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ORIGINAL CONTRIBUTION

The effect of almonds on inflammation and oxidative stress in Chinese patients with type 2 diabetes mellitus: a randomized crossover controlled feeding trial

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Abstract
Purpose Almond consumption is associated with ameliorations in obesity, hyperlipidemia, hypertension, and hyperglycemia. The hypothesis of this 12-week randomized, crossover, controlled feeding trial was that almond consumption would ameliorate inflammation and oxidative stress in Chinese patients with type 2 diabetes mellitus (T2DM) (9 M, 11 F; 58 years; BMI: 26 kg/m^2) with mild hyperlipidemia.
Methods After a 2-week run-in period, the patients were assigned to either a control NCEP step II diet (control diet) or almond diet for 4 weeks with a 2-week washout period between alternative diets. Almonds approximately at 56 g/day were added to the control diet to replace 20 % of total daily calorie intake.
Results As compared to the control diet, the almond diet decreased IL-6 by a median 10.3 % (95 % confidence intervals 5.2, 12.6 %), CRP by a median 10.3 % (−24.1, 40.5), and TNF-α by a median 15.7 % (−0.3, 29.9). The almond diet also decreased plasma protein carbonyl by a median 28.2 % (4.7, 38.2) as compared to the C diet but did not alter plasma malondialdehyde. The A diet enhanced the resistance of LDL against Cu^{2+}-induced oxidation by a median 16.3 % (7.4, 44.3) as compared to the C diet. Serum intercellular adhesion molecule-1 and vascular adhesion molecule-1 were not changed by both diets.
Conclusions Our results suggested that incorporation of almonds into a healthy diet could ameliorate inflammation and oxidative stress in patients with T2DM.

Keywords Almonds · Antioxidants · Inflammation · Oxidative stress · Type 2 diabetes mellitus

Abbreviations

T2DM	Type 2 diabetes mellitus
CVD	Cardiovascular disease
MDA	Malondialdehyde
CRP	C-reactive protein
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor-α
LDL	Low density protein
ICAM-1	Soluble intra-cellular adhesion molecule-1
VCAM-1	Soluble vascular adhesion molecule-1
FRAP	Ferric reducing antioxidant power assay

Introduction

Patients with type 2 diabetes mellitus (T2DM) are at a greater risk for a myriad of other health complications

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including cardiovascular disease (CVD), certain cancers, retinopathy, neuropathy, and nephropathy, and gallstone disease than those who do not have diabetes [1]. The increased risk for CVD that contributed to 75 % mortality among T2DM patients is mainly attributed to common pathologies shared in diabetes and CVD, that is, hyperglycemia, dyslipidemia, hypertension, inflammation, and oxidative stress [2–4]. The patients generally have enhanced oxidative stress because of generation of reactive oxidant species from hyperglycemia through a number of pathways, that is, glucose reduction to sorbitol and advanced glycation end products, impaired antioxidant defenses, compromised mitochondrial function, and activation of NADPH oxidase [5, 6]. Because the increased risk for CVD still exists even when the T2DM patients had tight control on blood glucose, identification of treatment approaches that address CVD risk factors may prove valid and beneficial to the patients beyond the management of blood glucose.

Lifestyle modification alongside medications has been recommended to be a cornerstone for the disease management for T2DM patients [7]. Lifestyle modification normally consisting of sustainable changes in dietary habits and physical activity not only help glucoregulation but also may prevent or delay the onset of developing chronic complications, especially CVD, by improving lipid profile and decreasing blood pressure and inflammation, oxidative stress. Dietary modifications that could benefit patients with diabetic condition could range from decreased intake of foods/nutrients proven to increase CVD risk to increased consumption of foods/nutrients with beneficial bioactions to decrease CVD risk.

A myriad of foods could be incorporated into diets for those who are at increased risk for CVD, for example, soy, fish oil, vegetables, and fruits. Recent clinical evidence shows almonds could be part of a heart healthy diet. A number of human interventions either with healthy or hypercholesterolemic subjects showed that almonds improved lipid profile, increased satiety, lowered postprandial glucose excursion, and decreased oxidative stress [8–15]. While clinical evidence obtained from studies with T2DM patients is scarce, Li et al. [16] and Cohen and Johnston [17] reported that almonds could improve glycemic control in patients with T2DM. In this study, using the samples collected in the Li et al. [16] trial, we further examine the extent by which incorporation of almonds into the National Cholesterol Education Program (NCEP) [18] step II diet improves oxidative stress and inflammation in T2DM patients.

