Beneficial Effect of Pistachio Consumption on Glucose Metabolism, Insulin Resistance, Inflammation, and Related Metabolic Risk Markers: A Randomized Clinical Trial

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OBJECTIVE

To examine whether a pistachio-rich diet reduces the prediabetes stage and improves its metabolic risk profile.

RESEARCH DESIGN AND METHODS

Prediabetic subjects were recruited to participate in this Spanish randomized clinical trial between 20 September 2011 and 4 February 2013. In a crossover manner, 54 subjects consumed two diets, each for 4 months: a pistachio-supplemented diet (PD) and a control diet (CD). A 2-week washout period separated study periods. Diets were isocaloric and matched for protein, fiber, and saturated fatty acids. A total of 55% of the CD calories came from carbohydrates and 30% from fat, whereas for the PD, these percentages were 50 and 35%, respectively (including 57 g/day of pistachios).

RESULTS

Fasting glucose, insulin, and HOMA of insulin resistance decreased significantly after the PD compared with the CD. Other cardiometabolic risk markers such as fibrinogen, oxidized LDL, and platelet factor 4 significantly decreased under the PD compared with the CD ($P < 0.05$), whereas glucagon-like peptide-1 increased. Interleukin-6 mRNA and resistin gene expression decreased by 9 and 6%, respectively, in lymphocytes after the pistachio intervention ($P < 0.05$, for PD vs. CD). SLC2A4 expression increased by 69% in CD ($P = 0.03$, for PD vs. CD). Cellular glucose uptake by lymphocytes decreased by 78.78% during the PD ($P = 0.01$, PD vs. CD).

CONCLUSIONS

Chronic pistachio consumption is emerging as a useful nutritional strategy for the prediabetic state. Data suggest that pistachios have a glucose- and insulin-lowering effect, promote a healthier metabolic profile, and reverse certain metabolic deleterious consequences of prediabetes.

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The global prevalence of impaired glucose tolerance (IGT) is estimated at 316 million people and is expected to increase to 471 million by 2035 (1). Data from the National Health and Nutrition Examination Survey (NHANES) suggest that impaired fasting glucose is twice as prevalent as IGT (2), suggesting that the prevalence of prediabetes (impaired fasting glucose plus IGT) may actually affect more than 900 million people globally (3). This metabolic condition is preventable and treatable if recognized early. If left untreated, however, the yearly progression rate to diabetes is estimated to be between 3.5 and 7.0%. This has far-reaching implications for morbidity and mortality (4). Reflecting the global burden of prediabetes, its high rate of progression to type 2 diabetes mellitus (T2DM), and the increased risk of micro- and macrovascular complications and death (5), its prevention and treatment is one of the major goals of public health strategies. This goal goes beyond just T2DM; it looks to prevent the risk of cardiovascular disease (CVD). Therefore, healthy lifestyles, including diet, stand out as effective strategies for reversing the prediabetic stage and its related complications (6). In fact, lifestyle interventions and the use of antidiabetic drugs in prediabetic subjects have been associated with a lower progression to diabetes (7,8) and a greater reduction in CVD risk (9).

Since the publication of the results of the Adventist Health Study, which showed that nut consumption was associated with a lower risk of coronary heart disease (10), a large body of evidence from epidemiological studies and controlled clinical trials has demonstrated the beneficial impact of nut consumption on health outcomes and total mortality (reviewed in Ros [11]) (12,13). The data consistently show nut consumption's cholesterol-lowering effect, and there is emerging evidence that it also benefits other cardioprotective mechanisms, such as endothelial function (14), and susceptibility to LDL cholesterol (LDL-c) oxidation and inflammatory processes (15). Nevertheless, the results of epidemiological studies on the impact of nut consumption on T2DM incidence, one of the major risk factors for CVD, are less conclusive (11,16,17). The results of several interventional studies examining the effects of nut-enriched diets on glycemic control and insulin sensitivity have also been inconsistent (reviewed in Bulló et al. [18]). Thus, whereas acute feeding studies reported reduced postprandial glucose and insulin excursions after nut consumption in healthy or T2DM subjects (19,20), medium- and long-term changes in fasting glucose or insulin sensitivity in response to nut diets are still controversial (21–23). Recently, it has been reported that acute consumption of pistachios can attenuate postprandial glucose levels when they are consumed with carbohydrates (24,25). It has also been demonstrated that chronic consumption of pistachios can improve blood glucose levels, LDL-c, and some inflammatory markers, but not fasting insulin, in healthy subjects and subjects with metabolic syndrome (14,26,27).

