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A moderate-fat diet containing pistachios improves emerging markers of cardiometabolic syndrome in healthy adults with elevated LDL levels

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Abstract

A randomised, cross-over, controlled-feeding study was conducted to evaluate the cholesterol-lowering effects of diets containing pistachios as a strategy for increasing total fat (TF) levels *v.* a control (step I) lower-fat diet. *Ex vivo* techniques were used to evaluate the effects of pistachio consumption on lipoprotein subclasses and functionality in individuals (*n* 28) with elevated LDL levels (≥2·86 mmol/l). The following test diets (SFA approximately 8 % and cholesterol <300 mg/d) were used: a control diet (25 % TF); a diet comprising one serving of pistachios per d (1PD; 30 % TF); a diet comprising two servings of pistachios per d (2PD; 34 % TF). A significant decrease in small and dense LDL (sdLDL) levels was observed following the 2PD dietary treatment *v.* the 1PD dietary treatment ($P=0·03$) and following the 2PD dietary treatment *v.* the control treatment ($P=0·001$). Furthermore, reductions in sdLDL levels were correlated with reductions in TAG levels (r 0·424, $P=0·025$) following the 2PD dietary treatment *v.* the control treatment. In addition, inclusion of pistachios increased the levels of functional α-1 ($P=0·073$) and α-2 ($P=0·056$) HDL particles. However, ATP-binding cassette transporter A1-mediated serum cholesterol efflux capacity ($P=0·016$) and global serum cholesterol efflux capacity ($P=0·076$) were only improved following the 2PD dietary treatment *v.* the 1PD dietary treatment when baseline C-reactive protein status was low ($<10^3$ μg/l). Moreover, a significant decrease in the TAG:HDL ratio was observed following the 2PD dietary treatment *v.* the control treatment ($P=0·036$). There was a significant increase in β-sitosterol levels ($P<0·0001$) with the inclusion of pistachios, confirming adherence to the study protocol. In conclusion, the inclusion of pistachios in a moderate-fat diet favourably affects the cardiometabolic profile in individuals with an increased risk of CVD.

Key words: Pistachios: Small and dense LDL: HDL subclasses: Insulin resistance: Cardiometabolic disease: C-reactive protein: Plant sterols

Cardiometabolic syndrome increases the risk of developing CVD and type 2 diabetes mellitus[1]. Epidemiological and clinical studies have demonstrated the beneficial effects of tree nut and peanut consumption on the risk of CVD and its co-morbidities[2–4]. Investigations of two independent cohorts of 76 464 women and 42 498 men have shown a 20 % reduction in total mortality in individuals who consumed nuts seven or more times a week *v.* those who did not consume nuts[5]. Reductions in CVD mortality (−25 %) and death due to heart disease (−29 %) have also been reported for individuals who consumed nuts five times a week *v.* those who did not consume nuts[5]. In addition, Jaceldo-Siegl *et al.*[4] demonstrated a significant inverse association ($P<0·01$) of high tree nut consumption with the prevalence of obesity in a group of 803 adults; a 46 % reduction in the prevalence of obesity was observed in high-tree nut consumers *v.* low-tree nut consumers.

Pistachios are a source of monounsaturated and polyunsaturated fats, as well as bioactive compounds, including plant sterols[6]. The report of the 2010 Dietary Guidelines Advisory Committee states that plant sterols may contribute to the cholesterol-lowering effect reported for plant-based diets; the sterols compete with dietary cholesterol for absorption within the intestinal lumen[7]. Subsequently, endogenous

Abbreviations: 1PD, one serving of pistachios per d; 2PD, two servings of pistachios per d; ABCA1, ATP-binding cassette transporter A1; CRP, C-reactive protein; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; sdLDL, small and dense LDL; TF, total fat.

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cholesterol synthesis is increased, which in turn up-regulates LDL receptor expression and LDL removal from the circulation, resulting in a decrease in LDL levels[8].

In contrast to low-fat diets, moderate-fat diets that include nuts and other plant-based foods reduce LDL levels and typically increase HDL levels[9,10]; additive effects have been observed with the inclusion of legumes, seeds, grains and nuts[10]. However, questions remain regarding the mechanistic effects of the bioactive components of tree nuts and peanuts on the emerging markers of CVD risk. Mechanisms by which bioactive components in pistachios affect these emerging markers are also not known.