Materials and methods

Subjects

Subjects were recruited from the Endocrine Clinic of the Taipei Medical University Hospital, Taiwan. They have been treated with T2DM condition by attending physicians in the clinic. Subjects were diagnosed with diabetic condition >5 years, aged between 40 and 70 years, with BMI = 24–35 kg/m^2, serum cholesterol >200 mg/dL or triglycerol >150 mg/dL, HbA1c 6.5–9 %, and regular use of oral hypoglycemic agents. Hypoglycemic medications used by the subjects included sulfonylureas (e.g., Glibenclamide) or meglitinides (e.g., mitiglinide) and biguanide (e.g., metformin). The other eligibility criteria included: (1) free of dietary restrictions/food allergies, (2) not receiving insulin therapy, (3) not using medications or supplementations known to alter lipid metabolism, (4) stable blood lipid and sugar levels within 3 months before study (routinely determined every 4 months in patients), (5) no clinical history of cardiovascular, hepatic, gastrointestinal, or renal disease (confirmed by their medical records), (6) no alcoholism, (7) no recent-history of smoking, and (8) normal liver and kidney function (serum aspartate aminotransferase and alanine aminotransferase <35 U/mL and serum creatinine <1.5 mg/dL, respectively). All women were postmenopausal. Thirty subjects were referred to the study by the physicians, and 8 were not qualified for the study because of not meeting all eligibility criteria.

After enrollment, participants were asked to stay with the same medications and refrain from nuts and supplements 1 month before and during the study. Three-day dietary records, including 2 week days and 1 weekend day,

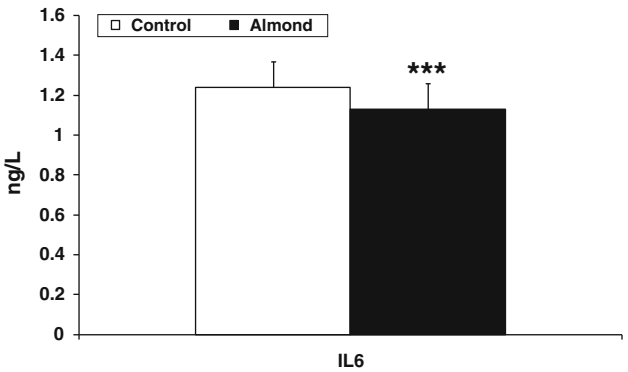

Fig. 1 Consumption of almond diet for 4 weeks decreased serum IL-6 of the T2DM patients. Data were expressed as mean ± SE. ***P value is ≤0.0001, tested by LSMEANS in the PROC GLM model

were collected weekly from the subjects to monitor dietary compliance and assess nutrient intakes. The study protocol was approved by the Institutional Review Board of the Taipei Medical University, and written consent was obtained from each participant prior to participation in the study.

Study design

In a 12-week randomized crossover, controlled feeding trial with a 2-week washout between alternative diets, subjects were assigned to receive control or almond diet for 4 weeks after a 2-week run-in period. Patients consumed meals prepared by the metabolic kitchen of Taipei Medical University Hospital during dietary interventions. During run-in and washout periods, the subjects consumed their habitual diets without nuts, and a daily diet diary was employed to monitor the compliance. A registered dietitian consulted all participants how to select appropriate foods during the run-in and wash out periods and prepare dietary records. Four overnight fasting blood samples and anthropometric data were collected from each participant before and after each dietary treatment phase.