To the best of our knowledge, to date, no studies have evaluated the chronic effect of nut intake on glucose metabolism and insulin resistance in the prediabetic state. The EPIRDEM (Effect of Pistachio Intake on Insulin Resistance and Type 2 Diabetes Mellitus) study is a randomized, controlled, crossover trial with a 4-month dietary intervention in prediabetic subjects that aimed to assess the effect of a pistachio-rich diet on glucose and insulin metabolism and other metabolic-related risk factors.

RESEARCH DESIGN AND METHODS

Participants

Eligible participants were community-living men and women between 25 and 65 years of age in Reus (Spain) with a BMI <35 kg/m^2 and fasting plasma glucose levels between 100 and 125 mg/dL. Subjects were excluded if they met one of the following criteria: *1*) diabetes or using oral antidiabetic drugs; *2*) alcohol, tobacco, or drug abuse; *3*) frequent consumption of nuts or known history of allergy to them; *4*) use of plant sterols, psyllium, fish oil supplements and multivitamins, vitamin E, or other antioxidant supplements; *5*) bad dentures, involving difficulty to chew pistachios; *6*) following a vegetarian or a hypocaloric diet to lose weight; *7*) being pregnant or wishing to become pregnant 9 months before or during the study or lactating 6 weeks before or during the study; *8*) significant liver, kidney, thyroid, or other endocrine diseases; or *9*) medical, dietary, or social conditions that hinder compliance to the intervention.

Participants were recruited from primary care centers affiliated with the Universitari Hospital of Sant Joan de Reus. Executed informed consent was obtained from all study participants. The institutional review board of the Universitari Hospital of Sant Joan de Reus approved the study protocol in September 2011. The trial was registered in ClinicalTrials.gov (National Institutes of Health) with identifier NCT01441921.

Study Design

The design of the crossover clinical trial is illustrated in Supplementary Fig. 1. A 15-day run-in period preceded the 4-month treatment period. A 2-week washout period separated the two crossover periods. 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 periods 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 preceding each study period. The isocaloric diet was individually calculated using World Health Organization equations adjusted by the estimated energy expenditure in physical-activity leisure time. After the 2-week run-in period, subjects were randomized to the control diet (CD) or the pistachio-supplemented diet (PD). The main characteristics of both intervention diets are shown in Supplementary Table 1. Participants allocated to the PD were supplemented with 2 ounces of pistachio (57 g/day) that was provided free to the subjects. The nuts were half roasted, and half roasted and salted. In the CD, the energy intake of other fatty foods, mostly olive oil, was adjusted to compensate for the energy from pistachios included in the PD. Participants were provided with detailed dietary instructions, including biweekly menus and seasonal recipes according to the type of diet, pistachio or control. A qualitative example of a daily menu is shown in Supplementary Table 2.

Measurements

Individual examinations were scheduled at baseline, after a 2-week run-in, and then monthly until the end of each intervention period.

Anthropometry, Body Composition, and Blood Pressure

Weight and waist circumference were determined at each visit with subjects wearing light clothes and no shoes. BMI was calculated. At the beginning and end of each 4-month intervention period, body composition was measured by bioelectrical impedance analysis (Human-Im-Scan; Dietosystem, Barcelona, Spain). Blood pressure was measured in the nondominant arm, using a validated semiautomatic oscillometer (HEM-705CP; OMRON, Hoofddorp, the Netherlands) in duplicate with a 5-min interval between each measurement.

