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Applied nutritional investigation

A comparison of *L*-carnitine and several cardiovascular-related biomarkers between healthy vegetarians and omnivores

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A B S T R A C T

Objective: A plant-based diet has been associated with a reduced risk of cardiovascular (CV) diseases. This study aimed to determine the levels and correlations of CV-related biomarkers and the beneficial role of dietary habits.
Methods: A total of 63 healthy vegetarians ($n = 32$) and omnivores ($n = 31$) were recruited. The baseline characteristics were recorded and measured (including lipid profiles, blood glucose, etc.). Liquid chromatography–mass spectrometry method was developed for the simultaneous determination of seven circulating CV-related biomarkers.
Results: *L*-carnitine (*L*-Car), *L*-methionine, and ascorbic acid (AA) were significantly higher in vegetarians than in omnivores. In the vegetarians, *L*-Car had a negative correlation with triacylglycerols ($P = 0.042$) and blood glucose ($P = 0.048$) and a positive correlation with high-density lipoprotein cholesterol ($P = 0.049$). *L*-Car was also positively correlated with *L*-lysine ($P = 0.009$), *L*-methionine ($P = 0.006$), and AA ($P = 0.035$). The vegetarians' AA also had a negative correlation with *L*-homocysteine ($P = 0.028$). In the omnivores, *L*-Car was negatively correlated with total cholesterol ($P = 0.008$), low-density lipoprotein cholesterol ($P = 0.004$), and high-density lipoprotein cholesterol ($P = 0.038$). Omnivores' body mass index was positively correlated with *L*-homocysteine ($P = 0.033$), and age was positively correlated with trimethylamine *N*-oxide ($P < 0.001$) and blood glucose ($P = 0.007$), but not in vegetarians.
Conclusions: Our results suggest that vegetarians have an elevated level of *L*-Car, which might be associated with endogenous biosynthesis and diet composition. Circulating *L*-Car might play an important role in CV protection, especially in vegetarians.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of disability and mortality globally [1]. People with CVDs or at high cardiovascular (CV) risk (because of the presence of one or more risk factors such as hypertension, diabetes, hyperlipidemia, or already established disease) need early detection and management using counseling. Nutrition may even be the most important factor in preventing premature CVD disability and mortality, surpassing other habits such as no smoking and physical activity [2]. The potentially beneficial role of plant-based diets in CV health has been increasingly recognized [3–5]. Plant-based diets are typically rich in fiber, dietary nitrates, and micronutrients such as magnesium, potassium, and antioxidants [6]. However, the advantages of the plant-based diet in CVDs are still mostly conceptually understood, although vegetarians generally have better CV risk profiles and serum homocysteine has been reported to be higher in vegetarians than in omnivores because of a

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relative vitamin B_{12} deficiency [7]. Therefore more CV-related biomarkers are needed for scientific research to confirm and should be easy to quantify in circulation [8].

L-Carnitine (β-hydroxy-γ-*N*-trimethylaminobutyric acid; *L*-Car) is a conditional nutrient that is necessary in humans for energy production through mitochondrial β-oxidation of long-chain fatty acids [9]. *L*-Car deficiency is associated with impaired fatty acid and glucose utilization and insulin sensitivity [10]. In humans, 75% of *L*-Car is obtained from the diets of animal origin [11]; thus the vegetarians obtain only negligible amounts [12]. The lost or insufficient *L*-Car is replenished by dietary *L*-Car (exogenous) and by endogenous synthesis [13]. Ringseis et al. [10] indicated that endogenous synthesis provides 90% of total body carnitine in strict vegetarians and approximately 25% in omnivores. Therefore a situation quite possible with vegetarians should have the maximally activated endogenous synthesis, bioavailability, and more efficient kidney reabsorption to compensate for the deficiency of dietary *L*-Car [14,15]. *L*-Car not obtained from food is synthesized endogenously from two essential amino acids, *L*-lysine (*L*-Lys) and *L*-methionine (*L*-Met), in the liver and kidneys [16]. Early studies indicated that vegetarians may be considered to have lower systemic *L*-Car because of a lack of *L*-Lys and *L*-Met [17,18]. Such apparently contradictory results can probably be attributed to geographic differences, notably to diet composition [19–21].

In the present study a liquid chromatography–mass spectrometry (LC-MS) method was developed for the simultaneous determination of seven circulating CV-related biomarkers included *L*-Car, *L*-Met, *L*-Lys, *L*-homocysteine (*L*-Hcy), methylmalonic acid (MMA), trimethylamine *N*-oxide (TMAO) and *L*-ascorbic acid (AA). The present study aimed to investigate the circulating levels of the seven CV-related biomarkers among Taiwanese healthy vegetarians and omnivores. We also identified their correlation with age, body mass index (BMI), blood glucose, and lipid profiles in both groups to provide more evidence of CV protection for plant-based diets.

Materials and methods

Reagents and chemicals

L-Car, *L*-Met, *L*-Lys, *L*-Hcy, MMA, TMAO, AA, sodium phosphate dibasic heptahydrate, and d3-*L*-Met were purchased from Sigma-Aldrich (St. Louis, MO, USA). The d9-TMAO (trimethylamine-d9 *N*-oxide) was obtained from Cambridge Isotope Laboratories (Tweksbury, MA, USA). Sulfadimethoxine (SDM) was purchased from Waters (Milford, MA, USA). Formic acid (FA) was obtained from Honeywell Fluka (Seelze, Germany). Methanol (MeOH) was purchased from Merck (Rahway, NJ, USA). Ethyl acetate was purchased from Avantor (Allentown, PA, USA). Novum Simplified liquid extraction (SLE) columns were obtained from Phenomenex (Torrance, CA, USA).