Diets

Meals for each patient were prepared to meet daily energy needs to maintain body weight within 2 kg of their initial value. We calculated energy need of each subject based on their daily activity level. For those who were less active, active, and very active, calories at 30, 35, and 40 kcal/kg/day were provided, respectively. Body weight was monitored weekly for adjustment of calorie intake if necessary. Based on the NCEP step II dietary guideline [18], the control diet was designed to provide daily calories from carbohydrate, protein, and fat at 56, 17, and 27 %, respectively. The percentages of calories from each macronutrient to total calories were comparable to those generally consumed by Taiwanese and similar to those consumed by the subjects during the run-in period. Calories from saturated and polyunsaturated fat were <7 and 10 %, respectively, and cholesterol content were <200 mg. A 5-day menu rotation was used to make food more appealing and palatable to the participants. The almond diet was prepared by incorporating roasted, unsalted whole almonds with skins into meals to replace 20 % calories of the control diet. Depending on the menus, almonds were incorporated into entrees or deserts or consumed as a snack. On average, a patient consumed 56 g/day almonds that were generously provided by the Almond Board of California. Almonds were sweet almonds containing small amounts of cyanogenic compounds [19]. The participants were provided with all foods needed during the dietary intervention. All meals were packaged for take-out. To assist with compliance assessment, each participant completed a daily food diary in which the patient recorded the study foods not eaten, non-study foods eaten, and beverages consumed. Nutrient compositions of the almond and control diets were calculated using the Nutritional Chamberlain Line, Nutritionist Edition, version 2002 (E-Kitchen Business Corp, Taiwan). The details on the mean nutrient intakes were reported in our previous report [16].

Sample preparation and storage

After an overnight fast, blood was drawn into vacutainers with and without anticoagulants (EDTA and NaF). Plasma was collected after centrifugation at 1,400×*g* for 10 min at 4 °C. Aliquots of plasma and serum were prepared and stored in −80 °C. NaF plasma was collected for glucose determination, EDTA plasma for malondialdehyde, protein carbonyl, and lag time of LDL oxidation, and serum for in vivo oxidized LDL, C-reactive protein, IL-6, TNF-α, ICAM-1, and VCAM-1.

Determination of inflammatory biomarkers

Soluble vascular adhesion molecule-1 (VCAM-1), soluble inter-cellular adhesion molecule-1 (ICAM-1), IL-6, and TNF-α all were determined using commercial ELISA assays obtained from R&D Systems (catalog #: DVC00, DCD540, HS600B, and HSTA00D, respectively) (Fig. 2). The assays were performed based on the respective manufacture protocols. The optical density of all assays was determined using a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA).

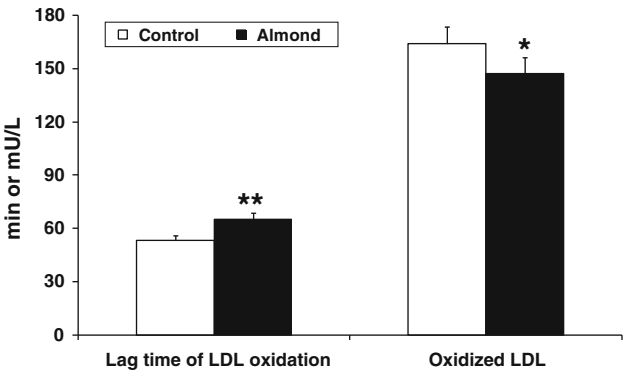

Fig. 2 Consumption of almond diet for 4 weeks increased lag time of Cu^{2+}-induced LDL oxidation and circulating oxidized LDL in the T2DM patients. Data were expressed as mean ± SE. *, **Means of the same analytes are significantly different with *P* value ≤0.05 and 0.01, respectively, tested by LSMEANS in the PROC GLM model

The quantitative immunological determination of serum CRP was performed on a Cobas Integra 800 System (Roche Diagnostics, Mannheim, Germany). Human CRP agglutinated with latex particles coated with monoclonal anti-CRP antibodies, and optic density was measured at 552 nm. The CRP concentration is expressed as mg/L.

Determination of biomarkers of oxidative stress

The resistance of LDL against Cu^{2+}-induced oxidation was determined based on the method of Chen et al. [20]. Briefly, LDL in EDTA plasma was collected using ultracentrifugation. After salt and EDTA removal, conjugated diene formation in LDL oxidized with Cu^{2+} was monitored at 234 nm for up to 3 h. The results are reported as lag time (min).

The concentration of oxidized LDL in serum was quantified using an ELISA kit (nr 10-1143-01; Mercodia, Uppsala, Sweden), based on an antibody directed against a conformational epitope in oxidized ApoB-100. The final result is reported as mU/L.

Plasma malondialdehyde (MDA) was analyzed by a reverse-phase HPLC method as described by Volpi and Tarugi [21]. Briefly, TBAR-MDA adduct was generated when plasma was incubated with TBAR for 30 min at 95 °C. The adduct was separated using a Varian C18 column and then quantified at 515 nm excitation and 553 nm emission. The final result is reported as μmol/L.

