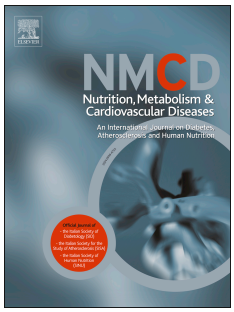
# Accepted Manuscript




Effect of pistachio consumption on plasma lipoprotein subclasses in pre-diabetic subjects

P. Hernández-Alonso, J. Salas-Salvadó, MD, PhD, M. Baldrich-Mora, R. Mallol, X. Correig, M. Bulló, PhD

PII: S0939-4753(15)00031-9

DOI: 10.1016/j.numecd.2015.01.013

Reference: NUMECD 1400

To appear in: *Nutrition, Metabolism and Cardiovascular Diseases*

Received Date: 16 December 2014

Revised Date: 15 January 2015

Accepted Date: 26 January 2015

Please cite this article as: Hernández-Alonso P, Salas-Salvadó J, Baldrich-Mora M, Mallol R, Correig X, Bulló M, Effect of pistachio consumption on plasma lipoprotein subclasses in pre-diabetic subjects, *Nutrition, Metabolism and Cardiovascular Diseases* (2015), doi: 10.1016/j.numecd.2015.01.013.

# Effect of pistachio consumption on plasma lipoprotein subclasses in pre-diabetic subjects

HERNÁNDEZ-ALONSO P.[a], SALAS-SALVADÓ J.[a,b*], BALDRICH-MORA M.[a,b], MALLOL R.[c], CORREIG X.[c], BULLÓ M.[a,b*].

[a]Human Nutrition Unit, Faculty of Medicine and Health Sciences, IISPV, Universitat Rovira i Virgili, Reus, Tarragona, Spain.

[b]CIBERobn Physiopathology of Obesity and Nutrition, Instituto de Salud Carlos III, Madrid, Spain.

[c]Metabolomic Platform, CIBERDEM (Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas), Universitat Rovira i Virgili, IISPV, Reus, Tarragona, Spain

[*]**Correspondence:** Mònica Bulló, PhD, and Jordi Salas-Salvadó, MD, PhD, Human Nutrition Unit, Faculty of Medicine, University of Rovira i Virgili, 43201 Reus, Spain Tel. +34 977759313 / Fax +34 977759322 – e-mail address: monica.bullo@urv.cat

**Short running title:** Pistachio intake and lipoprotein composition.

**Abstract word count:** 246

**Text word count: 3072** (including list of authors and their affiliations, corresponding author, acknowledgements and figure legends)

**Number of references:** 31

**Number of figure**: 1

**Number of tables**: 2

**Number of appendices: 1** (includes 2 figures plus 1 table)

**Keywords:** pistachios; lipoprotein; pre-diabetes; cardiovascular disease; dietary intervention; clinical trial.

**Abbreviations:** CV, cardiovascular; CD, control diet**;** DOSY, diffusion-ordered $^{1}$H NMR spectroscopy; DSTE, double stimulated echo; EPIRDEM, Effect of Pistachio Intake on Insulin Resistance and Type 2 Diabetes Mellitus; FID, finite impulse decay**;** HDL, high-density lipoprotein; IRAS, Insulin Resistance Atherosclerosis Study; ITT, intention-to-treat; LDL, low-density lipoprotein; LED, longitudinal eddy current delay; NMR, nuclear magnetic resonance; -P, particle**;** PD, pistachio diet; PLS, partial least-squares; PP, per protocol; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoprotein.

**Abstract**

***Background and Aims:*** Nuts have been demonstrated to improve several cardiovascular risk factors and the lipid profile in diabetic and pre-diabetic subjects. However, analysis of conventional serum lipid profiles does not completely explain the atherogenic risk associated with pre-diabetes. We therefore investigated whether chronic consumption of pistachio modifies the lipoprotein subclasses to a healthier profile in pre-diabetic subjects.

***Methods and Results:*** Randomized cross-over clinical trial in 54 subjects with pre-diabetes. Subjects consumed a pistachio-supplemented diet (PD, 50% carbohydrates, 33% fat, including 57g/d of pistachios daily) and a control diet (CD, 55% carbohydrates, 30% fat) for 4 months each, separated by a 2-week wash-out. Diets were isocaloric and matched for protein, fiber and saturated fatty acids. Nuclear magnetic resonance (NRM) was performed to determine changes in plasma lipoprotein subclasses. Small low-density lipoprotein particles (sLDL-P) significantly decreased after pistachio consumption compared to the nut-free diet (P=0.023). The non-high-density lipoprotein particles (non-HDL-P i.e. VLDL-P plus LDL-P) significantly decreased under the PD compared to CD (P=0.041). The percentage of sHDL-P increased by 2.23% after the PD compared with a reduction of 0.08% after the CD (P=0.014). Consequently, the overall size of HDL-P significantly decreased in the PD (P=0.007).

***Conclusion:*** Chronic pistachio consumption could modify the lipoprotein particle size and subclass concentrations independently of changes in total plasma lipid profile, which may help to explain the decreased risk of cardiovascular disease and mortality associated with those individuals who frequently consumed nuts.

***Registration Number:*** This study is registered at www.clinicaltrials.gov as NCT01441921.

## Introduction

Atherogenic dyslipidemia is a common feature of type 2 diabetes (T2DM) characterized by high levels of serum triglycerides, low HDL-cholesterol (HDL-C) concentrations and a relative increase in the number of small dense LDL particles (sLDL-P). Nonetheless, the lipid and lipoprotein profile often displays other abnormalities in the pre-diabetic stage, which may contribute to the subsequent increased risk of developing T2DM and cardiovascular (CV) disease. In this regard, large VLDL and small LDL particles have been related with a higher severity and incidence of coronary artery disease and type 2 diabetes [1]. However, results on HDL subfractions are more controversial. Thus, whereas some studies showed an association between small HDL-P and coronary risk [2], others found that small and medium-sized HDL particles were associated with a lower risk of total stroke [3].

While conventional cardiovascular prevention strategies focus on decreasing LDL-C concentrations increasing data suggests that preventive and therapeutic strategies could be focusing on lipoprotein subfractions abnormalities [4]. However, there is a lack of information on the potential modulatory effects of diet on lipoprotein subfractions and its effects on health and disease.

As far as nutritional factors are concerned, both epidemiological and clinical studies have provided a body of scientific evidence on the cardioprotective effects of nuts and their lipid-lowering properties. A pooled analysis of 25 clinical trials including different types of nuts showed a significant dose-related reduction in total cholesterol and LDL-C, but no effect on HDL-C or triglycerides (except in participants with hypertriglyceridemia) after nut consumption [5]. However, only one study has assessed the effect of nut consumption

on the composition and particle size of lipoprotein subfractions. This study showed beneficial changes in lipid distribution in lipoprotein subfractions after walnut consumption, and no changes in plasma lipid composition [6]. Compared to other nuts, pistachios have lower fat (mostly from poly- and monounsaturated fatty acids) and energy content, and higher levels of fiber (both soluble and insoluble), potassium, phytosterols, γ-tocopherol, vitamin K, xanthophyll and carotenoids thereby contributing to explain the beneficial relation between pistachio consumption and health-related outcomes [7].