In individuals with insulin resistance, there is increased synthesis of VLDL, which results in increased TAG levels[11] and decreased HDL levels[12]. The TAG:HDL ratio, a surrogate marker of insulin resistance, has been shown to predict an increased risk of hypertension and type 2 diabetes, as well as all-cause mortality in women with myocardial ischaemia[13–15]. In addition, decreased insulin sensitivity adversely affects LDL and HDL particle size. In a study carried out by Garvey *et al.*[16], insulin resistance was found to be significantly associated with a decrease in LDL and HDL particle size, as well as increases in the levels of small and dense LDL (sdLDL) and small HDL particles, and a reduction in the levels of large LDL particles. In a study carried out by Irving *et al.*[17] in non-diabetic subjects, low insulin sensitivity, cardiorespiratory fitness and truncal fat mass were all found to be associated with increases in the number of sdLDL and small HDL particles, as well as decreases in average LDL and HDL particle size; TAG was also found to be a strong predictor of lipoprotein levels and size. The effects of diet on lipoprotein particle size are important as sdLDL and the HDL subclasses have been shown to predict CVD risk better than LDL and HDL levels alone[18–21].

Elevated levels of sdLDL are associated with an increased risk of coronary artery disease[22]. sdLDL particles are highly atherogenic primarily due to their increased susceptibility to oxidation and their ease of entry into the arterial wall[23]. HDL are also heterogeneous and their subclasses are differentially associated with CVD risk[24–26]. Results obtained in the Framingham Offspring Study have demonstrated that low α-1 and α-2 HDL levels are stronger predictors of CHD risk than HDL levels alone[25]. Furthermore, the α-1 and α-2 subclasses of HDL are more functional in reverse cholesterol transport and promote atheroregression[27,28].

Recent advances in the development of *ex vivo* methodologies for assessing HDL functionality (serum cholesterol efflux capacity determination) provide an approach to evaluate the effects of pistachios on the lipid/lipoprotein profile and their association with reverse cholesterol transport. In the present study, we evaluated the effects of test diets that varied in the levels of total, monounsaturated and polyunsaturated fats provided by pistachios on the LDL and HDL subclasses, the TAG:HDL ratio, and serum cholesterol efflux capacity as a strategy for identifying mechanisms that explain how consumption of pistachios beneficially affects cardiometabolic status.

Experimental methods

Study design, participants and diet design

Plasma and serum samples obtained from the participants of our previous study[29] were used for the analyses described herein. Briefly, otherwise healthy, non-smoking men (n 10) and women (n 18) with elevated fasting LDL levels (≥ 2·86 mmol/l, 110 mg/dl) completed a three-period, randomised, cross-over, controlled-feeding study[29]. Previous sample size calculation[29] was based on effect sizes reported by Jenkins *et al.*[30] and Sabaté *et al.*[31]. Additional inclusion criteria were as follows: TAG levels < 3·94 mmol/l (349 mg/dl) and blood pressure < 160/90 mmHg; BMI between 21 and 35 kg/m^2; fasting blood glucose levels ≤ 6·93 mmol/l (125 mg/dl). The baseline characteristics of the participants are given in Table 1. The participants were found to be not insulin resistant based on the homeostasis model of assessment-estimated insulin resistance (HOMA-IR) index (< 2·5) and the TAG:HDL ratio (< 1·53). Exclusion from the study was based on the following: an inability to adhere to the study protocol; using blood pressure-lowering or cholesterol/lipid-lowering medications or cholesterol/blood pressure-lowering products (psyllium, fish oil, soya lecithin and phyto-oestrogens); being pregnant or wishing to become pregnant 6 months before or during the study; lactating 6 weeks before or during the study; experiencing weight loss ≥ 10 % body weight 6 months before the study; following vegetarian or weight-loss diets; having any of the following conditions: stroke, diabetes, liver disease, kidney disease or autoimmune diseases.

We used a controlled-feeding design and all meals were provided to the participants; energy levels were individualised for the maintenance of body weight. The macronutrient profile of the experimental diets is summarised in Table 2. After 2 weeks on a typical Western diet (35 % total fat (TF) and 11 % SFA), the participants were assigned to a balanced order sequence of three test diets. Each test diet was provided for 4 weeks followed by a 2-week compliance break. The following test diets were used: a lower-fat control diet (control; 25 % TF and 8 % SFA); a diet that provided 10 % of

Table 1. Baseline characteristics of the participants

(Least-squares (LS) means with their standard errors, n 28)

Characteristics	LS mean	SEM
Age (years)	48·0	1·5
BMI (kg/m^2)	26·8	0·7
Total cholesterol (mmol/l)	5·45	0·12
LDL (mmol/l)	3·46	0·11
HDL (mmol/l)	1·50	0·08
TAG (mmol/l)	1·15	0·09
Systolic blood pressure (mmHg)	111·9	2·1
Diastolic blood pressure (mmHg)	69·5	1·1
Fasting plasma glucose (mmol/l)	5·12	0·08
Fasting serum insulin (pmol/l)	64·4	5·6
HOMA-IR index*	2·1	1·1
TAG:HDL ratio	0·83	0·44

HOMA-IR, homeostasis model of assessment-estimated insulin resistance.
*HOMA-IR calculations were based on the method of Matthews *et al.*[35].