Plasma protein carbonyl was determined by a 2,4-dinitrophenolhydrazine (DNPH) spectrophotometric method [22]. Briefly, plasma was incubated with DNPH for 1 h at dark. After addition of trichloroacetic acid and centrifugation, the pellet was washed 3 times with ethanol/ethyl acetate solution. The absorbance at 370 nm was determined after the pellet was dissolved in 6 M guanidine hydrochloride. Protein carbonyl concentration was expressed as nmol/mg protein. Protein content was determined using a Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, USA).

Total phenolic content in plasma was measured by the Folin–Ciocalteau method [23]. Bound and conjugated phenolics were released with treatments of HCl and NaOH. After protein removal using metaphosphoric acid and acetone extraction, phenolics were determined by the assay. Results are expressed as gallic acid equivalents (GAE).

Total antioxidant capacity in plasma was determined using ferric reducing antioxidant power assay (FRAP) [24]. The FRAP assay determines the capability of antioxidants as reductants in a redox-linked colorimetric reaction of the reduction of Fe^{3+}-2,4,6-tri-pyridyl-S-triazine to a blue-colored Fe^{2+} complex at low pH which is measured at 593 nm. FRAP values are expressed as mmol Trolox equivalents (TE)/L.

Statistical analysis

Results were expressed as mean ± standard error (SE). Between-diet differences are expressed as median % change and 95 % confidence intervals (CI). A Student's paired *t* test was performed to evaluate the difference between the baseline and washout values of study outcomes, and the results showed that there were differences between them. A repeated measures analysis was performed to analyze significance between treatment using PROC GLM with treatment (almond vs. control), sequence (almond-control vs. control-almond), period (1 vs. 2), and subject as variables. As the effect of gender and its interaction with other independent variables were insignificant, they were excluded in the model. Further, LSMEANS was performed to evaluate significance in differences between almond and the control diet. Pearson's correlation test was performed using the data of difference in the end values of almond and control treatment. Significance was considered at $P \leq 0.05$ (two-tailed). All statistical analyses were performed using SAS® 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Participant characteristics, study diet, and study compliance

The details of the subject characteristics are described in our previous report [16]. Briefly, 22 participants signed consent forms and began the protocol, and 20 patients completed the study with full compliance. The baseline value of study biomarkers of inflammation and oxidative stress was shown in the Table 1.

The information on the study diets provided to the subjects during the intervention phases can be found in Li et al. [16]. Briefly, the study diet did not change body weight in the subjects. Calories from proteins were not different between the almond and control diets but from carbohydrates and fats were slightly different with the almond diet containing 9.9 % more fat calories. Since almonds are rich in α-tocopherol, the almond diet significantly enhanced plasma α-tocopherol concentration by 23 % from 25.8 to 31.8 μmol/L, indicating good compliance from the subjects [16].

Changes in serum biomarkers of inflammation

Five inflammation-related biomarkers were evaluated in the study (Table 2). The CRP value at the end of almond diet was significantly smaller than that at the end of the control diet, but the median percentage of decrease at

Table 1 Demographics and baseline values of blood biochemistries of the subjects

Subject number	20 (9 m/11 f)
Age (year)	58 ± 2
Diabetic history (year)	8 ± 1
Use of oral hypoglycemia medication (*n*)	20
Body mass index (kg/m^2)	26.0 ± 0.7
Systolic blood pressure (mmHg)	131.0 ± 3.7
Diastolic blood pressure (mmHg)	73.1 ± 2.6
Fasting glucose (mmol/L)	8.7 ± 0.7
Fasting insulin (μU/mL)	15.3 ± 2.1
C-Reactive protein (mg/L)	3.02 ± 0.44
IL-6 (ng/L)	1.32 ± 0.15
TNF-α (ng/L)	0.22 ± 0.02
ICAM-1 (μg/L)	316.3 ± 21.8
VCAM-1 (μg/L)	748.0 ± 21.3
Total antioxidant capacity (mmol/L)	1.89 ± 0.07
Total phenol (g GAE/L)	0.62 ± 0.01
Malondialdehyde (μmol/L)	2.45 ± 0.21
Protein carbonyl (μmol/L)	2.02 ± 0.16
Lag time of LDL oxidation (min)	52.4 ± 1.6
Oxidized LDL (mU/L)	162.6 ± 6.7