The aim of the present study is to evaluate the effect of chronic intake of pistachios on lipoprotein size and subclass concentration in pre-diabetic subjects as a potential mechanism for decreasing the cardiovascular risk associated with pre-diabetes.

## Methods

*Study characteristics*

The EPIRDEM (Effect of Pistachio Intake on Insulin Resistance and Type 2 Diabetes Mellitus) study is a randomized, controlled, cross-over trial with a four-month dietary intervention in each period conducted in pre-diabetic subjects. The institutional review board of the Universitary Hospital of Sant Joan de Reus approved the study protocol in September 2011. Executed informed consent was obtained from all study participants. The trial was registered in Clinical Trials (identifier NCT01441921).

*Study population*

Eligible participants were community-living men and women aged between 25 and 65 years, body mass index $\leq$ 35 kg/m$^2$ and pre-diabetes was considered when fasting plasma glucose levels were between 100 and 125 mg/dL. Subjects were excluded if they met one of the following criteria: a) diabetes mellitus or using oral anti-diabetic drugs; b) alcohol, tobacco or drug abuse; c) frequent consumption of nuts or known history of allergy to them; d) use of plant sterols, psyllium, fish oil supplements and multivitamins, vitamin E or other antioxidant supplements, e) bad dentition, involving difficulty to chew pistachios; f) following a vegetarian or a hypocaloric diet to lose weight; g) being pregnant or wishing to become pregnant 9 months before or during the study, lactating 6 weeks before or during the study; h) significant liver, kidney, thyroid or other endocrine diseases; i) medical, dietary or social conditions that hinder compliance with the intervention.

*Study design*

A 15-days run-in period proceeded the four-month treatment period. A 2-week wash-out period separated the 2 crossover sequences. At baseline, data on medical history, physical examination and fasting blood for biochemical analysis were collected. Subjects who met the inclusion criteria were randomly assigned to one of the two different intervention sequences, before the 15-days run-in period, using a computer-generated random-number table. They were instructed to follow a normocaloric diet that provided 50% of energy as carbohydrates, 15% as protein, and 35% as total fat during the 2 weeks proceeding each study period.

The isocaloric diet was individually calculated using WHO equations adjusted by the estimated energy expenditure in physical-activity leisure-time. After the 2-week run-in period, subjects were randomized to one of the two sequences: starting with a control diet (CD) followed by the pistachio supplemented diet (PD), or starting with the pistachio diet followed by the control diet. The main characteristics of both intervention diets have already been published [8]. Participants allocated to the pistachio diet (PD) were supplemented with 2 ounces of pistachio (57 grams/day, half roasted and half roasted and salted). In the control diet (CD), the energy intake of other fatty foods, mostly olive oil, was adjusted to compensate for the energy from pistachios included in the PD. Adherence to the intervention period was assessed by counting the empty sachets of pistachio administered and by measuring plasma lutein-zeaxanthin and γ-tocopherol levels as previously described [8].

*Data collection*

Medical and anthropometric measurements were collected at the beginning and at the end of each dietary period. Blood samples were also collected during the same visits after 12h of fasting.

*Lipoprotein analysis by NMR spectroscopy of plasma samples*

Lipoprotein analysis of plasma samples by 2D diffusion-ordered $^{1}$H NMR spectroscopy (DOSY) was performed using a previous protocol [9]. This protocol measures lipid concentrations (i.e., triglycerides and cholesterol), sizes and particle numbers for VLDL (38.6 to 81.9 nm), LDL (14.7 to 26.6 nm) and HDL (6.0 to 10.9 nm) classes, as well as the particle numbers of nine subclasses (namely large, medium and small VLDL, LDL and HDL, respectively). Briefly, 2D $^{1}$H NMR spectra were recorded on a BrukerAvance III 600 spectrometer at 310 K (Bruker BioSpin, Rheinstetten, Germany). We used the double stimulated echo (DSTE) pulse program with bipolar gradient pulses and a longitudinal eddy current delay (LED). The relaxation delay was 2 seconds, the finite impulse decays (FIDs) were collected into 64K complex data points and 32 scans were acquired for each sample. The gradient pulse strength was increased from 5 to 95% of the maximum strength of 53.5 Gauss cm$^{-1}$ in 32 steps. The squared gradient pulse strength was linearly distributed.

To determine lipoprotein size, the methyl signal was surface fitted with the numbers of functions so that the nine lipoprotein subclasses could be determined. The mean particle size of every main fraction was derived by averaging the NMR area of each fraction by its associated size. To obtain particle-weighted lipoprotein sizes, each NMR area was divided by its associated volume. The particle numbers of each lipoprotein main fraction were calculated by dividing the lipid volume by the particle volume of a given class. The lipid

volumes were determined by using common conversion factors to convert concentration units obtained from the partial least-squares (PLS) models into volume units. The relative areas of the lipoprotein components used to decompose the 2D spectra were used to derive the particle numbers of the nine lipoprotein subclasses.

*Statistical analysis*

The descriptive data of participants at baseline and differences during the intervention periods are shown as means and 95% confidence intervals (95% CI) for continuous variables, and number (%) for categorical variables. Differences in all variables were evaluated by analysis of variance (ANOVA), with intervention diet as the independent and repeated measures factor. Diet sequence (order of diet treatments) was analysed as an independent factor but as it was not significant it was not further considered. The differences in variable changes between dietary intervention periods were analysed by an ANCOVA test using baseline values as a covariate. All statistical analyses were conducted using intention-to-treat (ITT) and *per protocol* (PP) approaches. ITT analysis included all randomized participants and data was analyzed following the assumption that the drop outs (all of them attended only to the baseline visit) did not change their variables' values through the whole study. The PP analysis excluded participants who did not attend the last visit, and the results are shown in Supplement. The sample size estimation has been previously described [8]. All analyses were done using SPSS 20.0 (SPSS Inc, Chicago, IL). All tests were 2-sided, and significance was defined as $P < 0.05$.

**Results**

A total of 108 participants were assessed for eligibility, of whom 30 declined to participate and 24 did not meet the inclusion criteria. Fifty-four participants were randomly assigned to one of the two intervention sequences. During the pistachio period, 5 of the 54 randomized participants (9.25%) dropped out of the study for personal reasons (Figure S1, Supporting information). No gastrointestinal side effects were observed during the trial and no changes in medication were reported during the study. The baseline characteristics of the study participants are shown in Table 1. No significant differences were observed between dietary interventions at baseline in any of the analyzed parameters. As expected, plasma lutein-zeaxanthin and γ-tocopherol levels were significantly higher in the PD compared with the CD (already published data, [8]).