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Table 2. Macronutrient composition of the diets (*n* 28)

	Control	1PD*	2PD†
Energy (kJ)	8790‡	8790‡	8790‡
Protein (%)	15·4	16·7	16·9
CHO (%)	62·7	57·6	53·5
Fat (%)	25·4	29·6	34·3
SFA (%)	7·8	7·7	7·7
MUFA (%)	9·1	12·0	15·3
PUFA (%)	4·5	5·8	7·7
LA (%)	2·6	4·2	6·3
ALA (%)	0·4	0·3	0·4
Cholesterol (mg)	288·0	293·0	286·0
Phytosterols (mg/d)	37·5	103·0	321·0
Fibre (g)	32·8	33·3	35·9

CHO, carbohydrate; LA, linoleic acid; ALA, α-linolenic acid.
* 1PD represents one serving (32–63 g or 1·5 oz) of pistachios per d (10 % energy from pistachios).
† 2PD represents two servings (63–126 g or 3·0 oz) of pistachios per d (20 % energy from pistachios).
‡ Equivalent to 8786 kJ (2100 kcal) of dietary energy.

energy from pistachios (one serving of pistachios/d (1PD); 30 % TF and 8 % SFA); a diet that provided 20 % of energy from pistachios (two servings of pistachios/d (2PD); 34 % TF and 8 % SFA). Energy from carbohydrates was replaced with energy from pistachios. Salted, roasted pistachios (50 % of the daily dose) were consumed as snacks instead of baked potato chips and pretzels. Unsalted pistachios were incorporated into recipes.

Adherence to the study protocol was good, as demonstrated by daily compliance questionnaires. In addition, plasma β-sitosterol levels increased dose dependently with the inclusion of pistachios, consistent with dietary approximations, as β-sitosterol is the predominant sterol found in pistachios. The weight of the participants was recorded daily (Monday through Friday). Diets were isoenergetic, and there were no significant differences in pre-treatment and post-treatment means for either body weight ($P>0·05$) or BMI ($P>0·05$)[29]. No significant differences were also observed in body weight or BMI when comparing participants who consumed the control diet with those who consumed the pistachio diets ($P>0·05$)[29].

The participants were instructed to maintain the intensity, frequency and duration of their habitual physical activity for the duration of the study. The participants were required to complete daily and weekly monitoring forms that were verified by the study coordinator. The daily monitoring form contained a 'Comments' section where the participants could record any physical activity outside of their usual routine. They were then specifically asked about their deviations in physical activity and were reminded to maintain their usual physical activity regimen.

The present study was approved by the Institutional Review Board of The Pennsylvania State University. All participants provided signed informed consent. Among the participants, one was unable to adhere to the protocol and subsequently withdrew from the study.

Analytical methods

Lipoprotein subclasses. Lipoprotein subclass analyses were conducted at the Boston Heart Diagnostics laboratory (Framingham, MA, USA). For sdLDL analysis, large, buoyant and other apoB-containing lipoproteins were first removed from the plasma by filtration after the formation of aggregates with a polyanion and divalent cation-based reagent, and sdLDL levels were then determined using a Cobas 6000 analyser (Roche), with reagents obtained from Denka-Seiken Company Limited, as described previously[32]. The levels of HDL subclasses were determined by immunoblotting with prior separation using two-dimensional, non-denaturing PAGE as described previously[25]. Lipoprotein(a) levels were determined using ELISA as described elsewhere[33].

Measures of insulin sensitivity and inflammation. Methods used for determining fasting plasma glucose levels and fasting serum insulin levels have been described previously[29]. Briefly, fasting glucose levels were measured using an immobilised biosensor within the YSI 2300 STAT Plus Glucose & Lactate Analyzer (Yellow Springs Instruments Inc.). Insulin levels were measured using RIA with ^{125}I-labelled human insulin and a human insulin antiserum (Linco Research, Inc.). Serum C-reactive protein (CRP) levels were measured using latex-enhanced immunonephelometry (Quest Diagnostics; assay CV <8 %), and clinically significant limits were based on the 2003 AHA/CDC Scientific Statement on Markers of Inflammation and Cardiovascular Disease[34]. Fasting plasma glucose levels and fasting serum insulin levels were determined at the Penn State Hershey Medical Center (Hershey, PA, USA). HOMA-IR index calculations were based on the formula proposed by Matthews *et al.*[35] using conventional units.