Data were expressed as mean ± SE

Table 2 Changes in serum inflammatory biomarkers of the T2DM patients consuming either control or almond diet for 4 weeks

Biomarkers	Control diet	Almond diet	*P* value[a]
C-reactive protein (mg/L)	3.27 ± 0.79	1.98 ± 0.35	0.0455
TNF-α (ng/L)	0.20 ± 0.03	0.14 ± 0.02	0.1043
ICAM-1 (μg/L)	309.3 ± 16.2	317.2 ± 19.6	0.5666
VCAM-1 (μg/L)	796.6 ± 35.7	747.5 ± 18.8	0.1258

Data were expressed as mean ± SE

[a] *P* value for comparison between treatments tested by LSMEANS in the PROC GLM model

10.3 % (−24.1, 40.5) was not statistically significant. IL-6 value at the end of the almond diet was lower than that of the control diet with the median percentage of decrease by 10.3 % (5.2, 12.6) (Fig. 1). The almond diet as compared to the control diet tended to decrease TNF-α with the median percentage of decrease by 15.7 % (−0.3, 29.9). Two adhesion molecules, ICAM-1 and VCAM-1, were not altered by the almond diet.

Changes in biomarkers of antioxidant defense and oxidative stress in plasma

Total antioxidant capacity and total phenolic content in plasma were not altered by the almond diet (Table 3). Plasma MDA was also not altered by the almond diet.

Table 3 Changes in plasma biomarkers of oxidative stress and antioxidants of the T2DM patients consuming either control or almond diet for 4 weeks

Biomarkers	Control diet	Almond diet	*P* value[a]
Total antioxidant capacity (mmol/L)	2.02 ± 0.08	2.08 ± 0.10	0.6397
Total phenolic content (g GAE/L)	0.60 ± 0.01	0.61 ± 0.01	0.3875
Malondialdehyde (μmol/L)	2.36 ± 0.24	2.27 ± 0.23	0.4376
Protein carbonyl (μmol/L)	2.16 ± 0.23	1.59 ± 0.16	0.0003

Data were expressed as mean ± SEM

[a] *P* value for comparison between treatments tested by LSMEANS in the PROC GLM model

Plasma protein carbonyl was significantly diminished by 28.2 % (4.7, 38.2) by the almond diet as compared to the control diet. Lag time of Cu^{2+}-induced LDL oxidation was extended by 16.3 % (7.4, 44.3) by the almond diet as compared to the control diet (Fig. 2). While the almond diet significantly decreased the circulating oxidized LDL value as compared to the control, but the median percentage of decrease at 6.9 % (−2.0, 17.3) was insignificant (Fig. 2).

Correlations between circulating biomarkers

Pearson's partial correlation test was employed to assess the associations between study biomarkers in changes induced by the almond treatment as compared to the control. The change in CRP value between the diets was correlated with those of IL-6 and insulin with *r* values at 0.455 and 0.450, respectively ($P = 0.0437$ and 0.0468). A negative association was found between the changes of IL-6 and ICAM-1 ($r = -0.4473$, $P = 0.048$) and CRP and ICAM-1 ($r = -0.4334$, $P = 0.0562$). The change in TNF-α between the diets was associated with that of glucose ($r = 0.5229$, $P = 0.018$). The change in total antioxidant capacity determined by the FRAP assay was negatively correlated with that of insulin ($r = -0.5215$, $P = 0.0184$). Changes in body fat, HOMA, and insulin were not significantly correlated.

Discussion

Patients with T2DM have as much as a twofold–fourfold greater risk of atherosclerosis and CVD than those who do not have the condition [2, 25]. Given increased inflammation and oxidative stress present in T2DM patients, it can be a valid approach to incorporate foods with antioxidant and anti-inflammatory properties into their diets to avert deterioration of diabetes and development and progression of diabetes-related complications.

