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Research Article

Incorporation and metabolism of punicic acid in healthy young humans

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The objective of this study was to investigate the incorporation and metabolism of punicic acid (PA, *cis*9,*trans*11,*cis*13-18:3) in healthy young humans. The study was a randomized controlled trial. After 7 days adaptation with sunflower seed kernels supplementation, 30 subjects were then divided into the control and test group ($n = 15$). The test group was supplemented with *Trichosanthes kirilowii* (TK) seed kernels containing 3 g of PA per day in the form of triacylglycerols for 28 days. The control group was provided with sunflower seed kernels. After consumption of TK seeds containing 3 g PA per day for 28 days, the proportion of PA was increased from 0.00 to 0.47% in plasma and 0.00 to 0.37% in red blood cell membranes (RBCM), respectively. The proportion of *cis*9,*trans*11-18:2 was increased from 0.05 to 0.23% in plasma and 0.03 to 0.17% in RBCM after 28 days of intervention, respectively. Our results suggest that PA can be effectively incorporated into human plasma and RBCM, and is also associated with the increasing proportion of *cis*9,*trans*11-18:2 in humans, presumably as a result of metabolism by a saturation reaction. Edible TK seeds could be a potential dietary source of conjugated linoleic acids.

Keywords: Conjugated linoleic acid / Metabolism / Punicic acid

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1 Introduction

The conjugated fatty acids are the general term for a set of positional and geometric isomers of PUFAs with conjugated double bonds. The conjugated fatty acids occur naturally as diene, triene, and tetraene compounds [1]. Conjugated linoleic acids (CLA) with two conjugated double bonds are the conjugated fatty acid most extensively studied. CLA isomers have been reported to have many favorable physiological effects on health, including anticarcinogenic [2] and antiatherogenic action, regulation of body fat and lipid metabolism in both animals and humans [3–5]. A number of CLA isomers has been identified, however the two major isomers are the *cis*9,*trans*11-18:2 and *trans*10,-*cis*12-18:2, and these two isomers have been reported to exert different functional roles [3].

It has been reported that conjugated linolenic acids (CLnA), where three double bonds are conjugated together, have a cytotoxic effect on cultured human tumor cells [6–8], inhibit carcinogenesis [9–12] and alter the lipid metabolism in animals [13–16]. These findings have led to increased interest in CLnA. Five CLnA isomers occur in major seed oils of several plants: alpha-eleostearic acid (α-ESA, *cis*9,*trans*11,*trans*13-18:3), punicic acid (PA, *cis*9,-*trans*11,*cis*13-18:3), calendic acid (*trans*8,*trans*10,*cis*12-18:3), jacaric acid (*cis*8,*trans*10,*cis*12-18:3), and catalpic acid (*trans*9,*trans*11,*cis*13-18:3) [1]. Among the CLnA-containing seeds, only the seeds of *Trichosanthes kirilowii* Maxim. (TK seeds) from China are edible. The punicic acid-harboring TK seeds are a popular snack food in China.

It has been reported that α-ESA can be metabolized into *cis*9,*trans*11-18:2 in rats [17–19]. CLA also can be detected in liver of rats and mice fed with PA [12, 14, 15].

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Abbreviations: CLA, conjugated linoleic acid; **CLnA,** conjugated linolenic acid; **FAMEs,** Fatty acid methyl esters; **HDL-C,** high-density lipoprotein cholesterol; **LDL-C,** low-density lipoprotein cholesterol; **PA,** punicic acid; **RBCM,** red blood cell membranes; **TAG,** triacylglycerols; **TC,** total cholesterol; **TK,** *Trichosanthes kirilowi*

Our recent study also showed that PA can be incorporated and metabolized into *cis*9,*trans*11-18:2 in rat liver, plasma, adipose tissue, kidney, and brain [20]. However, there are no literature data on the incorporation and metabolism of PA in humans at present. PA, the *cis*9,*trans*11,*cis*13 isomers of CLnA, has been shown to exert several beneficial effects in animal and cell culture studies. Pomegranate seed oil rich in PA can suppress colon cancer in rats [12] and skin carcinogenesis in mice [21] and suppress proliferation, xenograft growth, and invasion of human prostate [22 – 24] and breast cancer cells [25]. PA was also shown to decrease the liver triacylglycerol accumulation *in vivo* [26], and reduce the apolipoprotein B100 secretion and triacylglycerol synthesis in HepG2 cell [27]. The present study was performed to determine the incorporation and metabolism of PA in human plasma and red blood cell membranes (RBCM) by using the unique material, TK seeds.