Dietary and Physical Activity Assessment

At the beginning of each intervention period, and every 2 months subsequently, dietary intake was estimated using the mean of 3-day dietary records including two workdays and a weekend day. Energy and nutrient intake were calculated using Spanish food composition tables (28). Adherence to the intervention period was assessed by counting the empty sachets of pistachio administered and measuring plasma lutein-zeaxanthin and γ-tocopherol levels with a liquid chromatograph coupled to a 6490 QqQ/MS (Agilent Technologies, Palo Alto, CA) as previously described. The detailed protocol is provided in Supplementary Methods 1.

Physical activity was evaluated using the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire (29). All participants received instructions to maintain constant physical activity during the study. A questionnaire was used to assess gastrointestinal side effects such as mouth symptoms; bloating, fullness, or indigestion; altered bowel habit; and any other diet-related symptoms.

Biological Samples, Collection, and Storage

Fasting blood samples were collected at baseline and at the end of each 4-month intervention period. Plasma fasting glucose and serum lipid profile were determined using standard enzymatic automated methods. LDL-c was estimated using the Friedewald formula in those subjects whose triglyceride levels were <400 mg/dL. Plasma tissue factor (TF) (AssayPro, St. Charles, MO), thromboxane B_2 (TXB2) (Cayman Chemical Company, Ann Arbor, MI), and oxidized LDL (ox-LDL) (Qayee Bio-Technology, Shanghai, China) were measured using ELISA commercial kits. Soluble receptor for advanced glycation end products (sRAGE) (Sigma-Aldrich, St. Louis, MO), interleukin-18 (IL-18) (Boster Biological Technology, Fremont, CA), and IL-6 (R&D Systems, Minneapolis, MN) were determined in serum using commercial ELISAs. Plasma fibrinogen, von Willebrand factor (vWF), platelet factor 4 (PF4), gastric inhibitory polypeptide (GIP), GLP-1, insulin, leptin, C-peptide, adiponectin, plasminogen activator inhibitor-1 (PAI-1), and resistin (RETN) were determined using a MILLIPLEX MAP Plex Kit (Merck Millipore, Billerica, MA). Both insulin resistance and insulin secretion were estimated by the HOMA of insulin resistance (HOMA-IR) and HOMA of β-cell function (HOMA-BCF) methods.

Cellular Glucose Uptake

Lymphocytes were obtained from blood, collected in heparin tubs, and incubated in a final concentration of 4×10^6 cells/well with 10 μmol/L of deoxy-D-glucose-2^{3}H(G) (2-NBDG) (Molecular Probes, Life Technologies, Eugene, OR) in RPMI 1640 (Gibco, Life Technologies, Frederick, MD), as previously described (30). After 30 min of incubation (37°C under 95% humidity, 5% CO_2, and atmospheric O_2 levels), the reaction was stopped by adding cold 1× PBS, and fluorescence was read (λ_{ex} = 485 nm; λ_{em} = 538 nm) (Fluoroskan Ascent; Thermo Fisher Scientific, Madrid, Spain).