Standards preparation and calibration curves

The standard stock solutions of *L*-Car, *L*-Met, *L*-Lys, *L*-Hcy, MMA, TMAO, AA, d3-*L*-Met, and SDM were prepared in 0.1 mg/mL and stored at −20°C. The different concentrations were prepared before use and further diluted with methanol. The calibration curves were made for *L*-Car, *L*-Met, *L*-Lys, *L*-Hcy, MMA, TMAO, and AA, respectively. The d3-*L*-Met and d9-TMAO were used for positive ion mode internal standards, and SDM was used for negative ion mode internal standard. The calibration curve formulas were revealed with the peak area ratio.

Human blood plasma collection and preparation

Healthy participants' plasma was collected from the dermatology clinic of the Buddhist Tzu-Chi General Hospital between May 2016 and April 2018. Participants' age range was 30 to 75 y. We collected a total of 63 human plasma samples and divided them into two groups: vegetarians and omnivores. We also excluded participants who were pregnant, smokers, alcoholics, and those addicted to drugs. We interviewed all the participants to confirm their preliminary lifestyle, diet habits, and vitamin B complex supplementation. The selection criteria also included being without diabetes, chronic kidney disease, hepatic disease, autoimmune disease, and cancer. The study was carried out in accordance with the Declaration of Helsinki, and the research project was also approved by the ethics committee of the Buddhist Tzu-Chi General Hospital. All participants signed consent forms and agreed to participate in the study.

Whole blood samples were collected in Vacutainer K2 E-EDTA tubes (BD Diagnostics, Franklin Lakes, NJ, USA) and centrifuged at 3000 rpm at room temperature (RT) for 10 min. The supernatant plasma was collected, and 1 N 1% acetic acid was added to prevent degradation. All plasma samples were stored at −80°C until analysis with a storage time of less than 1 year.

Human blood plasma extraction and determination

Results of triacylglycerols (TG), total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) was obtained from the Buddhist Tzu-Chi General Hospital. In plasma extraction, the plasma samples were thawed at RT for 10 to 20 min and transferred 100 μL plasma into other new 1.5-mL centrifuge tubes. Then, 100 μL of 50-mM sodium phosphate dibasic heptahydrate solution was added for half dilution and mixed briefly. The plasma samples were deproteinized by the addition of 3 parts of methanol and incubated at RT for 20 min. After centrifugation at 3,000 xg for 10 min at 4°C, the supernatant was collected. The sample was loaded onto a Novum SLE column and gentle pulse of vacuum until the sample moved into the filter valve. After a 5-min wait, 1.2 to 1.5 mL ethyl acetate was added for extraction. Nitrogen (N_2) gas was used to dry the sample, which was reconstituted in 100 μL methanol per internal standards. The sample was mixed thoroughly and transferred into LC-MS sample vials for 30 μL injection.

LC-MS conditions

Waters e2695 high-performance liquid chromatography system connected with a single quadrupole mass spectrometer (ACQUITY QDa, Waters Corp.) was used for the study. A Phenomenex Luna C18(2) column (5 μm, 250 × 4.60 mm, 100 Å) was used and combined with a guard cartridge system (KJ0-4282, Phenomenex). The temperature of the column was set at 40°C, and the flow rate of the mobile phase was set at 0.8 mL min^{-1}. The mobile phase was composed of A (99.9% ddH_2 O contain with 0.1% FA) and B (99.9% gradient grade MeOH and contain with 0.1% FA). The gradient of the mobile phase was started from 5% B to 70% B for 14 min and then held for 2 min and 70% B to 50% B for another 2 min. The MS-QDa detector settings were as follows: vaporization temperature 400°C, capillary voltage 0.8 kV, and sample cone 15.0 V. The single ion recording mode was used for MMA 117.1 *m/z*, AA 174.95 *m/z*, and SDM 309.3 *m/z* negative ion mode; and *L*-Lys 147.1 *m/z*, *L*-Met 150.1 *m/z*, *L*-Car 162.1 *m/z*, *L*-Hcy 136.1 *m/z*, TMAO 76.0 *m/z*, d9-TMAO 85.1 *m/z*, and d3-*L*-Met 154.1 *m/z* positive ion mode. The detection results were quantified by peak areas and compared with calibration curves obtained from standard solutions.

Statistical analysis

The SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA) statistical software packages were used for analyzing the data. Data are presented as the mean ± standard deviation. The parametric independent samples *t* test and the nonparametric Mann-Whitney *U* test were used to compare differences between the two groups. The normality of the distribution was determined using the Shapiro-Wilk test and the Pearson (for a normal distribution) or Spearman (for a non-normal distribution) correlation coefficient was used to analyze the relationship between the two groups. The level of statistical significance was set at a $P < 0.05$.

Results

Sample collection

A total of 63 participants (32 vegetarians and 31 omnivores) were involved in the analysis. The average age was 53.4 ± 11.1 y, and the vegetarian group was 65.6% female and 54.8% in the omnivore group. In the vegetarian group, the duration of vegetarianism was 13.1 ± 8.7 y, and all were lacto-ovo vegetarians.