Total LDL-P was non-significantly lowered in the PD diet than in the CD diet (mean (95% CI): -46.67 nM (-88.22, -5.12) and 20.66 nM (-23.62, 64.94), respectively, P=0.10) (Table 2). However, when the concentration of each LDL-P subclass (i.e. small, medium and large) was analysed, the concentration of small LDL-P was significantly lowered in the PD diet than in the CD diet (mean (95% CI): -28.07 nM (-60.43, 4.29) and 16.49 nM (-14.19, 47.18) respectively, P=0.02). However, results for total VLDL-P, total HDL-P concentrations and their relative subclasses, showed non-significant changes between intervention periods.

The mean size of HDL particles was significantly lowered in the PD diet than in the CD diet (mean (95% CI): -0.13 Å (-0.22, -0.03) and 0.02 Å (-0.07, 0.10), respectively, P=0.01), whereas no significant changes were observed for mean VLDL or LDL particle size.

Moreover, there were significant differences in non-HDL-P concentrations (i.e. sum of total VLDL-P and LDL-P) between the two periods: they were reduced in the PD period (mean

(95% CI): -36.02 nM (-77.56, 5.20)) and increased in the CD period (21.11 nM (-23.85, 66.06)); P=0.04. The proportion (expressed as percentage) of small-HDL particle concentrations from total HDL particles, was significantly higher after PD than after the control period (mean (95% CI): 2.23 % (0.57, 3.89) and -0.08 % (-1.66, 1.49), respectively, P=0.01), whereas the percentage of medium- and large-HDL particles was significantly lower. No significant differences in the proportion of small LDL-P were observed between intervention periods (Figure 1). The *Per Protocol* analysis showed similar results (Table S1 and Figure S2, Supporting information).

**Discussion**

In the present study, we have demonstrated that the chronic consumption of pistachios shifts the lipoprotein size and particle profile to a less atherogenic pattern, thus suggesting that pistachios may play a beneficial role in cardiovascular disease, even though they have no effect on the classic lipid factors of cardiovascular risk.

In hypercholesterolemic, normolipidemic and healthy subjects, it has been consistently reported that the regular intake of nuts has the beneficial effect on lipid profile of lowering serum LDL-C, without significantly affecting triglycerides or HDL-C [10]. However, these lipid-lowering properties attributed to nuts are more controversial in obese subjects or subjects who are resistant to insulin. In these subjects, whereas some authors have reported significant reductions in LDL-C and increases in HDL-C after nut consumption [11], others have failed to find significant changes in lipid profile [12]. In particular, consumption of pistachio has been reported to induce a significant reduction in total cholesterol (TC), TC/HDL-C ratio and LDL-C/HDL-C ratio [13–15] and a significant increase in plasma HDL-C [13,14] in healthy and hypercholesterolemic subjects. The effect of pistachio consumption on LDL-C concentrations is less consistent: they tend to decrease [13,15] but not always significantly [13]. In a previous publication conducted in the same population as the present study, we found no significant changes in either total cholesterol, LDL-C or HDL-C after pistachio consumption [8], supporting the hypothesis that obese or insulin-resistant subjects are less likely to have changes in their lipid profile [16].

In recent years, interest in the concentrations of lipoprotein subclass particles (small, medium, large) has been increasingly focused not on the total amount of cholesterol within these particles but on their potential effect on atherosclerosis and cardiovascular risk [4]. A decade ago, Liu et al. found that non-HDL-cholesterol (LDL-C plus VLDL-C) was strongly

associated with an increased risk of coronary heart disease [17], which was even greater than that attributed to LDL-C [18]. Unlike large LDL particles, small, dense LDL-P confers greater atherogenic risk because of its interaction with the arterial wall (e.g. increased residence time in the circulation, easy penetration into the sub-endothelial space and greater susceptibility to oxidative modification) [4]. Additionally, high levels of small, dense LDL-P have been positively correlated with microalbuminuria and negatively with glomerular filtration rate as predictors of diabetic nephropathy [19] and as emerging CV risk factors [20]. Otherwise, small HDL-P have been associated both with more non-calcified plaque or higher coronary risk but also with an atheroprotective role [21], whereas larger HDL-P have been associated with less non-calcified plaques and lower coronary risk [22]. Lipoprotein subclass abnormalities have also been related to insulin resistance and T2DM. Concentrations of small, dense atherogenic LDL particles are commonly higher in insulin-resistant subjects [23]. A study conducted on subjects with or without T2DM demonstrated that progressive insulin resistance was associated with an atherogenic profile characterized by an increase in VLDL size (mainly in large-VLDL particle concentrations), a decrease in LDL size (reflected by an increase in small- and a reduction in large-LDL), and a decrease in HDL size (by reduction in large- and an increase in small- HDL particles) [24]. The results found after 1 371 T2DM, impaired glucose-tolerant and normoglycemic participants were analyzed in the Insulin Resistance Atherosclerosis Study (IRAS) [25], were similar and suggested that dyslipidemia associated with insulin resistance or type 2 diabetes is not detected when the traditional lipid profile is evaluated. The IRAS study has also demonstrated for the first time that, independently of triglyceride and HDL-C concentrations, lipoprotein subclasses were positively associated with the incidence of T2DM at 5-year follow-up [1].

Although a considerable amount of research has been carried out on the conventional lipid profile, the modulatory effect of dietary fatty acids on the size and concentration of lipoprotein subclasses has been poorly analyzed. A lower consumption of saturated fatty acids (15%, 9% or 6.1%) for 4 weeks significantly decreased large HDL and increased small HDL subclasses [26]. A reduction in large, medium and small, dense LDL-P was observed after a portfolio diet including almonds (15g/day) for 4 weeks [27]. However, a significant decrease in the proportion of small LDL-P has been reported in normolipidemic subjects after 3 days on a high-fat diet (37% energy from fat) [28] and also 6 hours after a high-fat meal (83% energy from fat) [29]. To the best of our knowledge, only one study has evaluated the effect of nut consumption on the distribution and particle size of lipoprotein subclasses. The study was conducted in a small number of adults (n=18) with combined hyperlipidemia who consumed 48g of walnuts for 6 weeks. Cholesterol decreased, particularly in small-LDL-P and large-HDL particles, which suggested that the beneficial properties of nut intake on cardiovascular risk may be underlying by an additional mechanism [6]. In agreement with these results, our study demonstrates that chronic consumption of pistachios induces a significant decrease of small-LDL concentrations and HDL-P size, despite the absence of change in TC, LDL-C or HDL-C. The mean decrease observed in HDL-P size could be explained by the significant increase in the proportion of small HDL-P and the significant decrease in the proportion of medium and large HDL particle concentrations. Similarly, we found a significant decrease in non-HDL-particle concentrations, which are also strongly associated with cardiovascular risk [30].