Serum cholesterol efflux capacity. Cholesterol efflux capacity was determined at Vascular Strategies LLC. Serum HDL samples (apoB-depleted serum) were prepared from individual serum samples by precipitation of apoB-containing lipoproteins using polyethylene glycol. Briefly, for each serum sample, 100 parts serum were mixed with forty parts polyethylene glycol (20 %, v/v, in glycine buffer, pH 7·4). The samples were then incubated at room temperature for 20 min and centrifuged at 10 000 rpm for 30 min at 4 °C. The supernatant containing serum HDL was collected and used for the determination of cholesterol efflux capacity.

Cholesterol efflux capacities of serum HDL samples were determined as described in detail elsewhere[36,37]. In brief, global and ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol effluxes were measured using J774 mouse macrophage cells in the presence and/or absence of cyclic AMP. For all assays, the cells were pre-incubated with [^{3}H]cholesterol and acyl-CoA:cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma-Aldrich) overnight; cells were not preloaded with mass cholesterol. The cells were then incubated overnight in 0·2 % bovine serum albumin with or without 8-(*p*-chlorophenylthio)-cyclic AMP. After washing, the cells were incubated for 4 h with the serum HDL samples (apoB-depleted serum) added at 2·8 % (v/v). [^{3}H]Cholesterol released into serum after incubation with the

cells for 4 h was measured by liquid scintillation counting. Cholesterol efflux is expressed as the radiolabel released as a percentage of [^{3}H]cholesterol within the cells before the addition of serum. All efflux values were corrected by subtracting the small amount of radioactive cholesterol released from the cells incubated with serum-free media. ABCA1-dependent efflux from J774 cells was determined as the difference in efflux from cyclic AMP-treated cells and that from untreated cells.

Circulating sterols. The levels of β-sitosterol, campesterol, desmosterol and lathosterol were measured using GC as described previously[38]; analyses were carried out at the Boston Heart Diagnostics laboratory (Framingham, MA, USA). β-Sitosterol and campesterol were used as markers of dietary sterol absorption, while desmosterol and lathosterol were used as markers of endogenous cholesterol synthesis. As these sterols are primarily transported in the LDL fraction, their levels are expressed as a ratio to total cholesterol[39].

Statistical analysis

Data were tested for normality and transformed where appropriate. All data, except those obtained for α-2, α-3 and α-4 HDL, fasting serum insulin and lipoprotein(a), were log-transformed; data obtained for lipoprotein(a) were transformed using the square root scale. The effects of diet were analysed by comparing the levels of each endpoint at the end of the control treatment with levels measured after the consumption of the two pistachio diets. Treatment differences were analysed using mixed models (SAS version 9.2; SAS Institute Inc.). Diet, period and diet × period interaction were entered as fixed effects; subject was a random effect. No significant diet × period interactions were observed for any variable, except for α-4 HDL; no significant effects of diet were observed on this variable. Changes from baseline were calculated for the HOMA-IR index. We examined whether change in LDL or sdLDL levels was predicted by change in serum β-sitosterol levels for the 2PD dietary treatment group; change scores were calculated for this group (values recorded at the end of the dietary treatment − those recorded after the control treatment). We also estimated whether change in sdLDL levels was related to change in TAG levels; change scores were calculated for the 2PD dietary treatment group (values recorded at the end of the dietary treatment − those recorded after the control treatment). As CRP is a strong predictor of CVD and the metabolic syndrome[40,41], we further determined whether there was an association between CRP status (high: $\geq 10^3$ μg/l *v.* low: $< 10^3$ μg/l) at baseline and the effects of diets on cholesterol efflux capacity. Significant effects ($P \leq 0{\cdot}05$) were evaluated using Tukey's tests. Data are reported as least-squares means with their standard errors.