Linearity

The LC-MS gradient condition was modified from a previous study [22] and updated according to our ideas. The retention times of the analytes were approximately 2.528 min for *L*-Car, 2.383 min for *L*-Lys, 3.277 min for *L*-Met, 3.359 min for *L*-Hcy, 2.379 min for 3-MH

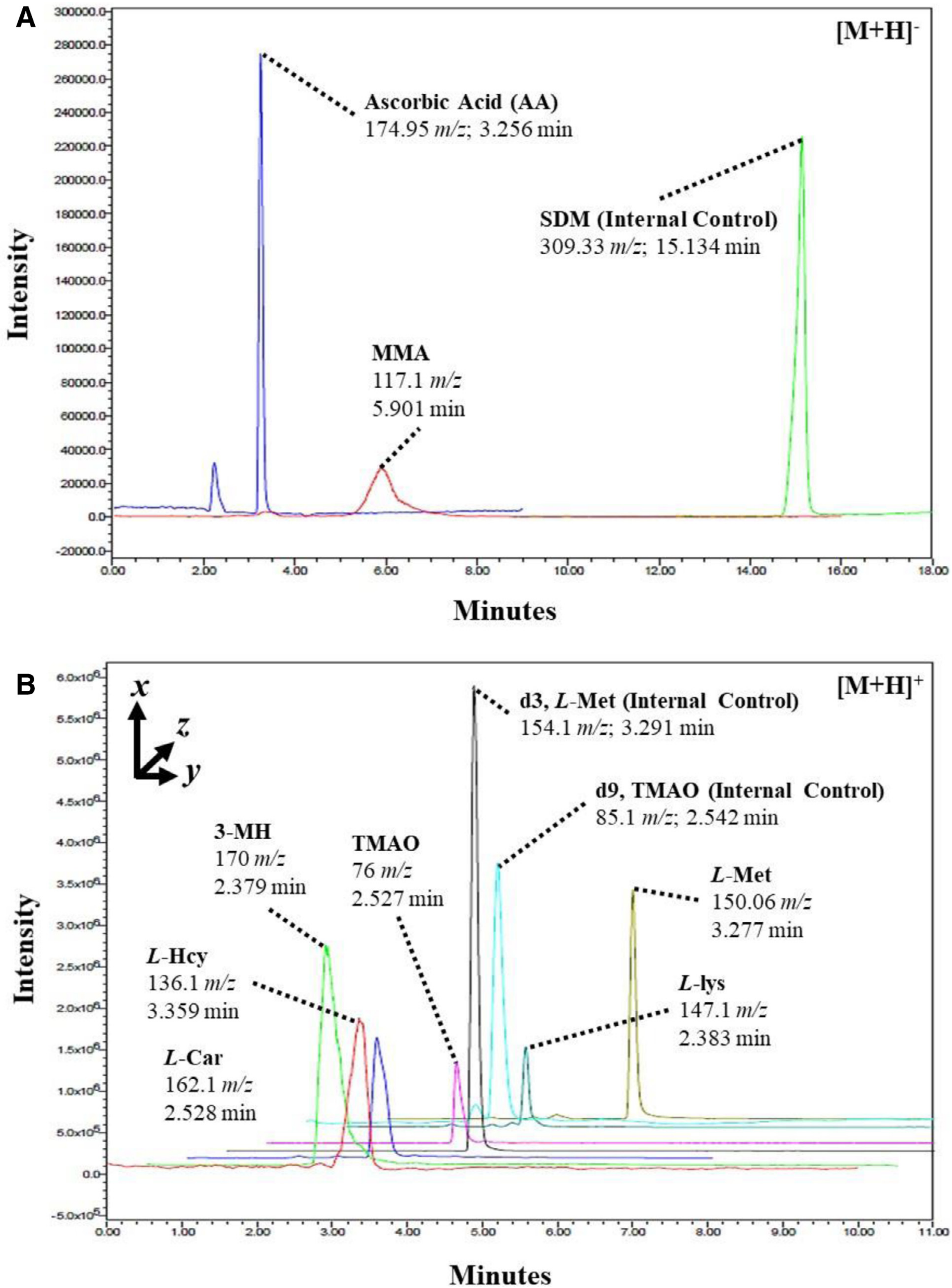

Fig. 1. Single ion recording (SIR) mode of LC-MS analysis. (A) Negative ion mode detection: MMA (117.1 *m/z*), AA (174.95 *m/z*) and d3, *L*-Met (154.1 *m/z*). (B) Positive ion mode detection: *L*-Met (150.06 *m/z*), *L*-lys (147.1 *m/z*), *L*-Hcy (136.1 *m/z*), 3-MH (170 *m/z*), *L*-Car (162.1 *m/z*), TMAO (76 *m/z*), d9, TMAO (85.1 *m/z*) and d3, *L*-Met (154.1 *m/z*). $[M+H]^-$, negative ion mode; $[M+H]^+$, positive ion mode; MMA, methylmalonic acid; SDM, sulfadimethoxine; *L*-Met, *L*-methionine; *L*-Lys, *L*-lysine; *L*-Hcy, *L*-homocysteine; 3-MH, 3-methylhistidine; *L*-Car, *L*-carnitine; TMAO, trimethylamine *N*-oxide.

(3-methylhistidine), 5.901 min for MMA, 2.527 min for TMAO, 3.256 min for AA, 15.134 min for SDM, 2.542 min for d9-TMAO, and 3.219 min for d3-*L*-Met. The calibration curves were further calculated, and all the determination coefficients (R^2) of linearity were ≥0.995. LC-MS single ion recording mode was used for single ion analysis, and the sample standard results are shown in Figure 1.