Several strengths and limitations of our study deserve comment. Among the strengths are its cross-over randomized design, its medium-term duration and the biochemical dietary compliance markers. We have also used NMR to analyse lipoprotein subfractions, which it

has been suggested as a better coronary heart disease estimator than non-denaturing polyacrylamide gradient gel electrophoresis [31]. Because data on HDL function was not measured, the implications of changes in HDL subtype distribution need to be further investigated. However, because the study focused on pre-diabetic subjects, the results cannot be extrapolated to healthy subjects or subjects with T2DM. Despite the cross-over design, other limitations could be related to the relatively heterogeneity of the subjects studied as the presence of dyslipidemia or the use of lipid-lowering treatments. Whether other nuts can modify lipoprotein subclasses in a similar way deserves further research.

In conclusion, the results of the present study suggest that the chronic consumption of pistachios has a beneficial effect on emergent cardiovascular risk factors, even though it has little or no effect on classical lipid risk markers. Further research is necessary to corroborate our results and to extend them in the knowledge of which subclasses are reliable markers of cardiovascular diseases and potential targets for effective therapies.

**Acknowledgements**

We thank the American Pistachio Growers (USA), and Paramount Farms for their funding support. None of the funding sources played a role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication. We are indebted to the participants in the study and the medical doctors and nursing staff of the SAGESSA group for their collaboration. We thank Carles Munné (Rovira i Virgili University, Reus, Spain) for his editorial assistance. J. Salas-Salvadó is a nonpaid member of the Scientific Advisory Council of the International Nut Council. P. Hernández-Alonso is the recipient of a pre-doctoral fellowship from the Generalitat de Catalunya's Department

of Universities [FI-DGR 2014]. M. Baldrich-Mora, R. Mallol, X. Correig and M. Bulló have nothing to declare.

**References**


[1] Festa A, Williams K, Hanley AJG, Otvos JD, Goff DC, Wagenknecht LE, et al. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. Circulation 2005;111:3465–72. doi:10.1161/CIRCULATIONAHA.104.512079.

[2] Martin SS, Jones SR, Toth PP. High-density lipoprotein subfractions: current views and clinical practice applications. Trends Endocrinol Metab 2014;25:329–36. doi:10.1016/j.tem.2014.05.005.

[3] Chei C-L, Yamagishi K, Kitamura A, Kiyama M, Imano H, Ohira T, et al. High-density lipoprotein subclasses and risk of stroke and its subtypes in Japanese population: the Circulatory Risk in Communities Study. Stroke 2013;44:327–33. doi:10.1161/STROKEAHA.112.674812.

[4] Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. QJM 2006;99:1–14. doi:10.1093/qjmed/hci154.

[5] Sabaté J, Oda K, Ros E. Nut consumption and blood lipid levels: a pooled analysis of 25 intervention trials. Arch Intern Med 2010;170:821–7. doi:10.1001/archinternmed.2010.79.

[6] Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. Am J Clin Nutr 2001;74:72–9.

[7] Bulló M, Juanola-Falgarona M, Hernández-Alonso P, Salas-Salvadó J. Nutrition attributes and health effects of pistachio nuts. Br J Nutr 2015;(in press).

[8] Hernández-Alonso P, Salas-Salvadó J, Baldrich-Mora M, Juanola-Falgarona M, Bulló M. Beneficial Effect of Pistachio Consumption on Glucose Metabolism, Insulin Resistance, Inflammation, and Related Metabolic Risk Markers: a Randomized Clinical Trial. Diabetes Care 2014;37:3098–105. doi:10.2337/dc14-1431.

[9] Mallol R, Amigo N, Rodriguez MA, Heras M, Vinaixa M, Plana N, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. J Lipid Res 2015:jlr.D050120 – . doi:10.1194/jlr.D050120.

[10] Banel DK, Hu FB. Effects of walnut consumption on blood lipids and other cardiovascular risk factors: a meta-analysis and systematic review. Am J Clin Nutr 2009;90:56–63. doi:10.3945/ajcn.2009.27457.

[11] Tapsell LC, Gillen LJ, Patch CS, Batterham M, Owen A, Baré M, et al. Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol

ratios in patients with type 2 diabetes. Diabetes Care 2004;27:2777–83. doi:10.2337/diacare.27.12.2777.

[12] Casas-Agustench P, López-Uriarte P, Bulló M, Ros E, Cabré-Vila JJ, Salas-Salvadó J. Effects of one serving of mixed nuts on serum lipids, insulin resistance and inflammatory markers in patients with the metabolic syndrome. Nutr Metab Cardiovasc Dis 2011;21:126–35. doi:10.1016/j.numecd.2009.08.005.

[13] Kocyigit A, Koylu AA, Keles H. Effects of pistachio nuts consumption on plasma lipid profile and oxidative status in healthy volunteers. Nutr Metab Cardiovasc Dis 2006;16:202–9. doi:10.1016/j.numecd.2005.08.004.

[14] Sheridan MJ, Cooper JN, Erario M, Cheifetz CE. Pistachio nut consumption and serum lipid levels. J Am Coll Nutr 2007;26:141–8.

[15] Sari I, Baltaci Y, Bagci C, Davutoglu V, Erel O, Celik H, et al. Effect of pistachio diet on lipid parameters, endothelial function, inflammation, and oxidative status: a prospective study. Nutrition 2010;26:399–404. doi:10.1016/j.nut.2009.05.023.

[16] Pihlajamäki J, Gylling H, Miettinen TA, Laakso M. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res 2004;45:507–12. doi:10.1194/jlr.M300368-JLR200.

[17] Liu J, Sempos C, Donahue RP, Dorn J, Trevisan M, Grundy SM. Joint distribution of non-HDL and LDL cholesterol and coronary heart disease risk prediction among individuals with and without diabetes. Diabetes Care 2005;28:1916–21.

[18] Liu J, Sempos CT, Donahue RP, Dorn J, Trevisan M, Grundy SM. Non-high-density lipoprotein and very-low-density lipoprotein cholesterol and their risk predictive values in coronary heart disease. Am J Cardiol 2006;98:1363–8. doi:10.1016/j.amjcard.2006.06.032.

[19] Abd-Allha E, Hassan BB, Abduo M, Omar SA, Sliem H. Small dense low-density lipoprotein as a potential risk factor of nephropathy in type 2 diabetes mellitus. Indian J Endocrinol Metab 2014;18:94–8. doi:10.4103/2230-8210.126585.

[20] Nagai K, Yamagata K, Ohkubo R, Saito C, Asahi K, Iseki K, et al. Annual decline in estimated glomerular filtration rate is a risk factor for cardiovascular events independent of proteinuria. Nephrology 2014;19:574–80. doi:10.1111/nep.12286.

[21] Rizzo M, Otvos J, Nikolic D, Montalto G, Toth PP, Banach M. Subfractions and subpopulations of HDL: an update. Curr Med Chem 2014;21:2881–91.

