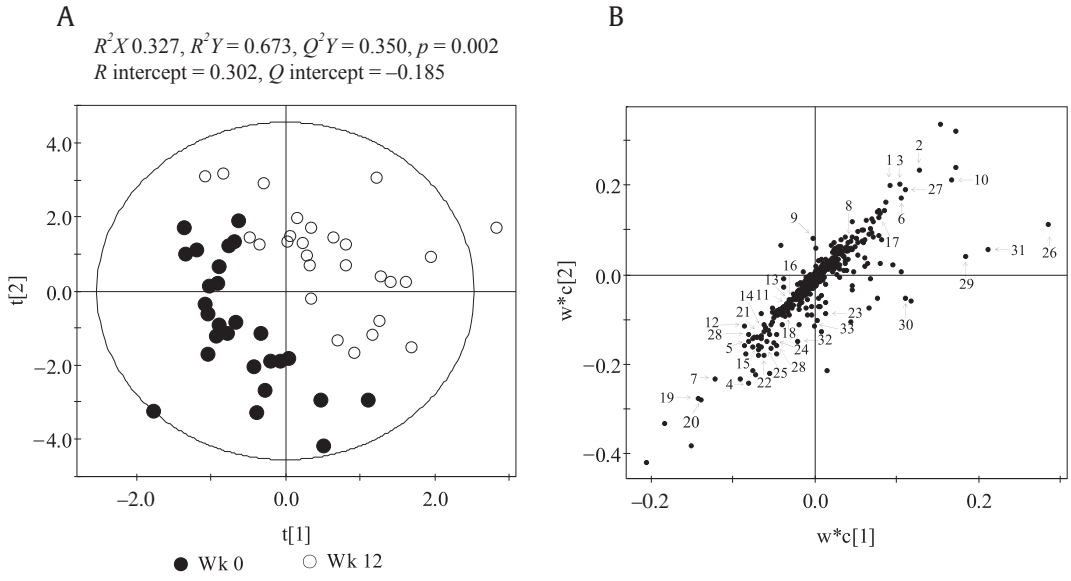

Fig. 6. Partial least-squares discriminant analysis (PLS-DA) score plot and S-plot. (A) PLS-DA scores plot obtained from the mass spectrometry data of the sera from study participants. Clear discrimination between the participants at Week 0 (open circle) and at Week 12 (filled circle) was obtained. (B) S-plot covariance [p] and reliability correlation [p_{corr}] from PLS-DA models. The numbers for the metabolites are as given in Table 2.

could be clearly differentiated by the primary or secondary components with the goodness of fit of the data. These results clearly indicate that the participants who consumed TP for 12 weeks experienced changes in the profiles of the serum metabolites.

The contribution of analyzed metabolites in the separation of sera of the 30 participants at 0 week and 12 weeks was determined by drawing the S-plot along the axes corresponding to the combined weight and reliability correlation (p_{corr}) (Fig. 6B). A higher or lower value of p_{corr} was associated with the greater degree of contribution made by these metabolites to the separation of the test samples. Positive p_{corr} values correspond to those with decreased serum levels due to TP consumption, whereas metabolites with negative values correspond to increased levels during the study period. All the 33 metabolites detected were marked in the S-plot, with the ones stipulated with numbers as the metabolites that were identified using MS–MS. Variable importance in the projection scores (VIP scores) and the normalized fold changes of the metabolites due to TP consumption are presented in Table 2. The 13 metabolites that showed significant increases in levels during TP treatment were xanthine (p = 0.004), phenylalanine (p = 0.02), palmitoleic acid (p = 0.000), palmitylcarnitine (p = 0.000), stearoylcarnitine (p = 0.003), palmitic acid (p = 0.005), acetylcarnitine (p = 0.007), argininosuccinic acid (p = 0.048), hypoxanthine (p = 0.031), butyrylcarnitine (p = 0.047), decanoylcarnitine (p = 0.014), methylmalonylcarnitine (p = 0.019), and LPC (C22:6) (p = 0.042). Six metabolites—valine (p = 0.02), lysine (p = 0.04),

Table 2
Identification of serum metabolites of study participants (non-TP and TP) analyzed using UPLC-Q-TOF-MS.