Rajaram et al. [26] recently reported in a randomized, controlled, crossover feeding study with 25 healthy Americans that almonds at doses to replace 10 or 20 % daily energy intake decreased serum CRP and E-selectin in a non-dose dependent manner. Consistently, we found that almonds incorporated into the NCEP step II diet to replace 20 % daily calories in Chinese T2DM patients significantly decreased CRP and IL-6 as compared to the NCEP II diet and had a tendency to decrease TNF-α and VCAM-1. CRP is an inflammatory biomarker and an established risk factor of CVD [27]. The circulating CRP level at 3.02 mg/L observed in our study much larger than the Chinese median level at 0.55 mg/L [28] was suggestive of enhanced inflammation in the study patients. The magnitude of CRP reduction by the almond diet was onefold larger than that reported in the Rajaram et al. [26] study with healthy Americans even though the almond dosage employed in our study at 56 g/day was 12 g smaller than their study. In contrast, Jenkins et al. [29] reported in a randomized, crossover 4-week clinical trial that almonds at dosage of 37 and 74 g/day did not alter circulating CRP value in patients with hyperlipidemia. Similarly, Salas-Salvadó et al. [30] did not find a significant association between nut consumption and CRP in a large cohort of asymptomatic subjects at high risk for CVD. From the same research group, an earlier intervention study (PREDIMED) also did not support that daily incorporation of 30 g mixed nuts into a typical Mediterranean diet benefited CRP level in 772 asymptomatic individuals at high cardiovascular risk [31]. Thus, the effect of almonds on CRP level warrants further investigations and the efficacy may be dependent upon demographics of subjects, dosage, and duration.

IL-6 and TNF-α are mediators for CRP synthesis in liver. An increased IL-6 level is associated with hyperglycemia, insulin resistance, and overt T2DM [32]. We found that the almond diet decreased plasma IL-6 concentration, in agreement with the PREDIMED study [31] that incorporation of 30 g/day mixed nuts into a typical Mediterranean diet significantly decreased IL-6 level in adults at high CVD risk as compared to a low fat diet. In contrast, Rajaram et al. [26] did not find almonds replacing 10 and 20 % total energy intake changed IL-6 levels in healthy adults. As IL-6 is an upstream mediator of CRP production, the decrease in IL-6 by the almond diet in our study was positively associated with that of CRP. Unlike the significant reductions in CRP and IL-6 by almonds, TNF-α level was only slightly decreased by the almond diet with *P* value at 0.10. Based on a power calculation, a total of 70 subjects will be required to detect the effect of almonds on the TNF-α values in the Chinese with T2DM. Even though there is an interactive relationship between TNF-α and CRP, there was no correlation, suggesting that TNF-α might not contribute significantly to the almond-induced reduction in the CRP value.

Elevated circulating levels of adhesion molecules, for example, e-selectin, ICAM-1, and VCAM-1, enable leukocyte attachment and intimal penetration and reduce NO responsiveness and are indicatives of endothelial dysfunction [33]. Further, the expression of ICAM and VCAM in human aortic endothelial cells could be activated by CRP [34], and their values were correlated to the maximal intimal-medial thickness [35]. The ICAM-1 and VCAM-1 values found in our study were comparable to those in Mexican T2DM patients [35]. Our study did not show there was an effect of almonds on ICAM-1 and VCAM-1. In contrast, Rajaram et al. [26] reported in a study with healthy subjects that almonds at a dose of 68 g/day decreased e-selectin. Further, the PREDIMED Study showed that 30 g/day mixed nuts decreased ICAM-1 and VCAM-1 in older subjects at a high risk for CVD [31]. We speculated that the measured adhesion molecules were not responsive to the almond nutrients, almond dosage is not adequate to induce the changes in Chinese patients with T2DM, or almond-induced improvements in diabetic condition is ineffective to decrease them.

Oxidative stress arises from hyperglycemia via various mechanisms and has been noted to coexist with insulin resistance in patients with T2DM [36, 37]. Ros et al. [38] summarized in a review paper that MUFA-rich nuts moderately improved biomarkers of oxidative stress. Almonds are rich in MUFA and lipophilic antioxidant α-tocopherol and contain antioxidant polyphenols [9, 11]. Our study showed that almonds decreased circulating oxidized LDL and enhanced LDL resistance against Cu^{2+}-induced oxidation. These two biomarkers reflect the local effect of almond antioxidants on LDL oxidation that plays a focal role in development of atherosclerosis. Our results are also in line with the Jenkins et al. [29] study in which consumption of 73 g/day almonds for 4 weeks decreased lipid peroxidation product in LDL in patients with hyperlipidemia. However, Hyson et al. [39] failed to demonstrate the antioxidant effect of almonds at 66 g/day for 6 weeks on LDL resistance against ex vivo oxidation in healthy adults. Plasma MDA, another biomarker of lipid peroxidation, was not altered by almonds even though plasma concentration of α-tocopherol was significantly elevated. This result is in contrast to the Jenkins et al. [40] and Li et al. [15] studies showing that almonds decreased lipid peroxidation products, MDA and $F_{2\alpha}$-isoprostanes in patients with hyperlipidemia and young male smokers, respectively. On the other hand, our study showed that protein carbonyl, an index of protein oxidation, was significantly decreased by almonds. Consistently, Jenkins et al. [19] reported in an acute study with healthy adults that 60 g almonds decreased postprandial protein oxidation based on the result of enhanced