Results

Lipoprotein subclasses

A significant effect of diet was observed on sdLDL levels ($P=0{\cdot}001$); compared with the control and 1PD diets, the 2PD diet significantly reduced sdLDL levels ($P=0{\cdot}001$ and $P=0{\cdot}03$, respectively; Table 3). Furthermore, reductions in sdLDL levels were positively associated with reductions in TAG levels following the 2PD dietary treatment *v.* the control treatment (r 0·42, $P=0{\cdot}03$). An increase in the levels of α-1 and α-2 HDL was observed with the inclusion of pistachios ($P=0{\cdot}073$ and $P=0{\cdot}056$, respectively; Table 3). No significant effects of diet were observed on preβ-1 HDL, α-3 HDL, α-4 HDL or lipoprotein(a) levels (Table 3); a significant diet × period interaction ($P=0{\cdot}007$) was observed for α-4 HDL levels.

Cardiometabolic measures

The assessment of the TAG:HDL ratio, a marker of insulin resistance (Table 4), revealed a significant effect of diet ($P=0{\cdot}041$), and a significant reduction was observed in this ratio following the 2PD dietary treatment *v.* the control treatment ($P=0{\cdot}036$); the pistachio diets minimised the increase in the TAG:HDL ratio from baseline compared with the lower-fat control diet[29]. However, no significant effect of diet was observed on the HOMA-IR index ($P=0{\cdot}71$; Table 4). Change in the HOMA-IR index from baseline also did not differ among the dietary treatment groups ($P=0{\cdot}994$). No significant differences were observed in either fasting plasma glucose levels or fasting serum insulin levels among the dietary treatment groups.

Table 3. Effects of pistachio inclusion on lipoprotein subclasses
(Least-squares (LS) means with their standard errors, *n* 28)

	Control		1PD*		2PD†					
Variables	LS mean	SE	LS mean	SE	LS mean	SE	*P* (diet)	*P*‡ (control *v.* 1PD)	*P*‡ (1PD *v.* 2PD)	*P*‡ (control *v.* 2PD)
sdLDL (mmol/l)	1·07	0·03	1·00	0·03	0·86	0·03	0·001	0·460	0·030	0·001
Preβ-1 HDL (mmol/l)	0·56	0·03	0·53	0·03	0·53	0·03	0·596	0·635	0·998	0·671
α-4 HDL (mmol/l)	0·22	0·01	0·23	0·01	0·22	0·01	0·457	0·523	0·523	1·000
α-3 HDL (mmol/l)	0·76	0·04	0·74	0·04	0·75	0·04	0·880	0·871	0·980	0·948
α-2 HDL (mmol/l)	1·41	0·06	1·39	0·06	1·50	0·06	0·056	0·868	0·059	0·167
α-1 HDL (mmol/l)	0·47	0·03	0·48	0·03	0·53	0·03	0·073	0·926	0·162	0·144
Lipoprotein(a) (μmol/l)	0·89	0·01	0·90	0·01	0·89	0·01	0·782	0·783	0·864	0·987

sdLDL, small and dense LDL.
* 1PD represents one serving (32–63 g or 1·5 oz) of pistachios per d (10 % energy from pistachios).
† 2PD represents two servings (63–126 g or 3·0 oz) of pistachios per d (20 % energy from pistachios).
‡ Tukey's adjustments were used for comparisons among the dietary treatment groups.

Table 4. Effects of pistachio inclusion on the TAG:HDL ratio, fasting plasma glucose levels, fasting serum insulin levels, and the homeostasis model assessment-estimated insulin resistance index (HOMA-IR)

(Least-squares (LS) means with their standard errors, *n* 28)

	Control		1PD*		2PD†					
Variables	LS mean	SE	LS mean	SE	LS mean	SE	*P* (diet)	*P*‡ (control *v.* 1PD)	*P*‡ (1PD *v.* 2PD)	*P*‡ (control *v.* 2PD)
TAG:HDL ratio	1·09	0·48	1·00	0·48	0·92	0·48	0·041	0·694	0·201	0·036
Fasting plasma glucose (mmol/l)	5·11	0·06	5·05	0·06	5·11	0·06	0·492	0·543	0·562	0·999
Fasting serum insulin (pmol/l)	65·3	5·6	66·0	5·6	66·0	5·6	0·971	0·969	0·996	0·987
HOMA-IR§	1·94	1·13	1·83	1·13	1·85	1·13	0·714	0·724	0·989	0·802

* 1PD represents one serving (32–63 g or 1·5 oz) of pistachios per d (10 % energy from pistachios).
† 2PD represents two servings (63–126 g or 3·0 oz) of pistachios per d (20 % energy from pistachios).
‡ Tukey's adjustments were used for comparisons among the dietary treatment groups.
§ HOMA-IR calculations were based on the method of Matthews *et al.*[35].