No.	Identity	Exact mass (M + H)	Actual mass (M + H)	Mass error (mDa)	*p*	VIP
1	Valine	118.0868	118.0841	−2.7	0.020	1.7
2	Benzoic acid	123.0434	123.0418	−1.6	0.010	2.13
3	Leucine/isoleucine	132.1025	132.1006	−1.9	0.040	1.8
4	Homocysteine	136.0432	136.0725	29.3	0.200	2.0
5	Hypoxanthine	137.0463	137.0443	−2	0.031	1.31
6	Lysine	147.0770	147.0426	−34.4	0.004	1.79
7	Xanthine	153.0413	153.0642	22.9	0.001	2.06
8	L-Carnitine	162.1130	162.1098	−3.2	0.276	1.0
9	Phenylalanine	166.0868	166.0817	−5.1	0.02	1.1
10	Uric acid	169.0362	169.0321	−4.1	0.057	2.67
11	Acetylcarnitine	204.1025	204.1357	33.2	0.007	0.64
12	Tryptophan	205.0977	205.0934	−4.3	0.413	1.41
13	Butyrylcarnitine	232.1549	232.1544	−0.5	0.047	0.8
14	C16:1 (palmitoleic acid)	255.1457	255.1579	12.2	0.000	1.05
15	C16:0 (palmitic acid)	257.1810	257.1723	−8.7	0.005	1.23
16	Methylmalonylcarnitine	262.1291	262.1506	21.5	0.019	0.71
17	Octanoyl carnitine	288.2170	288.2482	30.7	0.003	1.24
18	Argininosuccinic acid	291.1305	291.1369	6.4	0.048	0.46
19	Decanoylcarnitine	316.2488	316.2421	−6.7	0.014	2.45
20	Palmitylcarnitine	400.3427	400.3404	−2.3	0.000	2.42
21	Stearoylcarnitine	428.3740	428.3739	−0.1	0.003	1.20
22	LysoPC (C14:0)	468.3090	468.3047	−4.3	0.100	1.4
23	LysoPE (C18:2)	478.2934	478.3248	31.4	0.200	1.3
24	LysoPC (P16:0)	480.3454	480.3425	−2.9	0.205	1.3
25	LysoPE (C18:0)	482.3168	482.3232	6.4	0.081	1.2
26	LysoPC (C16:0)	496.3403	496.3337	−6.6	0.050	5.9
27	LysoPE (C20:4)	502.2934	502.3294	36	0.007	1.68
28	LysoPC (C18:3)	518.3247	518.3252	0.5	0.177	1.1
29	LysoPC (C18:2)	520.3403	520.3266	−13.7	0.070	4.0
30	LysoPC (C18:1)	522.3560	522.3422	−13.8	0.200	3.2
31	LysoPC (C18:0)	524.3716	524.3588	−12.8	0.244	4.6
32	LysoPC (C20:4)	544.3403	544.3331	−7.2	0.060	1.5
33	LysoPC (C22:6)	568.3403	568.3341	−7.2	0.042	1.4

lysoPC, lysophosphatidylcholine; lysoPE, lysophosphatidylethanolamine; UPLC-Q-TOF-MS, ultraperformance liquid chromatography-quadrupole-time of flight-mass spectrometry; VIP, variable importance in the projection scores.

benzoic acid ($p = 0.01$), leucine/isoleucine ($p = 0.04$), octanoylcarnitine ($p = 0.003$), and lysophosphatidylethanolamine (LPE) (C20:4) ($p = 0.007$)—were found to decrease during the same period. Uric acid ($p = 0.06$) and lysophosphatidylcholines (lysoPCs) containing C18:2 ($p = 0.07$), C16:0 ($p = 0.06$), and C20:4 ($p = 0.06$) showed insignificant variations, with fold changes in the range of 0.8−1.2 (Fig. 7). Palmitoleic acid, xanthine, palmitoylcarnitine, leucine/isoleucine, octanoylcarnitine, and LPE (C20:4) exhibited the highest changes in levels, with VIP values being >3.0. In particular, palmitoleic acid, xanthine, LPE (C20:4), and octanoylcarnitine were determined to be the most important parameters for discriminating samples.