Characteristics of the collection participants and plasma detection

The baseline characteristics in the blood biochemical test are shown in two groups. There were no significant differences in age, body mass index (BMI), blood glucose, TCH, TG, HDL-C, or LDL-C between vegetarians and omnivores (Table 1). However, no differences between the two groups were found for MMA, *L*-Lys, *L*-Hcy, and TMAO. Interestingly, we found that the levels of *L*-Car (4759.1 ± 1474.2 versus 3912.8 ± 1084.2 μg/L, $P = 0.012$), *L*-Met (5650.0 ± 1860.0 versus 4715.4 ± 1840.9 μg/L, $P = 0.049$), and AA (3809.9 ± 1390.6 versus 2864.2 ± 1249.0 μg/L, $P = 0.006$) were significantly higher in vegetarians than in omnivores (Table 2).

Correlations of L*-Car levels and lipid profiles*

The correlation of *L*-Car and lipid profiles are shown in Figure 2A–E. The level of circulating *L*-Car was indicated to have a significant negative correlation with TG ($r = -0.367$, $P = 0.042$) and a significant positive correlation with HDL-C ($r = 0.356$, $P = 0.049$) in vegetarians. The *L*-Car was revealed to have a significant negative correlation with TCH ($r = -0.471$, $P = 0.008$), HDL-C ($r = -0.375$, $P = 0.038$), and LDL-C ($r = -0.506$, $P = 0.004$) in omnivores. Furthermore, *L*-Car was also found to have a significant negative correlation with blood glucose ($r = -0.271$, $P = 0.048$) in the vegetarian group.

Correlations of L*-Lys,* L*-Met, and AA with* L*-Car levels*

The biosynthesis capacity and correlation of *L*-Lys, *L*-Met, and AA with endogenous *L*-Car levels were curious. We found that *L*-Car had a significant positive correlation with *L*-Lys ($r = 0.454$, $P = 0.009$) and *L*-Met ($r = 0.473$, $P = 0.006$) in the vegetarian group but not in the omnivore group. Our results also indicated a significant positive correlation between *L*-Car and AA ($r = 0.374$, $P = 0.035$) in vegetarians but not in the omnivores ($r = 0.190$, $P = 0.305$) (Fig. 3 A–C). A significant positive correlation of *L*-Car levels with *L*-Lys, *L*-Met, and AA in the vegetarian group indicates that a significant portion of the *L*-Car requirement is covered by endogenous synthesis.

Comparison with other CV-related markers in vegetarians and omnivores

We tried to use more cardiovascular-related markers to further evaluate vegetarians and omnivores. These results are shown in Figure 4A–D. We found that AA revealed a significant negative correlation with *L*-Hcy ($r = -0.388$, $P = 0.028$) in vegetarians but not in omnivores ($r = -0.194$, $P = 0.296$). The age of omnivores also had a significant positive correlation with TMAO ($r = 0.574$, $P < 0.001$) and blood glucose ($r = 0.479$, $P = 0.007$). However, these phenomena were not found in the vegetarian group. Moreover, the BMI levels had a positive correlation with *L*-Hcy ($r = 0.436$, $P = 0.033$) in omnivores but not in vegetarians ($r = 0.260$, $P = 0.182$).

Discussion

In this study we found that vitamin B12 is not deficient in Taiwanese vegetarians because the plasma MMA (marker of vitamin B12 insufficiency) [23] was in the normal range (<280 nmol/L) and there was no difference in omnivores. Hence, the level of *L*-Hcy was not significantly different between the two groups. The questionnaires further revealed that more than half of the vegetarians usually take vitamin B complex supplements. It seems that modern vegetarians in Taiwan have a more advanced nutritional knowledge.

Our data indicate that the vegetarians have higher plasma *L*-Car ($P = 0.012$), *L*-Met ($P = 0.049$), and AA ($P = 0.006$) levels than the

Table 1
Participant baseline characteristics

Characteristics	Vegetarians (*n* = 32) (male *n* = 11, female *n* = 21) mean ± SD	Omnivores (*n* = 31) (male *n* = 14, Female *n* = 17) mean ± SD	*P*
Age (y)	54.7 ± 9.3	51.8 ± 13.0	0.343
Body mass index (BMI, kg/m^2)	24.1 ± 2.7	25.1 ± 3.3	0.262
Duration of vegetarianism (y)	13.1±8.7	–	–
Non-smokers	100%	100%	–
Blood glucose (mg/dL)	97.3 ± 35.7	99.5 ± 34.9	0.206
Triacylglycerol (TG, mg/dL)	123.9 ± 59.1	127.1 ± 60.7	0.833
Total cholesterol (TCH, mg/dL)	171.4 ± 28.3	168.5 ± 31.3	0.700
High-density lipoprotein cholesterol (HDL-C, mg/dL)	55.5 ± 13.1	51.6 ± 10.4	0.206
Low-density lipoprotein cholesterol (LDL-C, mg/dL)	101.3 ± 27.9	98.7 ± 26.7	0.708

Table 2
Plasma markers detection by LC-MS

Biomarkers	Vegetarians (*n* = 32) Mean ± SD (μg/L)	Omnivores (*n* = 31) Mean ± SD (μg/L)	*P*
L-Carnitine (*L*-Car)	4759.1 ± 1474.2	3912.8 ± 1084.2	0.012*
Methylmalonic acid (MMA)	31.3 ± 23.4	32.9 ± 28.3	0.801
L-Lysine (*L*-Lys)	23453.7 ± 6001.4	22061.5 ± 6655.5	0.386
L-Methionine (*L*-Met)	5650.0 ± 1860.0	4715.4 ± 1840.9	0.049*
L-Homocysteine (*L*-Hcy)	951.7 ± 426.8	1021.0 ± 498.8	0.556
Trimethylamine *N*-oxide (TMAO)	281.6 ± 58.3	275.3 ± 68.5	0.699
Ascorbic acid (AA)	3809.9 ± 1390.6	2864.2 ± 1249.0	0.006†

LC-MS, liquid chromatography–mass spectrometry
*$P < 0.05$
†$P < 0.01$, and ‡$P < 0.001$ statistical significance.