2 Subjects and methods

2.1 Subjects

The project was approved by the Research Ethics Committee, School of Biosystem Engineering and Food Science, Zhejiang University (SBEFS2006005), and all subjects gave written informed consent prior to participation in the study. The exclusion criteria for the study were cigarette smoking, history of bleeding disorders, diabetes, cardiovascular disease, hypertension, hypercholesterolemia, regular intake of anti-inflammatory medications, and dietary supplements. Thirty young healthy volunteers (24 males, 6 females), aged 21 – 35 were recruited in Hangzhou, China. Physical activity was requested to be constant during the study period (no change from habitual physical activity). Baseline characteristics of the 30 subjects who completed the study did not differ between the groups (Table 1).

2.2 Study design and diets

The study consisted of a 7 day adaptation period and a 28 day intervention period. During the study period, the volunteers consumed their habitual diet but they were asked to refrain from eating ruminant-related products (meat and their products, milk, dairy products), thus minimizing their intake of CLA throughout the study.

During the adaptation period, all subjects consumed 23.4 g of sunflower seed kernels (12.5 g of fat mainly in the form of triacylglycerols (TAG) daily. The purpose of the adaptation period is to increase the baseline similarity of dietary fatty acids in subjects. Then subjects were randomly assigned to one of two groups (fifteen per group): the control group (thirteen males and two females) and the test group (eleven males and four females). Each test group subject consumed 20.8 g of TK seed kernels (12.5 g of fat with 3 g of PA) daily. The dose of PA was based on CLA used in human studies [3, 4]. Each control group subject consumed 23.4 g of sunflower seed kernels (12.5 g of fat mainly in the form of TAGs) daily. The fatty acid composition of TK seed kernels and sunflower seed kernels are shown in Table 2.

Table 1. Baseline characteristics of the subjects

	Control group	Test group
Age (years)	23.8 ± 3.5	25.9 ± 2.7
Body height (cm)	171.0 ± 8.3	166.5 ± 5.6
Body weight (kg)	62.9 ± 8.2	58.9 ± 7.9
BMI (kg/m^2)	21.5 ± 2.1	21.2 ± 2.0

All values are mean ± SD; $n = 15$ subjects. The control group contained thirteen males and two females and the test group contained eleven males and four females.

Table 2. Fatty acid composition of *Trichosanthes kirilowii* and sunflower seed oil

	g/100 g total fatty acids	
	Sunflower seed	TK seed
16:0	5.17	5.18
16:1n-7	0.08	ND
18:0	1.83	1.77
18:1n-9	11.36	28.01
18:2n-6	81.20	36.06
18:3n-3	0.16	ND
20:0	0.11	ND
20:1n-9	0.09	ND
Punicic acid	ND	24.26
Alpha-eleostearic acid	ND	3.25
Catalpic acid	ND	1.48

ND, not detected; TK, *Trichosanthes kirilowii*.

2.3 Nutrients intake

Each subject kept a 5 day diet record during the study period. Dietary energy and nutrient intake of each subject were assessed from the dietary record using the computer program "Diet analysis" (Cao Aihong, China).