The correlations between these major metabolites to some biochemical markers, such as MDA and HDL-C, were accessed using the Pearson correlation test (Fig. 8). Among the major metabolites with VIP values >3.0, the levels of xanthine and octanoylcarnitine were found to be reversely proportional to those of MDA and HDL-C levels, suggesting that TP was able to accelerate lipid catabolism without promoting oxidative stress, where both lipolysis and reactive oxygen species synthesis were facilitated.

3.3. Dyslipidemia

This study demonstrated that TP treatment for 12 weeks alleviated the metabolic disorders of the individuals with slightly elevated AST and ALT activities who had dyslipidemia, higher fasting hyperglycemia, and oxidative stress. In addition, in the metabolomics analysis of sera, levels of branch-chained amino acid (BCAA), such as valine and leucine/isoleucine, were decreased, whereas those of aromatic amino acids, such as tryptophan and phenylalanine, were increased. Total lysoPCs were reduced and acylcarnitine levels were increased in the circulation. Serum levels of xanthine and hypoxanthine, which are intermediates of purine degradation, were increased in the participants, although that of the final product, uric acid, was decreased. TP modulates fatty acid transport from the cytosol to the mitochondria for b-oxidation and amino acid and purine degradation in the circulation. The parameters that were changed on metabolomics analysis in the present study (i.e., BCAA, acylcarnitines, and phospholipids) have been identified as potential biomarkers for obesity, and the results indicated that they are also involved in lipid metabolism in the liver [31]. Thus, TP might improve lipid metabolism in the liver in participants with moderate liver damage.

Similar to unfermented turmeric, TP ameliorated dyslipidemia and oxidative stress, thereby decreasing the risk of atherosclerosis. Turmeric and its major polyphenol (curcumin) have been reported to have prevented and improved dyslipidemia and oxidative stress. Ejaz et al [32] and Weisberg et al [33] claimed that consuming curcumin may help alleviate metabolic disorders associated with obesity by suppressing adipose tissue angiogenesis as well as by increasing energy metabolic process of adipocytes. However, only a few studies have been conducted to determine the effects of TP on dyslipidemia, even though fermentation has been claimed to improve the bioactivity by removing glycosides attached to polyphenols in various herbs. TP administration (200 mg/kg and 500 mg/kg of body weight) resulted in alleviation of dyslipidemia in ob/ob mice; the TC and TG levels in the serum and liver significantly decreased compared to that observed after placebo administration, and HDL-C levels significantly increased [34]. In addition, TP also reduces lipid peroxidation and hepatotoxicity in rats with CCl4-induced liver diseases by enhancing the antioxidant capacity owing to increase in the activities of antioxidant enzymes such as catalase, glutathione-*S*-transferase, glutathione reductase, and glutathione peroxidase [22]. These results suggest that TP may improve dyslipidemia by ameliorating liver damage via the reduction of oxidative stress and the restoration of liver function-enhanced lipid metabolism.