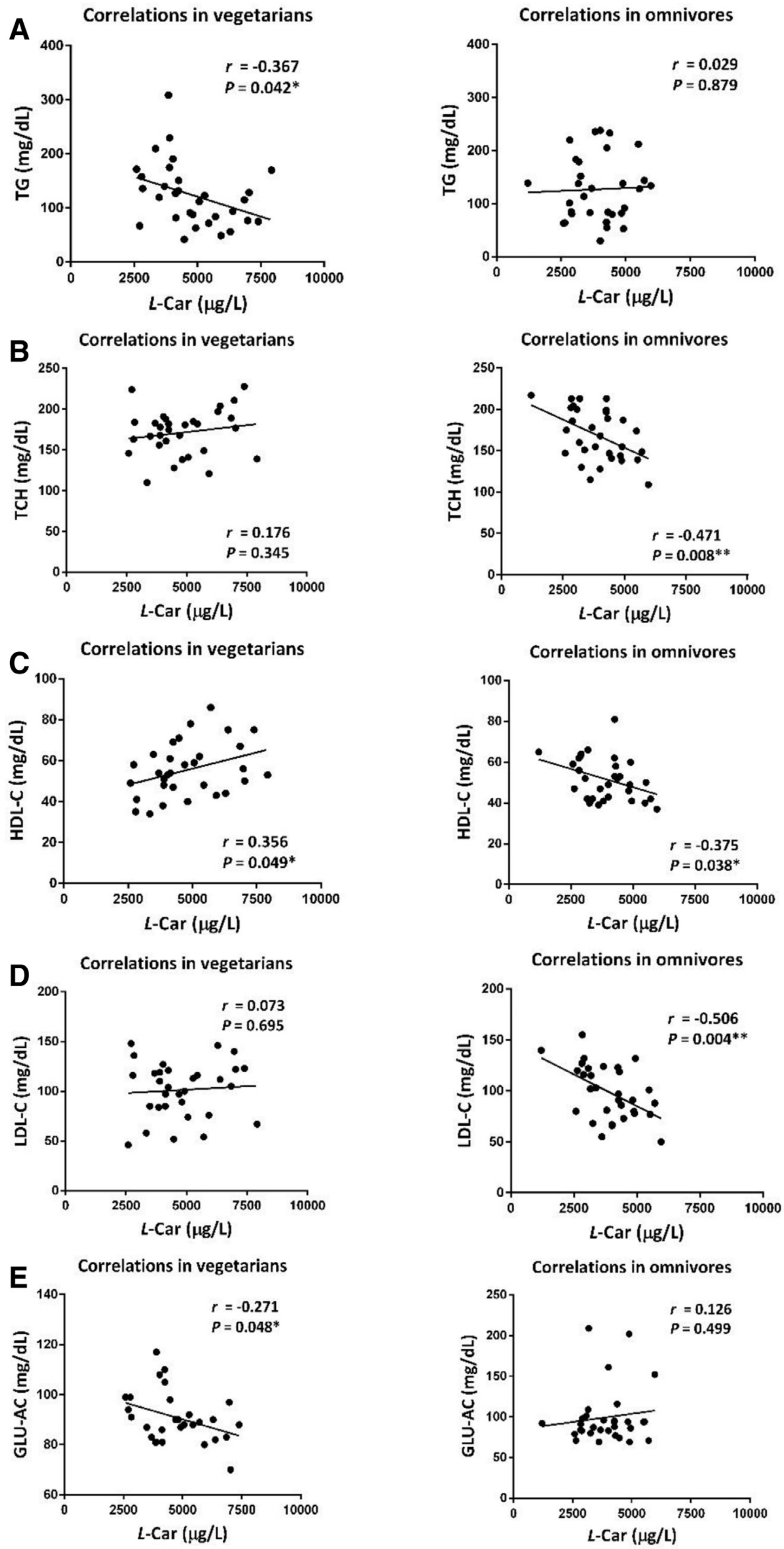

Fig. 2. Correlation of *L*-Car with lipid profiles and blood glucose. (A) Correlation of *L*-Car and TG, (B) correlation of *L*-Car and TCH, (C) correlation of *L*-Car and HDL-C, (E) correlation of *L*-Car and LDL-C, and (F) the correlation of *L*-Car and GLU-AC in vegetarians and omnivores. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. TG, triacylglycerol; TCH, total cholesterol; HDL-C, high-density lipoprotein cholesterol; *L*-Car, *L*-carnitine; LDL-C, low-density lipoprotein cholesterol; GLU-AC, the fasting blood glucose.