2.4 Blood specimen collections

Blood samples were collected at the end of adaptation period (day 0) and intervention period (day 28). Subjects attended the Hospital of Zhejiang University on one morning following an overnight fast (from 10:00 pm until the appointment). Subjects were allowed to sit relaxed for 10 min, weight and height were measured. Then venous blood (10 mL) was taken in EDTA tubes with 21-gauge needles. Blood samples were immediately placed on ice. Plasma was prepared during the 2 h after blood was drawn, aliquoted into separate tubes and stored at -70°C until analysis. After the plasma and platelets had been removed (20 min, $1200 \times g$), the red blood cells were dispersed in

Table 3. Daily energy and nutrient intake

	Control group		Test group	
	Day 0	Day 28	Day 0	Day 28
Total energy (kcal)	1767.7 ± 147.5	1798.8 ± 147.7	1727.7 ± 159.1	1719.8 ± 130.6
Carbohydrate (g)	232.9 ± 24.9	232.3 ± 26.6	233.2 ± 31.2	227.2 ± 23.5
Total fat (g)	62.8 ± 7.0	62.0 ± 5.3	61.8 ± 9.0	60.8 ± 5.9
Protein (g)	71.4 ± 11.0	70.5 ± 8.1	70.1 ± 23.5	69.4 ± 11.1
Carbohydrate (% of energy)	56.4 ± 2.2	56.4 ± 2.5	56.7 ± 5.1	56.3 ± 2.1
Fat (% of energy)	26.6 ± 3.3	26.5 ± 1.8	26.3 ± 2.8	26.5 ± 2.3
Protein (% of energy)	17.2 ± 2.2	17.2 ± 1.5	16.9 ± 5.4	17.2 ± 2.4

All values are mean ± SD; $n = 15$ subjects. The control group contained thirteen males and two females and the test group contained eleven males and four females.

0.9% saline and washed three times by centrifugation (20 min, 840 × *g*) and stored at −70°C until analysis [28].

2.5 Biochemical analyses

Plasma low-density lipoprotein cholesterol (LDL-C) and HDL cholesterol (HDL-C) concentrations were measured by enzymatic colorimetric procedures using commercially available kits (Wako, Osaka, Japan) on an auto-biochemical analyzer (Hitachi 7170, Hitachi, Tokyo, Japan). Plasma total cholesterol (TC) and TAG concentrations in plasma were measured using enzymatic procedures (CHOD-PAP and GPO-PAP, respectively; DiaSys Diagnostic Systems Co. Ltd., Shanghai, China) on the same auto analyzer.

2.6 Lipid extraction and fatty acid analysis

Total lipids were extracted from plasma and RBCM with chloroform : methanol (2:1 v/v) containing 50 mg/L of butylated hydroxytoluene (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) according to the method of Folch *et al.* [29]. The plasma fatty acids were converted to methyl ester by using 2 mL of 2% H_2SO_4-Methanol for 18 h at 50°C according to Burdge *et al.* [30] for plasma contains considerable amount of nonesterified fatty acids. This condition provides sufficient methylation but does not cause isomerization and oxidation of conjugated fatty acid to a significant extent. The fatty acids from RBCM were methylated according to Christie's method with minor modifications [31]. The methylation was done in 10-mL tubes with Teflon lined screw caps for 20 min at 55°C by using 2 mL of 0.5 N sodium methoxide in methanol. When cooled, the resulting methyl esters were extracted into n-hexane and dried on anhydrous Na_2SO_4. Before injected to GC, FAMEs were filtered by Sep-pak silica column (Altech, Associates Inc., Deerfield, IL, USA). The FAMEs were analyzed on a GC-14C capillary gas chromatograph (Shimadzu, Co. Ltd., Kyoto, Japan) with flame ionization detector connected with the N2010 Chromatography Data System (Zhida Information Technologies, Inc., Hangzhou, China). A DB-23 capillary column (60 × 0.25 mm id, 0.25 μm film thickness, Agilent technologies, Palo Alto, CA, USA) was used. The oven temperature was initially set at 100°C for 0 min, then increased to 220°C at 20°C/min and held for 15 min. Nitrogen was used as carrier gas. Injector (split mode) and detector temperature were both maintained at 270°C. Fatty acids were identified by comparison with standard mixtures of FAMEs, and the results were calculated using response factors derived from chromatograph standards of known composition (Nu-Chek-Prep, Elysian, MN, USA). The structure of *cis*9,*trans*11-18:2, the metabolite of PA, was identified by GC and GC-MS according to previously described methods [20].