3.4. Carnitine/acylcarnitine levels

L-Carnitine is mainly absorbed from the diet, but is also formed through biosynthesis in the human liver, kidneys, and brain. Other tissues receive L-carnitine from circulation [35]. L-Carnitine is a form of acylcarnitine that acts as the transporter of fatty acids into the mitochondria from the cytosol. Acylcarnitine is transferred into the mitochondria and changed into fatty acid, thereby producing energy. Carnitine also plays an important role in fatty acid transport from peroxisomes, where long-chain fatty acids are partially degraded [22]. L-Carnitine is important for utilizing fatty acids. The acylcarnitine profile is directly influenced by diet and metabolic status such as fasting [35]. Acylcarnitine is a product of incomplete fatty acid oxidation, and it is associated with the altered flux of fatty acid oxidation [36,37]. Although acylcarnitine is reported to increase during fasting and possibly in an insulin-resistant state, the findings are still controversial. Moreover, there are no correlations between acylcarnitine in the tissue and serum [35]. Serum acylcarnitine levels may not reflect the insulin-resistant state. In the present study, TP consumption led to increasing the ratio of acylcarnitines and carnitine by 1.55-fold in the serum as compared to that observed prior to TP consumption. The correlation study demonstrated that the serum levels of MDA were positively associated with those of octanoylcarnitine. These results suggested that

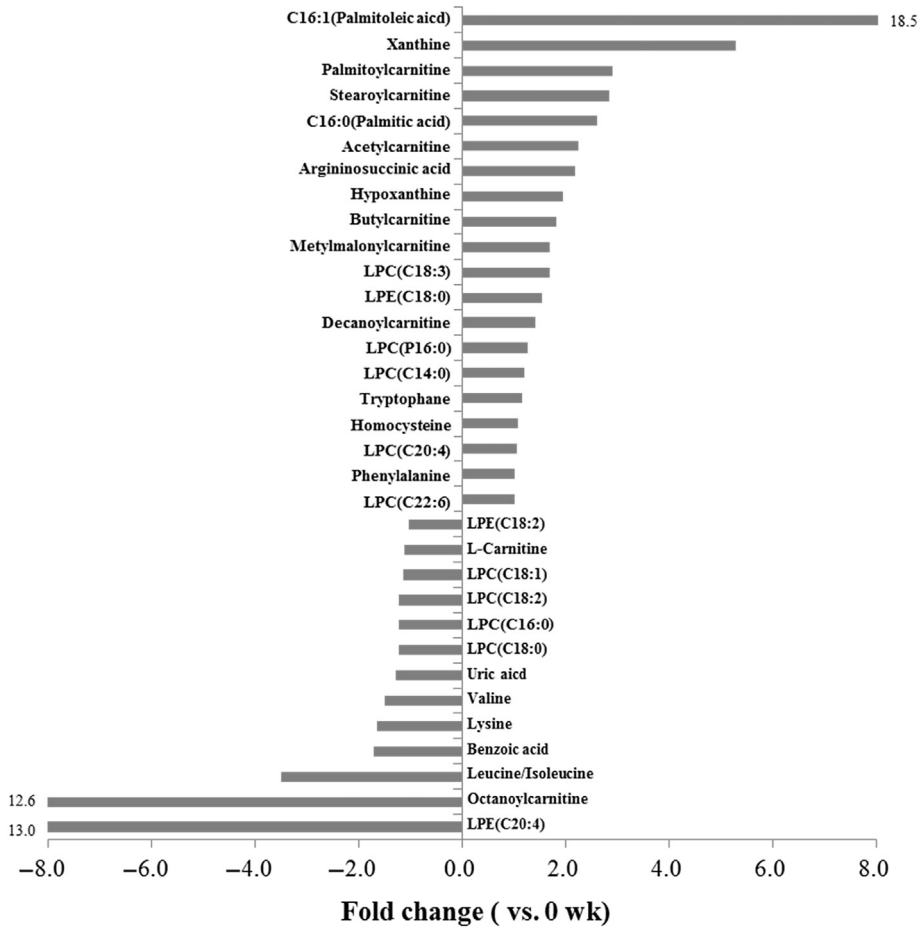

Fig. 7. The fold changes of serum metabolites in participants at 12 weeks were calculated against those at 0 week and are presented as positive and negative values. LPC, lysophosphatidylcholine; LPE, lysophophatidylethanolamine.

the serum levels of medium-chain acylcarnitine, but not long-chain acylcarnitine, may be associated with increasing oxidative stress and insulin resistance. Ejaz et al [32] demonstrated that curcumin-fed mice exhibited increased expression of CAP1 as well as decreased expression of PPAR-γ (peroxisome proliferator-activated receptor gamma) and CCAAT/enhancer binding protein-α, which might lead to an increase in β-oxidation and decrease in fatty acid synthesis. Thus, the elevation of serum acylcarnitine levels in the present study may be associated with the increase of fatty acid oxidation in a fasting state after TP consumption.