Gene Expression Analysis

Total RNA was extracted from blood samples using the Tempus Spin RNA Isolation Kit (Ambion, Madrid, Spain). The purity and quantification of the RNA were determined by spectrophotometry (NanoDrop; Thermo Fisher Scientific, Madrid, Spain). The retrotranscription step was performed using the high-capacity cDNA reverse transcription kit (Invitrogen, Madrid, Spain). Gene expression was analyzed using Taqman gene expression assays (AB, Madrid, Spain) in a 7900HT Fast Real-Time PCR System (AB). Primers-probes for the genes were selected as follows: TLR2 (Toll-like receptor 2) (Hs00152932_m1), TLR4 (Hs00152939_m1), SLC2A3 (solute carrier family 2, facilitated glucose transporter member 3) (Hs00359840_m1), SLC2A4 (Hs00168966_m1), IL-6 (Hs00985639_m1), and RETN (Hs00220767_m1) were analyzed. Genes tested as endogenous controls were selected from a pool of genes that Genevestigator (http://www.genevestigator.com/gv) gave as potential reference genes based on previous experiments on T2DM, and glucose or insulin impairment. Hence, ACTB (β-actin) (Hs99999903_m1), HPRT1 (hypoxanthine phosphoribosyltransferase 1) (Hs99999909_m1), RPL30 (ribosomal protein L30) (Hs00265497_m1), and YWHAZ (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ) (Hs00237047_m1) were initially tested as putative reference genes. HPRT1 and YWHAZ were finally selected as the best endogenous for our samples, so they were both used as reference genes for target gene normalization. Each quantitative PCR (qPCR) reaction contained 2 μL of 1:7 fold diluted cDNA, 0.5 μL of the validated TaqMan assay for target or reference gene, 5 μL of Universal TaqMan PCR master mix (Applied Biosystems, Madrid, Spain), and 2.5 μL RNase-free water (Ambion). All measurements were performed in duplicate, and qPCR data were acquired using Sequence detector software (SDS version 2.4; Applied Biosystems). Mean quantification cycle (Cq) values (also known as threshold cycle, Ct, values) were calculated as the average of two replicates if the SD was <0.5; otherwise the sample was repeated in the qPCR experiment. Normalized expression was calculated for individual samples using the $2^{-\Delta Cq}$ method (ExpressionSuite Software v1.0.3). Changes in expression were shown as the ratio between final and baseline values. The nontemplate sample did not produce a signal in any assay, and internal control showed a mean variation between independent plates of <8% for all the genes tested.

Statistical Analysis

Descriptive data of participants at baseline and differences during the intervention periods are shown as means and 95% CIs for continuous variables and number (%) for categorical variables. Differences in all variables were evaluated by ANOVA, with intervention diet as the independent and repeated measures factor. Diet sequence (order of diet treatments) was analyzed as independent factor, but as it was not significant, it was not further considered. The differences in

variable changes between dietary intervention periods were analyzed by an ANCOVA test using baseline values as a covariate. All statistical analyses were conducted using intent-to-treat (ITT) and per protocol (PP) approaches. ITT analysis included all randomized participants at least fulfilling all baseline measurements. The PP analysis excluded participants who did not attend the last visit, and results are shown in the Supplementary Data. The sample size was calculated considering changes in HOMA-IR as a primary end point. Assuming an α error of 0.05 and 90% power, we required a sample size of 40 subjects to identify significant differences in HOMA-IR similar to those observed in a previous study by our group (31). We finally decided on a sample of 54 individuals to compensate for an expected 17% loss in participants. All analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL). All tests were two-sided, and significance was defined as $P < 0.05$.

RESULTS

Study Participants

The study flowchart is shown in Supplementary Fig. 2. A total of 108 participants were assessed for eligibility. Of these, 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. No gastrointestinal side effects were observed during the trial. The baseline characteristics of the study participants are shown in Table 1. No changes in medication were reported during the study. No significant differences were observed between dietary interventions at baseline in any of the analyzed parameters.

Dietary Compliance

As expected, plasma lutein-zeaxanthin levels significantly increased (Table 2) during the PD compared with the CD (mean 222.53 nmol/L [95% CI 150.89, 294.16] and −16.65 nmol/L [−63.46, 30.17], respectively; $P < 0.001$) (Table 2). The results were similar for γ-tocopherol (684.53 nmol/L [469.97, 899.09] and −99.76 nmol/L [−264.84, 65.33], respectively; $P < 0.001$).

Glucose Metabolism and Lipid Profile

In the ITT analysis, glucose and insulin circulating levels decreased significantly ($P < 0.001$) in the PD intervention (Table 2) compared with the CD. Subsequently, HOMA-IR decreased during the PD intervention compared with the CD (mean −0.69 [95% CI −1.07, −0.31] and 0.97 [0.49, 1.44], respectively; $P < 0.001$). No significant changes, however, were observed in HOMA-BCF and glycated hemoglobin (HbA_{1c}) between PD and CD. Although lipid profile did not change significantly between groups, LDL-c showed a nonsignificant reduction after pistachio intervention compared with the increase observed in the CD period (mean −4.00 mg/dL [95% CI −9.03, 1.03] and 1.20 mg/dL [−4.35, 6.74], respectively; $P = 0.16$). The PP results were similar to those analyzed by the ITT approach (Supplementary Table 3).