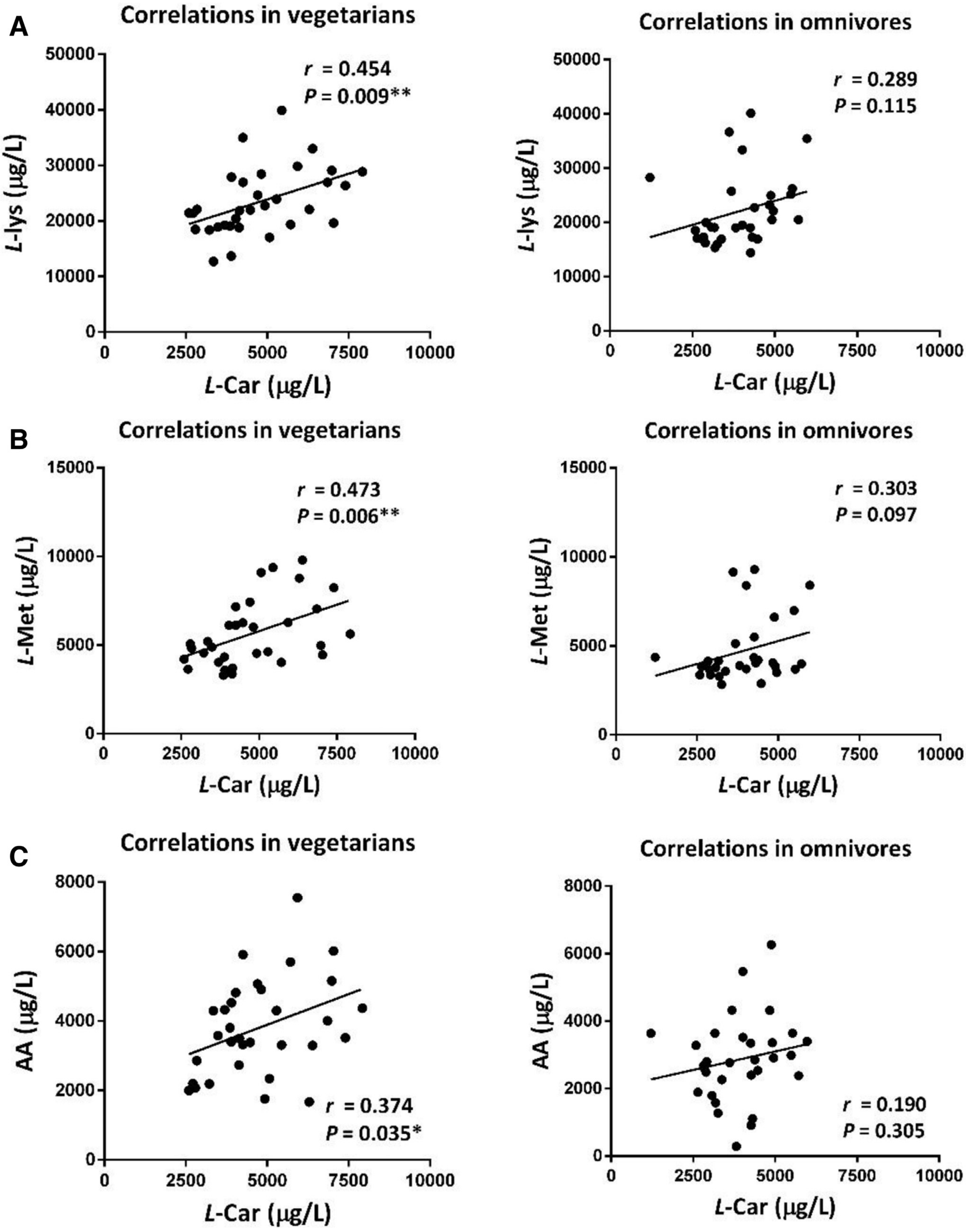

Fig. 3. Correlations of *L*-Car with *L*-Lys, *L*-Met, and AA. (A) Correlation of *L*-Car and *L*-Lys, (B) correlation of *L*-Car and *L*-Met, and (C) the correlation of *L*-Car and AA in vegetarians and omnivores. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *L*-Car, *L*-carnitine; *L*-Lys, *L*-lysine; *L*-Met, *L*-methionine; AA, ascorbic acid.