[22] Voros S, Joshi P, Qian Z, Rinehart S, Vazquez-Figueroa JG, Anderson H, et al. Apoprotein B, small-dense LDL and impaired HDL remodeling is associated with

larger plaque burden and more noncalcified plaque as assessed by coronary CT angiography and intravascular ultrasound with radiofrequency backscatter: results from the ATLANTA I . J Am Heart Assoc 2013;2:e000344. doi:10.1161/JAHA.113.000344.

[23] Friedlander Y, Kidron M, Caslake M, Lamb T, McConnell M, Bar-On H. Low density lipoprotein particle size and risk factors of insulin resistance syndrome. Atherosclerosis 2000;148:141–9.

[24] Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes 2003;52:453–62.

[25] Goff DC, D'Agostino RB, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the Insulin Resistance Atherosclerosis Study. Metabolism 2005;54:264–70. doi:10.1016/j.metabol.2004.09.002.

[26] Berglund L, Oliver EH, Fontanez N, Holleran S, Matthews K, Roheim PS, et al. HDL-subpopulation patterns in response to reductions in dietary total and saturated fat intakes in healthy subjects. Am J Clin Nutr 1999;70:992–1000.

[27] Lamarche B, Desroches S, Jenkins DJA, Kendall CWC, Marchie A, Faulkner D, et al. Combined effects of a dietary portfolio of plant sterols, vegetable protein, viscous fibre and almonds on LDL particle size. Br J Nutr 2004;92:657–63.

[28] Guay V, Lamarche B, Charest A, Tremblay AJ, Couture P. Effect of short-term low- and high-fat diets on low-density lipoprotein particle size in normolipidemic subjects. Metabolism 2012;61:76–83. doi:10.1016/j.metabol.2011.06.002.

[29] Wojczynski MK, Glasser SP, Oberman A, Kabagambe EK, Hopkins PN, Tsai MY, et al. High-fat meal effect on LDL, HDL, and VLDL particle size and number in the Genetics of Lipid-Lowering Drugs and Diet Network (GOLDN): an interventional study. Lipids Health Dis 2011;10:181. doi:10.1186/1476-511X-10-181.

[30] Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009;302:1993–2000. doi:10.1001/jama.2009.1619.

[31] Arsenault BJ, Lemieux I, Després J-P, Wareham NJ, Stroes ESG, Kastelein JJP, et al. Comparison between gradient gel electrophoresis and nuclear magnetic resonance spectroscopy in estimating coronary heart disease risk associated with LDL and HDL particle size. Clin Chem 2010;56:789–98. doi:10.1373/clinchem.2009.140939.

**Appendix A. Supplementary data:**

**Table S1.** Baseline and changes after intervention period in lipoprotein concentration and size.

**Figure S1.** Flowchart of study participants.

**Figure S2.** Changes (expressed as percentages) in the proportion of lipoprotein subclasses (small, medium or large) related to the total lipoprotein particle concentration (HDL-P, LDL-P or VLDL-P) according to intervention period.

**Tables**

**Table 1.** Baseline characteristics of the study population

| Variable | Subjects (n=54) |
|---|---|
| Female sex, n (%) | 25 (46) |
| Age (years) | 55 (53.4, 56.8) |
| Weight (kg) | 77.6 (74.8, 80.3) |
| Body mass index (kg/m$^2$) | 28.9 (28.2, 29.6) |
| Waist circumference (cm) | 94.7 (92.8, 96.6) |
| Systolic blood pressure (mmHg) | 134 (130, 137) |
| Diastolic blood pressure (mmHg) | 81 (79, 83) |
| Total cholesterol (mg/dL) | 213.13 (205.00, 221.26) |
| LDL-C (mg/dL) | 135.66 (127.65, 143.67) |
| HDL-C (mg/dL) | 54.47 (50.83, 58.11) |
| Triglycerides (mg/dL) | 115.34 (102.68, 128.00) |
| VLDL-P (nM) | 41.62 (36.07, 47.18) |
| LDL-P (nM) | 1210.40 (1147.26, 1273.54) |
| HDL-P (µM) | 30.34 (29.13, 31.56) |
| Fasting plasma glucose (mg/dL) | 112.80 (108.51, 117.08) |
| Fasting plasma insulin (mU/mL) | 12.19 (10.59, 13.79) |
| HOMA-IR | 3.50 (2.97, 4.02) |
| Glycated $HbA_{1c}$ (%) | 5.92 (5.81, 6.03) |
| Glycated $HbA_{1c}$ (mmol/mol) | 41.20 (40.01, 42.38) |
| Dyslipidemia, n (%) | 27 (50) |
| Hypertension, n (%) | 23 (42.6) |
| Statins, n (%) | 5 (9.3) |
| Fibrates, n (%) | 2 (3.7) |
| Angiotensin converter enzyme inhibitors, n (%) | 6 (11.1) |
| Beta-blockers and other antihypertensive drugs, n (%) | 13 (24.1) |
| Leisure-time physical activity (Kcal/day) | 347 (307, 387) |

Data are given as mean (95% CI) or number (%). VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; -C, cholesterol; -P, particle; HOMA-IR, homeostatic model assessment of insulin resistance; $HbA_{1c}$, Glycated hemoglobin.