Serum cholesterol efflux capacity

No significant effects of diet were observed on ABCA1-mediated serum cholesterol efflux capacity ($P=0·213$) or global serum cholesterol efflux capacity ($P=0·553$; Table 5). After adjusting for baseline CRP status, however, significant diet × CRP status interactions were observed for both ABCA1-mediated serum cholesterol efflux capacity ($P=0·016$) and global serum cholesterol efflux capacity ($P=0·029$) (Table 6). A significant difference was observed in ABCA1-mediated serum cholesterol efflux capacity between participants consuming the 2PD diet (9·89 (SE 0·75) %) and those consuming the 1PD diet (7·35 (SE 0·74) %) within the low-CRP status group ($P=0·0158$). A difference was also observed in global cholesterol efflux capacity between participants consuming the 2PD diet (17·55 (SE 1·06) %) and those consuming the 1PD diet (14·73 (SE 1·06) %) within the low-CRP status group ($P=0·076$).

Circulating sterols

Significant effects of diet were observed on β-sitosterol ($P<0·0001$) and campesterol ($P=0·009$) levels. A significant increase in β-sitosterol levels was observed following the 1PD and 2PD dietary treatments *v.* the control treatment ($P<0·0001$ for both; Table 7). A significant increase in β-sitosterol levels was also observed following the 2PD dietary treatment *v.* the 1PD dietary treatment ($P=0·0002$). A significant decrease in campesterol levels was observed following the 2PD dietary treatment *v.* the control treatment ($P=0·009$) and following the 1PD dietary treatment *v.* the control treatment ($P=0·051$) (Table 7). Given the significant reductions in sdLDL levels observed in the present study and reductions in LDL levels reported previously ($P<0·001$)[29], we also evaluated whether increasing levels of β-sitosterol played a role in these reductions. Pearson's correlation analyses revealed no significant associations between reductions in sdLDL (r 0·26, $P=0·20$) and LDL (r 0·30, $P=0·13$) levels and increases in β-sitosterol levels on comparing the control and 2PD dietary treatment groups. No significant differences were observed in desmosterol ($P=0·14$) or lathosterol ($P=0·15$) levels among the dietary treatment groups (Table 7).

Discussion

In the present study, the inclusion of pistachios in a heart-healthy diet as a strategy for increasing TF levels was found to favourably reduce sdLDL levels. The reduction in sdLDL levels would be expected to confer an additional benefit with regard to CVD risk beyond the reduction in LDL levels. Our previous findings showed the beneficial effects of pistachio consumption on lipid and lipoprotein profiles (Supplementary Table 1); specifically the 1PD and 2PD diets decreased LDL levels by −9 and −12 %, respectively[29]. sdLDL particles are associated with elevated TAG levels and lower HDL levels[19,42], which are typically observed in individuals with dyslipidaemia and insulin resistance. We had previously reported a significant reduction in TAG levels with the inclusion of pistachios (1·40 (SE 0·09) mmol/l for the lower-fat control diet, 1·28 (SE 0·09) mmol/l for the 1PD diet,

Table 5. Effects of pistachio inclusion on ATP-binding cassette transporter A1 (ABCA1)-mediated serum cholesterol efflux capacity (ABCA1 efflux) and global serum cholesterol efflux capacity (global efflux)

(Least-squares (LS) means with their standard errors, *n* 28)

	Control		1PD*		2PD†					
Variables (%)	LS mean	SE	LS mean	SE	LS mean	SE	*P* (diet)	*P*‡ (control *v.* 1PD)	*P*‡ (1PD *v.* 2PD)	*P*‡ (control *v.* 2PD)
ABCA1 efflux	8·55	0·58	7·63	0·58	8·59	0·58	0·213	0·293	0·263	0·998
Global efflux§	15·72	1·05	15·12	1·05	15·94	1·05	0·553	0·720	0·547	0·958

* 1PD represents one serving (32–63 g or 1·5 oz) of pistachios per d (10 % energy from pistachios).
† 2PD represents two servings (63–126 g or 3·0 oz) of pistachios per d (20 % energy from pistachios).
‡ Tukey's adjustments were used for comparisons among the dietary treatment groups.
§ Diet×period interaction ($P=0·023$).