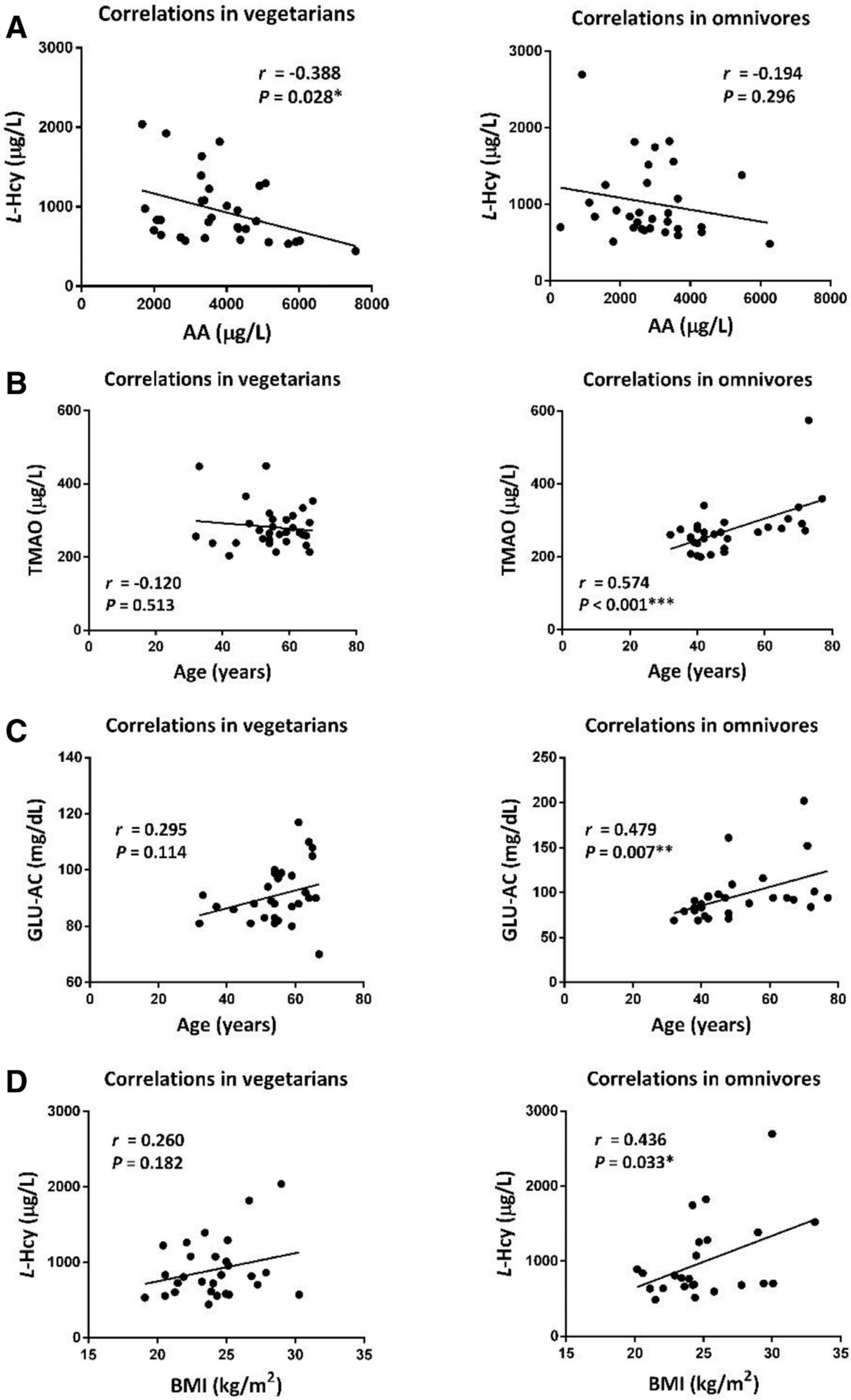

Fig. 4. Correlation of other CVD-related markers in vegetarians and omnivores. (A) Correlation of AA and *L*-Hcy, (B) Correlation of age and TMAO, (C) correlation of age and GLU-AC, (D) and the correlation of BMI and *L*-Hcy in vegetarians and omnivores. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *L*-Hcy, *L*-homocysteine; AA, ascorbic acid; TMAO, trimethylamine *N*-oxide; GLU-AC, the fasting blood glucose; BMI, body mass index.

omnivores. The TG, TCH, HDL-C, and LDL-C had no significant difference between the two groups. BMI, blood glucose, MMA, *L*-Lys, and TMAO were not significantly different between the two groups. Interestingly, we found that Taiwanese vegetarians not only had sufficient *L*-Car but also had higher *L*-Car than omnivores (4759.1 ± 1474.2 versus 3912.8 ± 1084.2 μg/L, $P = 0.012$). This could be attributed to several reasons. First, we found plasma *L*-Met and AA levels in Taiwanese vegetarians were higher than in omnivores. Except for *L*-Lys, *L*-Met and AA are involved in endogenous *L*-Car synthesis and self-regulated to further compensate for lipid metabolism in vegetarians [16,24]. In our results, *L*-Car was significantly positively associated with *L*-Lys, *L*-Met, and AA in vegetarians but not in omnivores. These results are similar to previous studies [17,25]. Second, a situation quite possible with vegetarians is that they should have the maximally activated endogenous *L*-Car synthesis, bioavailability, and more efficient kidney reabsorption to compensate for the deficiency of dietary *L*-Car [14,15]. Moreover, Blancquaert et al. [26] indicated that endogenous *L*-Car has a slow turnover rate (300–500 μmol/d) and might be maintained with homeostasis in vegetarianism. Third, from the geographic difference in vegetarian diet, most Taiwanese vegetarians follow the diet either for health purposes or religious beliefs, such as in the Buddhist population. Most Taiwanese vegetarians eat dairy products and eggs in addition to plant-based food (lacto-ovo vegetarians) [19] and have a large intake of soybean products, which are the major substitutes for animal products [27]. Moreover, exogenous *L*-Car has been found naturally in dairy and fermented soybean products [28,29]. Fourth, previous nutritional survey research indicated that Taiwanese vegetarians consumed fewer calories, more carbohydrates, and less protein and fat [27]. Luci et al. [30] indicated that endogenous *L*-Car biosynthesis was increased in a caloric restriction rat model.