**Table 2.** Baseline and changes in lipoprotein concentration and size after the intervention period

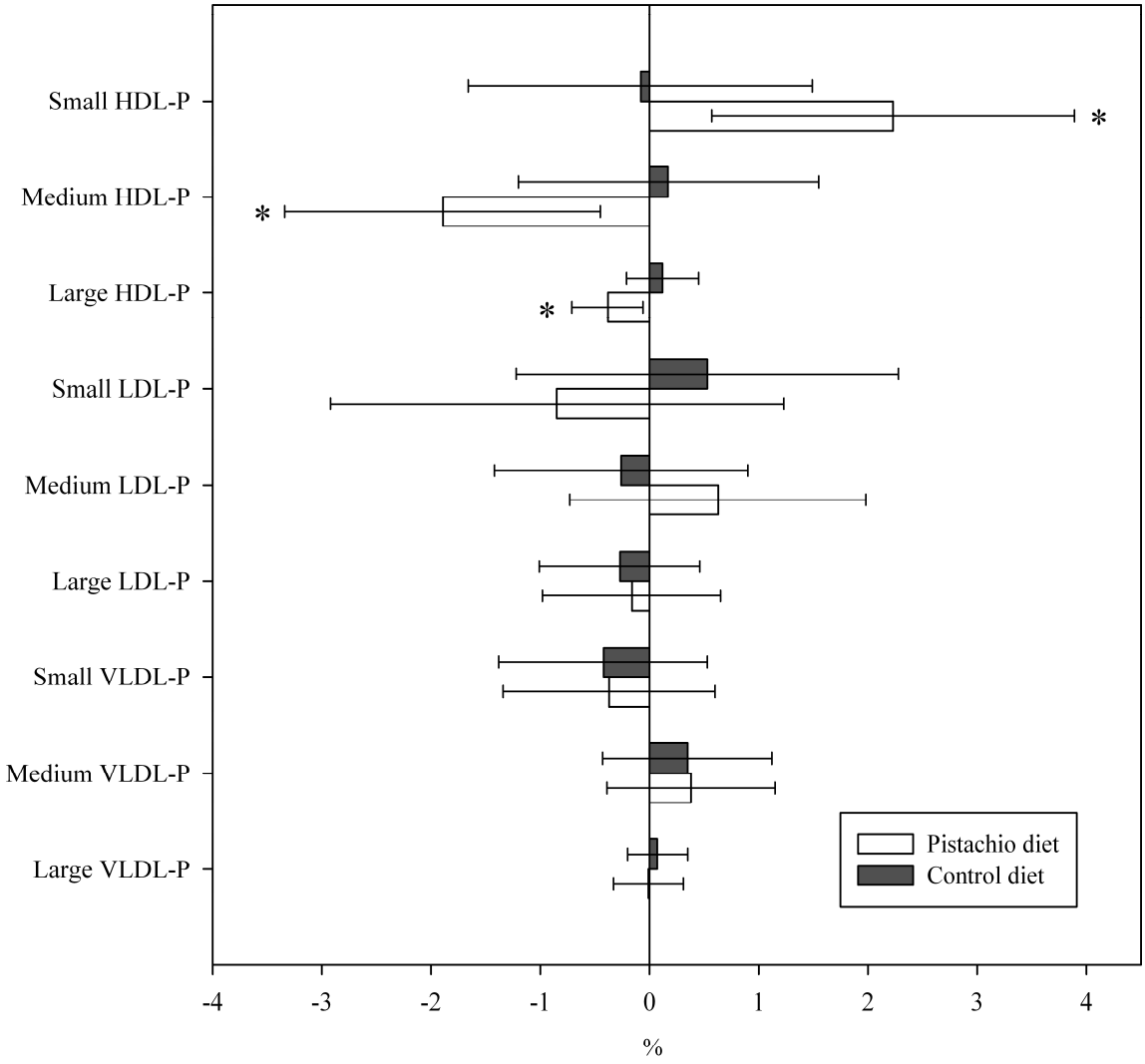
| | Pistachio diet | | Control diet | | Treatment effect |
|---|---|---|---|---|---|
| **Characteristics** | **Baseline** | **Change** | **Baseline** | **Change** | **P-value** |
| Total VLDL-P (nM) | 46.24 (39.74, 52.73) | -0.58 (-5.12, 3.95) | 42.12 (36.61, 47.64) | 2.27 (-3.36, 7.90) | 0.33 |
| Large VLDL-P (nM) | 1.42 (1.06, 1.78) | -0.06 (-0.33, 0.21) | 1.10 (0.87, 1.34) | 0.22 (-0.20, 0.65) | 0.23 |
| Medium VLDL-P (nM) | 7.09 (5.64, 8.54) | -0.11 (-1.09, 0.87) | 6.04 (4.89, 7.18) | 0.75 (-0.47, 1.97) | 0.25 |
| Small VLDL-P (nM) | 37.72 (32.93, 42.51) | -0.41 (-3.81, 2.98) | 34.98 (30.73, 39.22) | 1.35 (-2.73, 5.43) | 0.39 |
| Total LDL-P (nM) | 1236.37 (1160.70, 1312.04) | -46.67 (-88.22, -5.12) | 1219.11 (1154.56, 1283.67) | 20.66 (-23.62, 64.94) | 0.10 |
| Large LDL-P (nM) | 131.36 (113.49, 149.23) | -8.15 (-18.99, 2.69) | 133.98 (120.49, 147.47) | 1.80 (-8.13, 11.73) | 0.30 |
| Medium LDL-P (nM) | 463.62 (421.63, 505.62) | -10.45 (-31.39, 10.49) | 462.91 (431.15, 494.68) | 2.37 (-21.26, 26.01) | 0.49 |
| Small LDL-P (nM) | 647.68 (604.74, 690.61) | -28.07 (-60.43, 4.29) | 620.60 (581.13, 660.06) | 16.49 (-14.19, 47.18) | 0.02 |
| Total HDL-P (µM) | 30.32 (29.02, 31.62) | 1.18 (-0.33, 2.70) | 30.72 (29.46, 31.98) | 0.70 (-0.28, 1.68) | 0.55 |
| Large HDL-P (µM) | 1.14 (0.83, 1.44) | -0.08 (-0.18, 0.03) | 1.04 (0.79, 1.29) | 0.05 (-0.06, 0.17) | 0.09 |
| Medium HDL-P (µM) | 6.69 (5.77, 7.61) | -0.28 (-0.76, 0.20) | 6.44 (5.73, 7.15) | 0.13 (-0.25, 0.51) | 0.13 |
| Small HDL-P (µM) | 22.58 (21.24, 23.91) | 1.57 (0.22, 2.91) | 23.34 (22.19, 24.49) | 0.56 (-0.53, 1.65) | 0.18 |
| Particle size (Å) | | | | | |
| VLDL | 200.59 (199.15, 202.04) | 0.27 (-0.77, 1.31) | 199.58 (198.38, 200.78) | 0.41 (-0.58, 1.39) | 0.83 |
| LDL | 99.20 (98.85, 99.54) | 0.09 (-0.16, 0.35) | 99.46 (99.20, 99.71) | -0.15 (-0.40, 0.11) | 0.17 |
| HDL | 40.30 (40.09, 40.51)* | -0.13 (-0.22, -0.03) | 40.21 (40.05, 40.37) | 0.02 (-0.07, 0.10) | 0.01 |
| Non-HDL-P (nM) | 1282.61 (1206.53, 1358.69) | -36.02 (-77.56, 5.52) | 1261.35 (1195.00, 1327.70) | 21.11 (-23.85, 66.06) | 0.04 |
| Total-P/HDL-P ratio | 1.04 (1.04, 1.05) | -0.003 (-0.005, -0.0006) | 1.04 (1.04, 1.05) | -0.0004 (-0.002, -0.002) | 0.09 |
| LDL-P/HDL-P ratio | 0.04 (0.04, 0.05) | -0.003 (-0.005, -0.0005) | 0.04 (0.04, 0.04) | -0.003 (-0.005, -0.0007) | 0.86 |

Intention-to-treat analysis, n=54. All values are means (95% CI). Intra-group analysis was assessed by the paired t-test. Basal-adjusted changes between groups were analysed using adjusted ANOVA of repeated measurements. * Significant difference ($P<0.05$) between baseline and end of a particular intervention period. -P, particle; -C, cholesterol; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

**Figure legends**

**Figure 1.** Changes (expressed as percentages) in the proportion of lipoprotein subclasses (small, medium or large) related to the total lipoprotein particle concentration (HDL-P, LDL-P or VLDL-P) according to intervention period.