2.7 Statistical analysis

Statistical analysis was performed using the SPSS package program version 11.5 (SPSS inc. Chicago, IL, USA). Effects of PA and treatment period on plasma and RBCM fatty acid composition were determined by repeated measures ANOVA. The General Linear Model was used to determine the difference in *cis*9,*trans*11-18:2 and PA between the two groups at day 28, with day 0 values used as the covariance. The values are reported as means with their SDs for all results. Differences were considered significant at $p < 0.05$.

3 Results

3.1 Daily intake of energy and nutrients

All 30 subjects successfully completed the study. No side effects were reported. There were no significant differences in daily consumption of energy or macronutrients between the control group and test groups during the adaptation and intervention period (Table 3). No significant differences between the test and the control groups in plasma lipids profile at day 0 and day 28 were seen (Table 4).

Table 4. Effect of *Trichosanthes kirilowii* seed kernels rich in punicic acid on plasma lipids profile

	Control group		Test group		*P*-Value[a]		
	Day 0	Day 28	Day 0	Day 28	Time	Treatment	Treatment × Time
TAG (mmol/L)	1.07 ± 0.65	1.00 ± 0.51	0.69 ± 0.29	0.65 ± 0.20	0.380	0.026	0.813
TC (mmol/L)	3.76 ± 0.67	3.92 ± 0.89	3.73 ± 0.71	3.69 ± 0.69	0.444	0.634	0.218
HDL-C (mmol/L)	1.29 ± 0.30	1.28 ± 0.35	1.39 ± 0.27	1.38 ± 0.29	0.804	0.376	0.866
LDL-C (mmol/L)	2.28 ± 0.49	2.34 ± 0.66	2.27 ± 0.53	2.15 ± 0.47	0.614	0.607	0.166
Ratio of LDL-C to HDL-C	1.85 ± 0.56	1.95 ± 0.79	1.66 ± 0.36	1.60 ± 0.34	0.750	0.166	0.207
Ratio of TC to HDL-C	3.02 ± 0.74	3.22 ± 1.02	2.72 ± 0.45	2.73 ± 0.43	0.212	0.118	0.249

All values are mean ± SD; $n = 15$ subjects. The control group contained thirteen males and two females and the test group contained eleven males and four females. TC, total cholesterol; TAG, triacylglycerols; LDL-C, low-density lipoprotein cholesterol; HDL-C, HDL cholesterol.
a) Repeated measures ANOVA.

Table 5. Fatty acid composition of human plasma (% total fatty acids)