3.5. LysoPC levels

LysoPC and lysophosphatidylethanolamine (lysoPE) are produced as the intermediates during the formation or breakdown of phosphatidylcholines and phosphatidylethanolamines, respectively. LysoPC and lysoPE are closely associated with certain disorders such as endothelial dysfunction, inflammation, and obesity [38–40]. The levels of both total lysoPC and lysoPE were decreased by approximately 20% in the sera of the participants after TP consumption. In the present study, the serum levels of lysoPCs 18:1, 18:2, 18:0, and LPE 20:4 were decreased, whereas that of lysoPCs 22:6, 20:4, 14:0, 16:0, 18:3, and LPE 18:2 were slightly increased, in ascending order. Similar to the results of our study, studies on obese men [41] and monozygotic twins [42] revealed that the plasma lysoPC level is associated with obesity. Moreover, other studies showed that these hydrolyzed phospholipid levels and obesity were unrelated, and thus, further studies with human volunteers are necessary to fully ascertain the role of these compounds in obesity [43]. Thus, TP improved lipid metabolism considering lysoPC and lysoPE levels in circulation.

3.6. Serum BCAA levels

Circulating levels of BCAAs tend to increase in individuals with obesity, insulin resistance, or type 2 diabetes mellitus [43]. This may be related to increased insulin resistance. Insulin resistance might promote aminoacidemia by increasing the protein degradation that insulin normally suppresses, and/or by eliciting an impairment of efficient BCAA oxidative metabolism in some tissues [44,45]. The accumulation of mitotoxic metabolites, possibly BCAA, promotes β-cell mitochondrial dysfunction, stress signaling, and apoptosis associated with type 2 diabetes [46]. The metabolism of BCAAs is also affected in other diseases associated with insulin resistance, including kidney and liver dysfunction [46]. The present study demonstrated that the levels of BCAAs, notably valine and leucine/isoleucine, were decreased in participants consuming TP, although those of aromatic amino acids such as phenylalanine and tryptophan increased. According to Nie and Henriksson [47], the metabolism of BCAA increased during strenuous exercise and the level of alphaketocaproic acid increased from the transamination of leucine in both muscles and blood. In addition, serum leucine levels were positively correlated with serum levels of MDA. Thus, this BCAA change after TP