Intention-to-treat analysis, n=54. Values are means (95% CI). Changes between groups were analysed using ANOVA of repeated measurements.* stands for significant differences ($P<0.05$) in changes between pistachio diet and control diet. HDL-P, high-density lipoprotein particle; LDL-P, low-density lipoprotein particle; VLDL-P, very low-density lipoprotein particle.


Small HDL-P
Medium HDL-P
Large HDL-P
Small LDL-P
Medium LDL-P
Large LDL-P
Small VLDL-P
Medium VLDL-P
Large VLDL-P
Pistachio diet
Control diet
-4
-3
-2
-1
0
1
2
3
4
%

**Highlights**

- We examined the effect of pistachio intake in lipoprotein subclasses
- Pistachio intake improves the lipoprotein profile in pre-diabetic subjects
- We suggest it as a dietary tool for managing other prevalent chronic diseases

**Supplementary data**

for

***"Effect of pistachio consumption on plasma lipoprotein subclasses in pre-diabetic subjects"***

by

Hernández-Alonso P., Salas-Salvadó J., Baldrich-Mora M., Mallol R., Correig X., Bulló M.

**Table S1.** Baseline and changes after intervention period in lipoprotein concentration and size.

**Figure S1.** Flowchart of study participants.

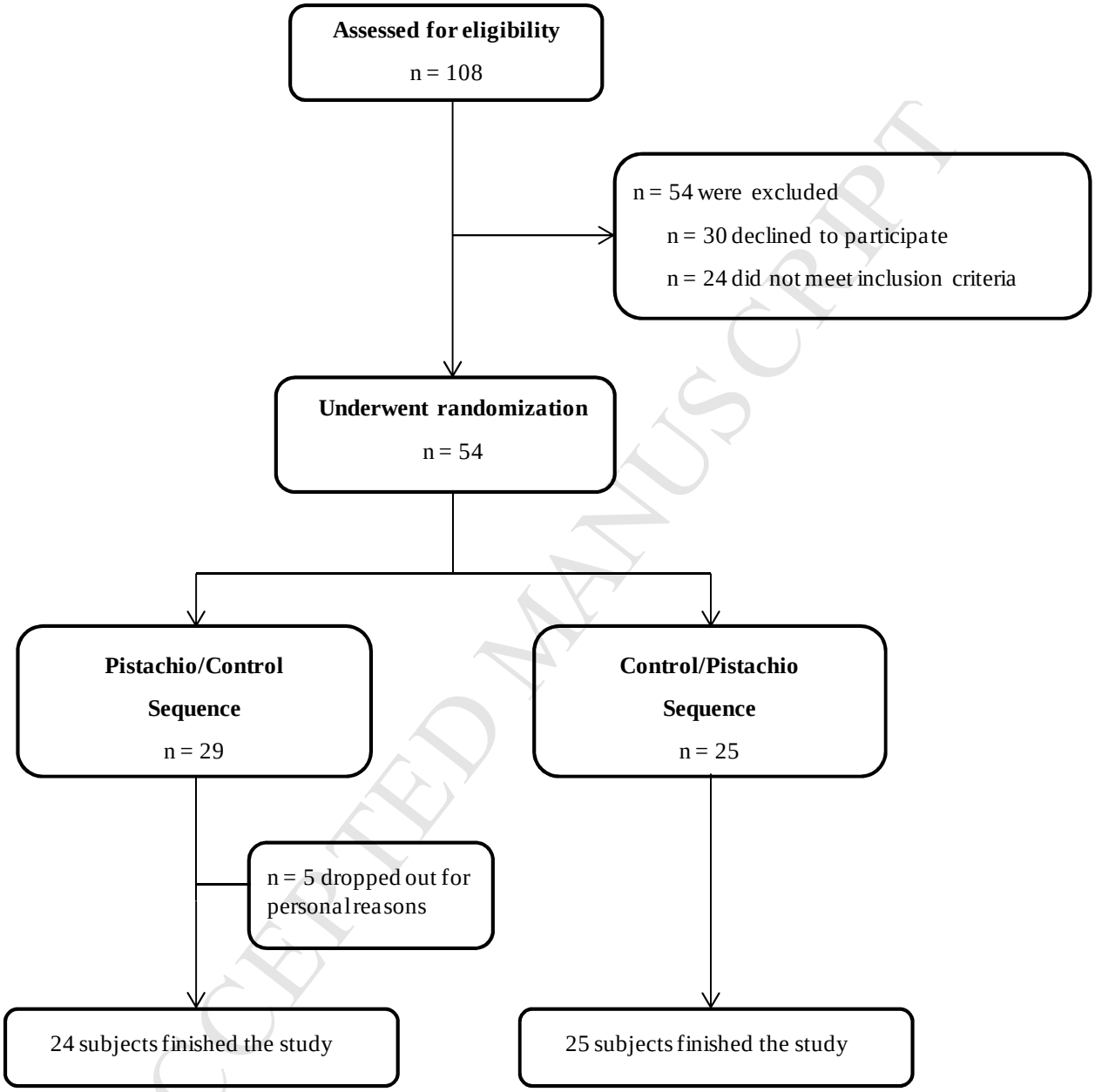
**Figure S2.** Changes (expressed as percentages) in the proportion of lipoprotein subclasses (small, medium or large) related to the total lipoprotein particle concentration (HDL-P, LDL-P or VLDL-P) according to intervention period.