	Control group		Test group		*P*-Value[a]		
	Day 0	Day 28	Day 0	Day 28	Time	Treatment	Treatment × Time
14:0	0.54 ± 0.06	0.50 ± 0.07	0.46 ± 0.04	0.43 ± 0.03	0.383	0.290	0.891
16:0	19.90 ± 0.31	19.64 ± 0.77	20.55 ± 0.41	19.84 ± 0.36	0.308	0.425	0.626
16:1n-7	1.45 ± 0.16	1.77 ± 0.14	1.33 ± 0.14	1.35 ± 0.15	0.225	0.106	0.268
18:0	7.47 ± 0.43	6.53 ± 0.36	7.16 ± 0.21	6.53 ± 0.20	0.005	0.555	0.555
18:1n-9	16.15 ± 0.50	17.62 ± 0.81	16.14 ± 0.50	16.36 ± 0.55	0.125	0.347	0.252
18:2n-6	40.42 ± 0.79	38.62 ± 1.01	40.38 ± 0.79	40.76 ± 1.02	0.354	0.323	0.161
18:3n-6	0.31 ± 0.04	0.42 ± 0.05	0.27 ± 0.04	0.33 ± 0.06	0.042	0.225	0.521
18:3n-3	1.05 ± 0.06	1.05 ± 0.13	1.02 ± 0.06	0.84 ± 0.06	0.291	0.185	0.307
*cis*9,*trans*11-18:2	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.23 ± 0.03[b]	0.000	0.000	0.000
20:0	0.08 ± 0.01	0.13 ± 0.04	0.12 ± 0.05	0.09 ± 0.01	0.760	0.964	0.196
20:1n-9	0.29 ± 0.03	0.30 ± 0.05	0.31 ± 0.07	0.24 ± 0.02	0.515	0.741	0.341
20:2n-6	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.28 ± 0.01	0.000	0.072	0.032
20:3n-6	1.01 ± 0.06	1.12 ± 0.10	0.98 ± 0.10	0.84 ± 0.06	0.788	0.139	0.047
20:4n-6	7.18 ± 0.29	7.75 ± 0.63	7.12 ± 0.32	7.64 ± 0.26	0.151	0.848	0.939
20:5n-3	0.80 ± 0.09	0.78 ± 0.08	0.61 ± 0.10	0.70 ± 0.10	0.697	0.205	0.536
Punicic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.47 ± 0.04[b]	0.000	0.000	0.000
22:4n-6	0.29 ± 0.05	0.22 ± 0.03	0.25 ± 0.02	0.20 ± 0.02	0.129	0.333	0.772
22:5n-6	0.26 ± 0.05	0.25 ± 0.06	0.17 ± 0.02	0.18 ± 0.01	0.930	0.137	0.687
22:5n-3	0.45 ± 0.04	0.47 ± 0.07	0.43 ± 0.02	0.43 ± 0.02	0.840	0.350	0.820
22:6n-3	1.99 ± 0.11	2.01 ± 0.14	1.98 ± 0.13	2.19 ± 0.16	0.244	0.600	0.363

All values are mean ± SD; $n = 15$ subjects. The control group contained thirteen males and two females and the test group contained eleven males and four females.
a) Repeated measures ANOVA.
b) $p < 0.001$ versus day 0 in the test group and versus the control group.

3.2 The incorporation of PA into plasma and RBCM

No PA was detected in plasma and RBCM at day 0 in both control and test groups. After consumption of TK seeds containing 3 g PA per day for 28 days, the proportion of PA was increased to 0.47 and 0.37% (% total fatty acids) in plasma and RBCM in the test group ($p < 0.001$), whereas that was unchanged in the control group (Tables 5 and 6). These results showed that PA was incorporated into the plasma and RBCM in humans after 28 days of intervention in the test group.

3.3 The metabolism of PA into *cis*9,*trans*11-18:2 in plasma and RBCM

As shown in Tables 5 and 6, there were no significant differences in the fatty acid compositions of plasma and RBCM between two groups, with the exception of *cis*9,*trans*11-18:2 and PA. There were no significant differences in the proportion of *cis*9,*trans*11-18:2 in plasma at day 0 between two groups. The proportions of *cis*9,*trans*11-18:2 were 0.06 and 0.05% at day 0 and day 28 in the control group, respectively. The proportion of *cis*9,*trans*11-18:2 in plasma was

Table 6. Fatty acid composition of RBCMs (% total fatty acids)