In vegetarian participants we found that *L*-Car had a negative correlation with TG ($r = -0.367$, $P = 0.042$) and a positive correlation with HDL-C ($r = 0.356$, $P = 0.049$), but the *L*-Car had a negative correlation with TCH ($r = -0.471$, $P = 0.008$), HDL-C ($r = -0.375$, $P = 0.038$), and LDL-C ($r = -0.506$, $P = 0.004$) in omnivores, further suggesting that *L*-Car may play an important role in CV risk regulation. Some studies have already indicated that the antioxidant [31] and antiinflammatory effects of *L*-Car occur via reactive oxygen species suppression and further inhibit the nuclear factor κ–light-chain-enhancer of activated B cells signaling pathway [32,33]. A previous study involving a rat model of *L*-Car deficiency found that systemic endogenous *L*-Car deficiency can lead to abnormalities in myocardial function [34]. *L*-Car has been found to upregulate HDL-C in patients with coronary artery disease after supplementation (1000 mg/d) [35,36]. Malaguarnera et al. [37] also found that after a high dose (2000 mg/d) of *L*-Car supplementation, the levels of TCH, TG, LDL-C, and oxidized LDL were decreased and the level of HDL-C was increased in patients with type 2 diabetes. Nevertheless, there were four people with higher blood glucose levels (>120 mg/dL) among omnivore participants. To answer the doubt that it may further affect the correlation between *L*-Car and blood glucose, we also tried to remove their data and further made the new correlation. According to this result, the correlation between *L*-Car and blood glucose still has a non-significant difference even after removing these four participants ($r = -0.060$, $P = 0.765$). On the other hand, our results suggest a negative correlation between *L*-Car and blood glucose ($P = 0.048$) in vegetarians. These results suggest that endogenous *L*-Car might play an important role in CV protection, especially in vegetarians.

TMAO is an intestinal microbiota–dependent metabolite formed from dietary trimethylamine-containing nutrients, including *L*-Car, choline, and phosphatidylcholine [38]. High concentrations of TMAO have been related to several vascular risks, but the role of TMAO in atherosclerosis remains controversial [39]. Therefore, to avoid the doubt that high levels of *L*-Car may have caused an increased TMAO concentration, we also monitored plasma TMAO. In both groups, plasma TMAO was low and did not expose high atherosclerosis risk in healthy participants (lower than hemodialysis patients after *L*-Car supplementation, approximately 41.2 mg/L) [40].

Furthermore, the AA level was also significantly higher in the vegetarians than in the omnivores. AA also had a positive correlation with *L*-Car and a negative correlation with *L*-Hcy in vegetarians. These results suggest that the vegetarians may have better antioxidant activity and free radical scavenging capacity than omnivores. On the other hand, the correlations among age versus TMAO, age versus blood glucose, and BMI versus *L*-Hcy revealed a positive correlation in omnivores but not in vegetarians. As age or body weight increases, meat-based diet habits may further promote CVD risk.

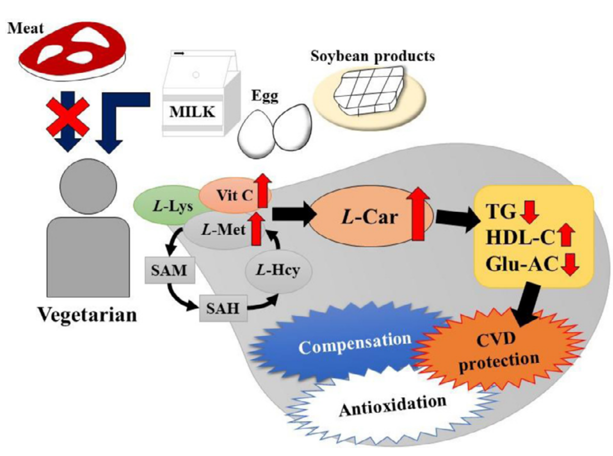

Fig. 5. Schematic diagram of vegetarian diets and CVD protection. Without animal protein intake, the exogenous *L*-Car might decrease. *L*-Car might be produced by *L*-Lys and *L*-Met and further transformed via the methionine cycle in the circulatory system. The high *L*-Car level has been measured in blood plasma, revealing a compensatory effect in vegetarians. The level of *L*-ascorbic acid (AA) was also significantly higher in vegetarians than in omnivores. Therefore, most of the *L*-Lys, *L*-Met, and AA might be involved in *L*-Car synthesis and further increase the *L*-Car level and antioxidant capacity in vegetarians. *L*-Car revealed the beneficial effects of lipid profile regulation and further contributes to CVD prevention in vegetarians. *L*-Lys, *L*-lysine; *L*-Met, *L*-methionine; *L*-Hcy, *L*-homocysteine; SAH, S-adenosyl-*L*-homocysteine; SAM, S-adenosyl-*L*-methionine; Vit C, ascorbic acid; *L*-Car, *L*-carnitine; TG, triacylglycerol; HDL-C, high-density lipoprotein cholesterol; GLU-AC, the fasting blood glucose; CVD, cardiovascular disease.

Several limitations of this study should be considered. A small group of vegetarians was studied and all were lacto-ovo vegetarians but relatively homogeneous, with the same food culture. Nutritional supplement consumed by vegetarians, and mentioned in this study is vitamin B complex, but the detailed composition is not clear. Although we found some important correlations of *L*-Car in this study, the comparison of *L*-Car with other cardiovascular-related biomarkers remains to be determined.

Conclusions

The present study found significantly higher plasma *L*-Car levels in Taiwanese lacto-ovo vegetarians than in omnivores. Higher levels of *L*-Car in the vegetarians might be associated with more efficient endogenous biosynthesis and a large intake diet of soybean products (Fig. 5). Therefore *L*-Car might play an important role in CV protection, especially in vegetarians.

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