Lymphocyte Glucose Uptake

A significant decrease in cellular glucose transport was observed in lymphocytes after a PD compared with the CD (−78.78% [95% CI 127.46, −30.10] and 15.86% [−34.55, 66.27], respectively; $P = 0.01$) (Fig. 1*A*). Results by the PP approach provided equivalent results (Supplementary Fig. 3*A*).

Inflammation and Metabolic Risk Markers

Table 3 depicts baseline inflammatory and metabolic risk markers and changes during the intervention. No significant differences were observed in these parameters at baseline according to the sequence of randomization. After the PD, fibrinogen and PF-4 significantly decreased in comparison with the CD. In contrast, GLP-1 increased during the PD intervention compared with the CD. There were no different effects between diets for the other inflammatory and metabolic biomarkers analyzed, although all changes were in the expected direction. Although nonsignificant, ox-LDL decreased during the PD but increased during the CD ($P = 0.10$). In the PP approach, ox-LDL decreased significantly during the PD intervention but increased during the CD (−7.22 ng/mL [95% CI −21.28, 6.83] and 11.24 ng/mL [2.82, 19.66], respectively; $P = 0.03$) (Supplementary Table 4).

Gene Expression in Peripheral Lymphocytes

The gene expression data (Fig. 1*B*) show that IL-6 mRNA decreased by a significant 9% in the PD in comparison with

Table 1—Baseline characteristics of the study population

Variable	Whole population	Male	Female
Subjects, *n* (%)	54	29 (53.7)	25 (46.3)
Age (years)	55 (53.4, 56.8)	54.5 (52.1, 56.9)	55.7 (53.1, 58.3)
Weight (kg)	77.6 (74.8, 80.3)	82.7 (79.0, 86.4)	71.6 (68.7, 74.5)*
BMI (kg/m^2)	28.9 (28.2, 29.6)	28.4 (27.4, 29.5)	29.4 (28.4, 30.3)
Waist circumference (cm)	94.7 (92.8, 96.6)	96.9 (94.2, 99.6)	92.1 (89.7, 94.5)*
Systolic blood pressure (mmHg)	134 (130, 137)	137 (132, 142)	130 (125, 136)
Diastolic blood pressure (mmHg)	81 (79, 83)	82 (78, 85)	81 (78, 83)
Dyslipidemia, *n* (%)	27 (50)	15 (51.7)	12 (48.0)
Hypertension, *n* (%)	23 (42.6)	11 (37.9)	12 (48.0)
Statins, *n* (%)	5 (9.3)	2 (6.9)	3 (12.0)
Fibrates, *n* (%)	2 (3.7)	2 (6.9)	0 (0.0)
ACE inhibitors, *n* (%)	6 (11.1)	2 (6.9)	4 (16.0)
β-Blockers and other antihypertensive drugs, *n* (%)	13 (24.1)	7 (24.1)	6 (24.0)
Leisure-time physical activity (kcal/day)	347 (307, 387)	328 (267, 387)	370 (317, 423)

Data are given as mean (95% CI) or number (%). *Significant differences between sexes.

Table 2—Baseline and changes after intervention period in anthropometric and biochemical parameters