	Control group		Test group		*P*-Value[a]		
	Day 0	Day 28	Day 0	Day 28	Time	Treatment	Treatment × Time
14:0	0.18 ± 0.06	0.17 ± 0.07	0.19 ± 0.10	0.16 ± 0.11	0.325	0.896	0.485
16:0	20.79 ± 1.43	18.99 ± 1.13	20.44 ± 0.83	18.91 ± 1.18	0.000	0.951	0.951
16:1n-7	0.26 ± 0.07	0.22 ± 0.06	0.24 ± 0.08	0.20 ± 0.10	0.064	0.768	0.768
18:0	13.29 ± 0.65	13.81 ± 1.12	13.44 ± 0.80	13.67 ± 1.23	0.090	0.988	0.514
18:1n-9	12.86 ± 0.63	12.66 ± 0.79	12.99 ± 1.03	12.59 ± 0.71	0.008	0.899	0.369
18:2n-6	15.83 ± 2.03	16.09 ± 2.24	16.12 ± 1.65	16.25 ± 1.58	0.391	0.733	0.781
18:3n-6	0.08 ± 0.04	0.08 ± 0.07	0.09 ± 0.05	0.06 ± 0.02	0.106	0.695	0.214
18:3n-3	0.19 ± 0.04	0.16 ± 0.04	0.21 ± 0.07	0.16 ± 0.03	0.005	0.407	0.485
*cis*9,*trans*11-18:2	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.17 ± 0.07[b]	0.000	0.000	0.000
20:0	0.23 ± 0.55	0.11 ± 0.17	0.08 ± 0.05	0.09 ± 0.07	0.462	0.252	0.391
20:1n-9	1.47 ± 0.89	1.00 ± 0.37	1.37 ± 0.55	0.90 ± 0.25	0.001	0.543	0.996
20:2n-6	0.42 ± 0.04	0.43 ± 0.06	0.44 ± 0.06	0.40 ± 0.05	0.246	0.874	0.007
20:3n-6	1.61 ± 0.21	1.66 ± 0.25	1.50 ± 0.34	1.34 ± 0.29	0.147	0.030	0.015
20:4n-6	17.59 ± 1.19	18.21 ± 1.72	17.68 ± 0.89	18.58 ± 1.39	0.006	0.580	0.582
20:3n-3	0.18 ± 0.07	0.15 ± 0.03	0.17 ± 0.07	0.14 ± 0.05	0.063	0.531	0.947
22:0	0.07 ± 0.03	0.12 ± 0.07	0.10 ± 0.10	0.17 ± 0.09	0.007	0.261	0.765
Punicic acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.15[b]	0.000	0.000	0.000
20:5n-3	0.54 ± 0.10	0.87 ± 0.73	0.75 ± 0.73	0.90 ± 0.96	0.077	0.597	0.500
22:4n-6	4.09 ± 0.56	4.15 ± 0.63	3.97 ± 0.44	4.19 ± 0.56	0.074	0.846	0.315
22:5n-6	0.93 ± 0.18	0.96 ± 0.15	0.87 ± 0.15	0.92 ± 0.13	0.039	0.335	0.468
24:1n-9	2.23 ± 0.10	2.25 ± 0.28	2.18 ± 0.63	2.45 ± 0.33	0.115	0.508	0.188
22:6n-3	5.65 ± 0.86	6.03 ± 1.25	5.38 ± 1.20	6.00 ± 1.16	0.007	0.687	0.502

All values are mean ± SD; $n = 15$ subjects. The control group contained thirteen males and two females and the test group contained eleven males and four females.

a) Repeated measures ANOVA.

b) $p < 0.001$ versus day 0 in the test group and versus the control group.

0.06% at day 0, whereas that was increased to 0.23% after 28 days of intervention in the test group ($p < 0.001$).

Trace of *cis*9,*trans*11-18:2 was detected, account 0.03% of total fatty acid in the RBCM at day 0 in both the control and test groups. The proportion of *cis*9,*trans*11-18:2 in RBCM was increased by six-fold to 0.17% in the test group after 28 days of intervention ($p < 0.001$), whereas that was unchanged in the control group.

4 Discussion

The incorporation and metabolism of PA in humans were investigated in the present study. To our knowledge, this is the first study demonstrating the incorporation and metabolism of CLnA isomer in humans using a unique food product, TK seeds. TK seeds have long been used as a snack food in China without known adverse effects.

The present work showed that the PA was incorporated into the plasma and RBCM in humans after supplementation with 3 g per day of PA for 28 days. Similar results were observed that conjugated fatty acid other than PA can be incorporated into plasma and cellular lipids in humans [32, 33].