plasma total thiol, an inverse measure of protein oxidation. Collectively, our results support that the incorporation of almonds into a healthy diet diminishes oxidative stress in patients with a diabetic condition. However, it is noted that not all study biomarkers were markedly modulated by almond constituents. This inconsistency underscores the differences in mechanism of detection of assays and efficacy of antioxidative actions of absorbed almond constituents in sites of disease lesions or susceptible molecules. For example, increased in vivo oxidized LDL and plasma MDA both could illustrate enhanced oxidative stress while it shall be noted that oxidized LDL can be formed through mechanisms distinctively different from MDA. Oxidized LDL detected by the ELISA assay monitors the formation of MDA-, 4-hydroxynonenal-, and carboxymethyl-lysine adducts in apo-B100 and MDA is a breakdown product of lipid peroxidation induced by reactive oxygen species. While much remains to be learned about the most effective ways to assess oxidative stress in vivo, a balanced approach examining both the generation of reactive species (including their "footprint" biomarkers) and antioxidant defenses is necessary to examine the role of free radicals and antioxidants in biology and medicine.

Potential mechanisms by which almonds decreased inflammation in T2DM patients should be multifaceted and remain to be elucidated in future studies even though improved glycemic control, increased plasma α-tocopherol, and increased MUFA intake might all be underlying contributors [41–44]. As our study was not designed to differentiate contribution of individual almond constituents to functional end points, all positive findings shall be a consequence of synergistic work of all almond nutrients, for example, α-tocopherol, magnesium, fiber, monounsaturated fat, arginine, and polyphenols. Although mechanism(s) regulating inflammation in T2DM patients has not yet been clearly established, oxidative stress induced by hyperglycemia might partially play an underlying factor via NFκB pathway [45]. However, it is worth noting that antioxidant rich fruit, berries, and vegetables were proved unable to improve the glycemic control or lower the level of oxidative stress or inflammation in the T2DM [44] and intensive insulin therapy improved hyperglycemia but not inflammation in T2DM patients [46]. It could be speculated that almond antioxidants might not have a direct involvement in regulation of inflammation, supported by no correlations between biomarkers of oxidative stress and inflammation. Therefore, almond nutrients other than antioxidants such as magnesium, fiber, arginine, and MUFA might play a bigger role in regulation of inflammation [47–49]. Weight loss, including fat loss, has been convincingly found to decrease inflammation [50]; the decrease in body fat after almond consumption may also attenuate inflammation.

Our previous report shows that the incorporation of 56 g/day almonds into the NCEP step 2 diet improved glycemic control and lipid profiles in Chinese patients with T2DM. In this report, we further illustrate the benefits of almond consumption in inflammation and oxidative stress in the same cohort. Inflammation and oxidative stress are two important factors of development and progression of diabetes-related complications, for example, CVD. As therapeutic approaches that target to attenuate inflammation in the T2DM have been recognized [50], healthy foods, such as nuts, may play an adjunct role in medical treatment plans for the patients. Therefore, incorporation of almonds into a healthy diet represents a reasonable nutrition strategy in patients with T2DM to decrease the risk of complications, in particular in the patients with a long history of diabetes and increased risk for CVD. Further studies with almonds are warranted to examine whether such benefits are dependent upon background diet, lifestyle, and ethnicity, as well as mechanism(s) contributing to improving glycemic control, oxidative stress, and inflammation. We are currently conducting a more robust study [larger sample size ($n = 40$) and longer intervention (3 months)] to confirm our findings.

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Conflict of interest None.

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