**Table S1.** Baseline and changes after intervention period in lipoprotein concentration and size.

| | Pistachio Diet | | Control diet | | Treatment Effect |
|---|---|---|---|---|---|
| **Characteristics** | **Baseline** | **Change** | **Baseline** | **Change** | **P-value** |
| Total VLDL-P (nM) | 46.24 (39.74, 52.73) | -0.07 (-5.05, 4.92) | 42.63 (36.57, 48.69) | 2.50 (-3.72, 8.72) | 0.42 |
| Large VLDL-P (nM) | 1.42 (1.06, 1.78) | -0.06 (-0.35, 0.24) | 1.08 (0.83, 1.34) | 0.25 (-0.23, 0.72) | 0.24 |
| Medium VLDL-P (nM) | 7.09 (5.64, 8.54) | -0.07 (-1.15, 1.01) | 6.02 (4.76, 7.28) | 0.82 (-0.53, 2.18) | 0.28 |
| Small VLDL-P (nM) | 37.72 (32.93, 42.51) | 0.05 (-3.67, 3.78) | 35.53 (30.87, 40.18) | 1.49 (-3.02, 5.99) | 0.53 |
| Total LDL-P (nM) | 1236.37 (1160.70, 1312.04) | -52.14 (-97.74, -6.54) | 1213.04 (1144.42, 1281.65) | 23.05 (-26.21, 72.31) | 0.09 |
| Large LDL-P (nM) | 131.36 (113.49, 149.23) | -8.05 (-20.01, 3.92) | 136.54 (122.13, 150.95) | 2.11 (-8.95, 13.17) | 0.35 |
| Medium LDL-P (nM) | 463.62 (421.63, 505.62) | -11.20 (-34.29, 11.89) | 463.86 (429.02, 498.70) | 2.63 (-23.67, 28.94) | 0.50 |
| Small LDL-P (nM) | 647.68 (604.74, 690.61) | -32.89 (-68.37, 2.59) | 610.42 (570.29, 650.55) | 18.31 (-15.89, 52.51) | 0.02 |
| Total HDL-P (µM) | 30.32 (29.02, 31.62) | 1.28 (-0.39, 2.95) | 30.71 (29.34, 32.08) | 0.77 (-0.30, 1.85) | 0.56 |
| Large HDL-P (µM) | 1.14 (0.83, 1.44) | -0.09 (-0.20, 0.03) | 1.06 (0.79, 1.34) | 0.06 (-0.07, 0.19) | 0.09 |
| Medium HDL-P (µM) | 6.69 (5.77, 7.61) | -0.30 (-0.83, 0.23) | 6.48 (5.71, 7.24) | 0.14 (-0.28, 0.57) | 0.15 |
| Small HDL-P (µM) | 22.58 (21.24, 23.91) | 1.66 (0.19, 3.13) | 23.28 (22.07, 24.50) | 0.62 (-0.58, 1.82) | 0.21 |
| Particle size (Å) | | | | | |
| VLDL | 200.59 (199.15, 202.04) | 0.16 (-0.99, 1.30) | 199.11 (197.90, 200.33) | 0.45 (-0.64, 1.54) | 0.68 |
| LDL | 99.20 (98.85, 99.54) | 0.12 (-0.16, 0.40) | 99.52 (99.25, 99.79) | -0.16 (-0.44, 0.12) | 0.15 |
| HDL | 40.30 (40.09, 40.51)* | -0.14 (-0.24, -0.04) | 40.22 (40.04, 40.39) | 0.02 (-0.08, 0.11) | 0.01 |
| Non-HDL-P (nM) | 1282.61 (1206.53, 1358.69) | -40.37 (-85.97, 5.22) | 1255.80 (1185.03, 1326.57) | 23.38 (-26.61, 73.37) | 0.04 |
| Total-P/HDL-P ratio | 1.04 (1.04, 1.05) | -0.003 (-0.005, -0.0006) | 1.04 (1.04, 1.05) | -0.0004 (-0.003, -0.002) | 0.09 |
| LDL-P/HDL-P ratio | 0.04 (0.04, 0.05) | -0.003 (-0.005, -0.0005) | 0.04 (0.04, 0.04) | -0.0004 (-0.003, -0.002) | 0.10 |

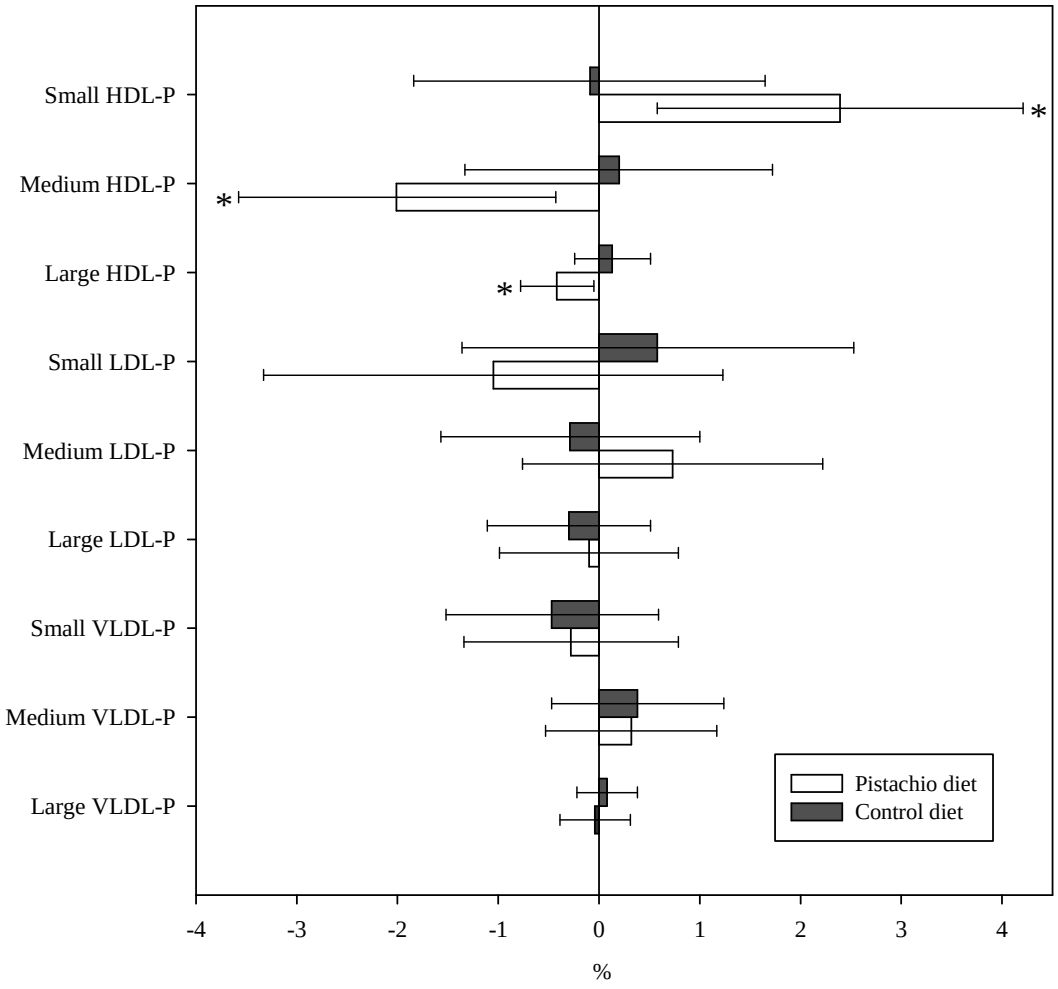
Per protocol analysis, n=49. All values are means (95% CI). Intra-group analysis was assessed by the paired t-test. Basal-adjusted changes between groups were analysed using adjusted ANOVA of repeated measurements. * Significant difference (P<0.05) between baseline and end of a particular intervention period. -P, particle; -C, cholesterol; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

**Figure S1.** Flowchart of study participants.




EPIRDEM Flow chart. Crossover design.

**Figure S2.** Changes (expressed as percentages) in the proportion of lipoprotein subclasses (small, medium or large) related to the total lipoprotein particle concentration (HDL-P, LDL-P or VLDL-P) according to intervention period.




*Per protocol* analysis, n=49. Values are means (95% CI). Changes between groups were analysed using ANOVA of repeated measurements.* stands for significant differences (P<0.05) in changes between pistachio diet and control diet. HDL-P, high-density lipoprotein particle; LDL-P, low-density lipoprotein particle; VLDL-P, very low-density lipoprotein particle.