Table 6. Moderation of the effects of pistachio inclusion on ATP-binding cassette transporter A1 (ABCA1)-mediated serum cholesterol efflux capacity (ABCA1 efflux) and global serum cholesterol efflux capacity (global efflux) by C-reactive protein (CRP) status (*n* 28)

Variables (%)	*P* (diet)	*P* (diet×period)	*P* (diet×CRP status)*
ABCA1 efflux	0·322	0·176	0·016†
Global efflux	0·764	0·044	0·029‡

* Test for an interaction between pistachio inclusion and baseline CRP status (high: $\geq 10^3$ μg/l or low: $< 10^3$ μg/l) for ABCA1-mediated and global serum cholesterol efflux capacities.
† Significant difference between those consuming two servings/d (9·89 (SE 0·75) %) and those consuming one serving/d (7·35 (SE 0·74) %) within the low-CRP status group (*P*=0·016).
‡ Trend for a difference between those consuming two servings/d (17·55 (SE 1·06) %) and those consuming one serving/d (14·73 (SE 1·06) %) within the low-CRP status group (*P*=0·076).

and 1·20 (SE 0·09) mmol/l for the 2PD diet)[29]; the pistachio diets minimised the increase in TAG levels from baseline (1·15 (SE 0·09) mmol/l) compared with the lower-fat control diet. In the present study, we found a significant correlation between reductions in both sdLDL and TAG levels, indicating a beneficial shift in the metabolic profile.

With a decrease in insulin sensitivity, a predominance of sdLDL particles is typically associated with a high TAG:HDL ratio[43]. In comparison with the lower-fat control diet, the pistachio diets resulted in decreases in both sdLDL levels and the TAG:HDL ratio; they also reduced increases in the TAG:HDL ratio from baseline when compared with the lower-fat control diet. Although the TAG:HDL ratio was still in the normal range in all participants (<1·53), further reductions may confer additional cardioprotective benefits. Our findings are important because we studied generally healthy individuals with elevated LDL levels who did not present with insulin resistance (as assessed by HOMA-IR scores) and yet observed beneficial effects on sdLDL levels and the TAG:HDL ratio in response to a moderate-fat diet containing pistachios. The TAG:HDL ratio is important because it incorporates two of the criteria for the metabolic syndrome and has been shown to predict insulin resistance, incident hypertension and diabetes[13,15,44]. The results of the present study demonstrate improvements in the TAG:HDL ratio, indicating that progression to insulin resistance can be slowed by consumption of a moderate-fat, heart-healthy diet containing pistachios.

Studies have shown that moderate-fat diets that contain tree nuts or peanuts increase HDL levels when compared with lower-fat diets[9]. In our previous study, we had also found beneficial effects on HDL levels with the inclusion of pistachios in the diet; there was a significant difference in HDL levels between those who consumed the 1PD diet and those who consumed the 2PD diet; there were no differences between those who consumed either the 1PD or the 2PD diet and those who consumed the lower-fat control diet. However, it must be noted that the mean HDL level at baseline in all participants was approximately 1·50 mmol/l, which is moderately high. The inclusion of pistachios (2PD) in the diet further attenuated decreases in HDL levels from baseline when compared with the lower-fat control diet[29]. Interestingly, we observed trends in the effects of diet on α-1 and α-2 HDL levels. These larger, cholesterol-rich HDL particles have been shown to enhance reverse cholesterol transport to promote atheroregression *v.* the relatively small and immature subclasses, which do not contribute to the regression of an atherosclerotic plaque[28]. An *ex vivo* assay of serum cholesterol efflux capacity did not reveal differences among the dietary treatment groups. However, previous studies have indicated that circulating levels of CRP can predict the risk of atherosclerotic plaque formation and thrombotic events[40,41]. When the effect of baseline CRP status was taken into account, we found significant increases in both ABCA1-mediated and global serum cholesterol efflux capacities following the 2PD dietary treatment *v.* the 1PD dietary treatment when baseline CRP values were $< 10^3$ μg/l. These results indicate that HDL functionality may be increased with the inclusion of pistachios in the absence of low-grade inflammation. As such, effects of diet on cholesterol efflux capacity may be blunted in individuals with chronic systemic inflammation. Future clinical studies should consider inflammatory status for a more accurate description of the cardioprotective effects conferred by nut consumption.

There were no significant effects of diet on lipoprotein(a), indicating that dietary fat from pistachios may not influence this cardiometabolic marker. Although elevated lipoprotein(a) levels (≥1·07 μmol/l) are associated with an increased risk of CVD, and modestly associated with insulin resistance[45], levels observed in the present study (lower-fat control, 0·89 (SE 0·01) μmol/l; 1PD, 0·90 (SE 0·01) μmol/l; 2PD, 0·89 (SE 0·01) μmol/l) were below the cut-off associated with an increased risk.