Previous studies in rats by Tsuzuki *et al*. [17 – 19] observed that PA can be converted into *cis*9,*trans*11-18:2. They investigated the absorption and metabolism of PA using a lipid absorption assay in lymph from the thoracic duct and found that the majority of PA was slowly absorbed in an unchanged state in rat intestine and part of the absorbed PA was quickly converted into CLA in rat intestine [17]. Our study further showed that PA can be converted into *cis*9,*trans*11-18:2 in various rat tissues, such as liver, plasma, adipose tissue, kidney, and brain [20]. In the present study, we showed that PA can be incorporated into plasma and RBCM and partial of PA can also be metabolized into *cis*9,*trans*11-18:2 in humans. The data obtained in the present study will provide further information on the conversion of PA into CLA *in vivo*.

The mechanism of conversion of PA into *cis*9,*trans*11-18:2 *in vivo* is not clear. A Δ13-saturation reaction which converted CLnA into *cis*9,*trans*11-18:2 in rats and mice was proposed by Tsuzuki *et al*. [17]. The present study strongly suggests that PA can also be metabolized into *cis*9,*trans*11-18:2 in humans. It is possible that PA was converted into *cis*9,*trans*11-18:2 in humans in a similar pathway with in animals. However, the enzyme that catalyzed the reaction of Δ13-saturation in animals and humans is not identified so far, although Tsuzuki *et al*. [17, 19] suggested that an NADPH-dependent enzyme which is either a novel enzyme recognizing conjugated trienoic acid or the enzyme active in the Leukotriene B4 reductive pathway could carried this Δ13-saturation reaction in rats and mice. Further studies are

warranted to provide a clearer understanding of the mechanism of conversion of PA into CLA in humans and animals.

In the present study, subjects consumed 3 g per day of PA for 28 days, the proportion of *cis*9,*trans*11-18:2 in plasma was significantly increased from 0.06 to 0.23% and in the RBCM from 0.03 to 0.17% ($p < 0.001$), respectively. In other studies, when subjects consumed a similar amount of CLA mixtures (50:50% *cis*9,*trans*11:*trans*10,*cis*12 isomers) for 8 wk, the proportion of *cis*9,*trans*11-18:2 in plasma was significantly increased from 0.23 to 0.71% [34], and in another study from 0.33 to 0.62% [35]. Different baseline levels of CLA between the present study and the other studies might be caused by low consumption of ruminant meat and related products in Chinese population. In regard to the proportion of CLA in human plasma and RBCM, the consumption of these seeds rich in PA could compensate the relative low consumption of ruminant meat and related products in Chinese food.

The observation that PA could be converted into *cis*9,*trans*11-18:2 has gained increased importance since it has been demonstrated that *cis*9,*trans*11-18:2 can exert many biological activities [2 – 5]. The *cis*9,*trans*11-CLA was naturally only found in limited sources such as ruminant with a very low level [36]. A mixture of positional and geometrical isomers has been obtained by alkaline isomerized linoleic acid-rich oil, which is commercially used as a health supplement CLA. On the other hand, PA is present in some plant seeds, such as TK and pomegranate in large amounts, and they can be purified relatively easily. Furthermore, TK seed kernels, a natural resource containing PA can be ingested directly. To our knowledge, TK seed kernels have long been used as a snack food in China and is the only natural edible food source containing PA. Therefore, natural sources of PA, such as edible TK seeds, could be a potential dietary source of tissue CLA, presumably as a result of conversion of PA into *cis*9,*trans*11-18:2 in humans. The present study showed that intake of TK seeds can increase the proportion of *cis*9,*trans*11-18:2 in humans, thereby it could be an effective way to increase the proportion of CLA in humans.

In conclusion, PA can be effectively incorporated into human plasma and RBCM, and also is associated with the increasing proportion of *cis*9,*trans*11-18:2 in humans. Edible TK seeds could be a potential dietary source of CLA, as a result of conversion of PA into *cis*9,*trans*11-18:2 in humans.

The authors have declared no conflict of interest.

5 References

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