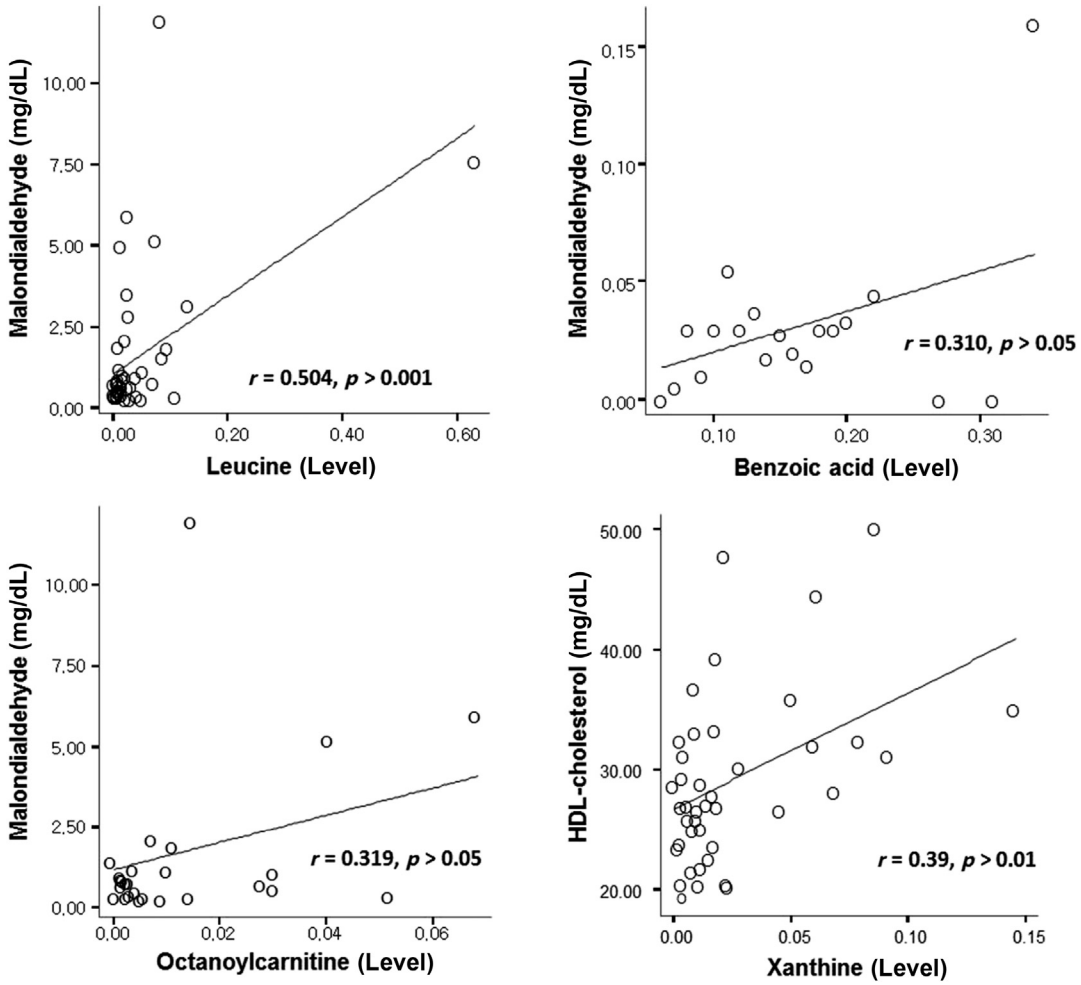

Fig. 8. Relationship between the changes in major metabolite levels by Pearson's correlation analysis. The changes in lyso-phosphatidylcholine (lysoPC) 16:0 levels were positively related to those of lysoPC 15:0 ($r = 0.90$, $p < 0.001$) and lysoPC 18:0 ($r = 0.76$, $p < 0.001$). r, correlation coefficient.

consumption may be related to the reduction in insulin resistance and oxidative stress by improving liver function, and it may be associated with increased metabolic rates.

3.7. Xanthine metabolism

The present study showed that TP led to a decrease in the levels of uric acid whereas those of hypoxanthine and xanthine increased. Serum hypoxanthine levels are associated with exercising, as reported by Syuko et al [48]. The results of the study by Syuko et al [48] are in accordance with our results; the participants experienced a marginal decrease in body weight, percent body fat, and blood TG levels. A previous study has demonstrated that uric acid causes mitochondrial oxidative stress that stimulates fat accumulation independent of excessive caloric intake [49]. Therefore, high levels of uric acid in blood are associated with the development of obesity [50] and dyslipidemia [51], besides hypertension [52]. Tsushima et al [53] also revealed that uric acid production and its levels in blood were elevated in obesity-induced mice. Thus, increased levels of hypoxanthine and xanthine with concordant decreased levels of uric acid in the present study suggest that TP elevated the metabolic state of subjects with reducing oxidative stress.

The consumption of TP normalized dyslipidemia and serum MDA levels in individuals with mild liver dysfunction. These changes were associated with the serum metabolite patterns; the levels of LysoPC, LysoPE, and BCAA were decreased in participants after TP consumption. These metabolic changes by consuming TP represented the increase of metabolic activities with decreased oxidative stress, which might be similar to conducting regular exercise.

Conflicts of interest

The authors have no conflicts of interest.

Acknowledgments

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