Table 7. Effects of pistachio inclusion on the levels of β-sitosterol and campesterol (markers of sterol absorption) and desmosterol and lathosterol (markers of endogenous cholesterol synthesis)

(Least-squares (LS) means with their standard errors, *n* 28)

Variables ($\times 10^{-4}$ mol/mmol total cholesterol)	Control		1PD*		2PD†		*P* (diet)	*P*‡ (control *v.* 1PD)	*P*‡ (1PD *v.* 2PD)	*P*‡ (control *v.* 2PD)
	LS mean	SE	LS mean	SE	LS mean	SE				
β-Sitosterol	120·9	1·1	139·1	1·1	154·2	1·1	<0·0001	<0·0001	0·0002	<0·0001
Campesterol	144·0	1·1	129·9	1·1	126·0	1·1	0·009	0·051	0·755	0·009
Desmosterol	63·2	1·2	87·1	1·2	46·9	1·3	0·137	0·366	0·115	0·384
Lathosterol	109·3	1·1	116·4	1·1	123·7	1·1	0·150	0·616	0·643	0·127

* 1PD represents one serving (32–63 g or 1·5 oz) of pistachios per d (10 % energy from pistachios).
† 2PD represents two servings (63–126 g or 3·0 oz) of pistachios per d (20 % energy from pistachios).
‡ Tukey's adjustments were used for comparisons among the dietary treatment groups.

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As has been discussed, the 1PD and 2PD diets significantly decreased LDL levels (−9 and −12 %, respectively) compared with the lower-fat control diet[29]. In the present study, β-sitosterol levels were elevated in the 1PD and 2PD dietary treatment groups *v.* the control group. In addition, β-sitosterol levels were inversely associated with campesterol levels in participants consuming the pistachio diets. Pistachios provide 200 mg of β-sitosterol and 10 mg of campesterol per 100 g[7], which account for the shift in the plasma sterol profile. Thus, increases in β-sitosterol levels with the inclusion of pistachios confirm participants' adherence to the study protocol. Despite elevated β-sitosterol levels, decreased cholesterol absorption[46,47] probably was not the primary factor contributing to the LDL-lowering effect observed following the 1PD and 2PD dietary treatments, as there were no significant associations between reductions in LDL and sdLDL levels and increases in β-sitosterol levels. This is not unexpected as the 1PD and 2PD diets provided only 103 and 321 mg phytosterols per d, which are appreciably less than the recommended intake of 2–3 g sterols/stanols per d for cholesterol-lowering effects to be observed[48]. Nonetheless, it is the combination of β-sitosterol levels and the fatty acid profile of the pistachio diets that most probably explains the cholesterol-lowering effects observed in the present study. Furthermore, the inclusion of pistachios may aid in the maintenance of whole-body cholesterol homeostasis[49], as evidenced by the absence of a change in desmosterol and lathosterol levels.

The present study has a few limitations. The duration of the study may have been too short to detect shifts in the lipoprotein subclass profile, particularly in the HDL subclasses. Other limitations include the lack of plasma samples for baseline lipoprotein subclass determinations, as well as waist circumference measurements for conclusive assessment of cardiometabolic status. Had we access to these data, we could have investigated the changes in subclass levels from baseline following the dietary treatments and evaluated how reductions in sdLDL levels and the TAG:HDL ratio are related to any change in waist circumference in participants over the course of the study. In addition, we observed effects of diet on cholesterol efflux capacity only in participants with low CRP status. Although this was not accounted for *a priori*, these results indicate that the traditional model for dietary treatment effects may not be as successful as in individuals with chronic systemic inflammation. Future studies should monitor the inflammatory status of participants at baseline and over the course of the study.

The present study provides new insights into the role of pistachios and their bioactive components that affect the markers of cardiometabolic syndrome. Collectively, we showed that inclusion of pistachios at 10–20 % energy per d as part of a heart-healthy, cholesterol-lowering diet decreases sdLDL levels, reduces the TAG:HDL ratio, modestly increases the levels of the functional HDL subclasses, and has beneficial effects on cholesterol efflux capacity (only in the lower-CRP status group), which in aggregate would be expected to decrease the risk of cardiometabolic syndrome. Based on our findings, we conclude that the inclusion of pistachios in a moderate-fat diet may prevent and slow the transition to CVD and diabetes. Further studies are required to directly evaluate whether the consumption of a heart-healthy, moderate-fat diet (that contains pistachios or other healthy fats) can prevent the onset of insulin resistance in individuals at an increased risk of cardiometabolic syndrome.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114514001561

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