	PD		CD		Treatment effect
Characteristics	Baseline	Change	Baseline	Change	*P* value
Waist circumference (cm)	94.19 (92.27, 96.11)	0.63 (−0.02, 1.29)	94.80 (92.88, 96.72)	−0.44 (−1.30, 0.43)	0.08
Weight (kg)	77.29 (74.46, 80.14)	0.40 (−0.08, 0.08)	77.74 (74.93, 80.54)	0.21 (−0.74, 0.32)	0.07
BMI (kg/m^2)	28.76 (28.03, 29.49)	0.12 (−0.06, 0.30)	28.90 (28.21, 29.59)	−0.07 (−0.28, 0.12)	0.12
Systolic blood pressure (mmHg)	133.89 (129.75, 138.03)	−3.64 (−6.23, −1.06)	132.17 (128.70, 135.64)	−1.47 (−4.40, 1.46)	0.22
Diastolic blood pressure (mmHg)	80.48 (78.33, 82.63)	0.19 (−1.25, 1.61)	79.94 (77.80, 82.07)	−0.25 (−1.59, 1.10)	0.75
Fasting plasma glucose (mg/dL)	116.24 (112.37, 120.11)	−5.17 (−8.14, −2.19)*	108.06 (104.27, 111.84)	6.72 (4.38, 9.07)	<0.001
HbA_{1c} (%)	5.92 (5.82, 6.02)	−0.03 (−0.12, 0.05)	5.87 (5.75, 5.99)	0.03 (−0.03, 0.10)	0.13
HbA_{1c} (mmol/mol)	41.18 (40.10, 42.27)	−0.46 (−1.38, 0.46)	40.66 (39.36, 41.96)	0.32 (−0.47, 1.11)	0.14
Fasting plasma insulin (mU/mL)	14.36 (12.65, 16.07)	−2.04 (−3.17, −0.92)*	11.44 (9.81, 13.07)	2.51 (1.02, 4.00)	<0.001
HOMA-IR	4.22 (3.66, 4.77)	−0.69 (−1.07, −0.31)*	3.10 (2.64, 3.56)	0.97 (0.49, 1.44)	<0.001
HOMA-BCF	98.22 (86.35, 110.09)	−3.46 (−11.45, 4.53)	96.76 (78.45, 115.07)	−0.25 (−9.65, 9.16)	0.62
Total cholesterol (mg/dL)	217.44 (208.10, 226.79)	−3.74 (−9.20, 1.72)	213.83 (205.48, 222.19)	2.11 (−4.41, 8.63)	0.15
HDL-c (mg/dL)	54.28 (50.43, 58.13)	1.33 (−1.65, 4.32)	54.42 (51.00, 57.83)	1.34 (−0.71, 3.39)	0.96
LDL-c (mg/dL)	137.93 (128.18, 147.67)	−4.00 (−9.03, 1.03)	136.77 (128.85, 144.70)	1.20 (−4.35, 6.74)	0.16
VLDL-c (mg/dL)	25.19 (22.23, 28.14)	−1.04 (−3.20, 1.13)	22.74 (20.32, 25.15)	0.36 (−1.65, 2.38)	0.28
Total cholesterol/ HDL-c ratio	4.29 (3.87, 4.72)	−0.19 (−0.38, −0.01)	4.14 (3.80, 4.47)	−0.05 (−0.25, 0.15)	0.31
LDL-c/HDL-c ratio	2.78 (2.39, 3.17)	−0.15 (−0.32, 0.02)	2.68 (2.40, 2.96)	−0.04 (−0.20, 0.11)	0.33
Triglycerides (mg/dL)	125.81 (111.07, 140.56)	−4.96 (−15.72, 5.79)	113.89 (101.70, 126.07)	7.47 (−7.87, 22.81)	0.15
Lutein-zeaxanthin (nmol/L)	452.90 (399.86, 505.95)*	222.53 (150.89, 294.16)	465.01 (408.27, 521.75)	−16.65 (−63.46, 30.17)	<0.001
γ-Tocopherol (nmol/L)	625.49 (464.20, 786.78)*	684.53 (469.97, 899.09)	755.06 (603.04, 907.09)	−99.76 (−264.84, 65.33)	<0.001

ITT analysis, *n* = 54. All values are means (95% CI). Intragroup analysis was assessed by the paired Student *t* test. Basal-adjusted changes between groups were analyzed using adjusted ANOVA of repeated measurements. HDL-c, HDL cholesterol. *Significant difference ($P < 0.05$) between baseline and end of a particular intervention period.

the CD ($P = 0.004$). After the PD, RETN gene expression was also lower than after the PD (6%, $P = 0.04$). Facilitated glucose transporter gene expression assessed by SLC2A3 and SLC2A4 showed different patterns. SLC2A3 expression showed no significant changes between groups, whereas SLC2A4 showed a significant 69% increase during the CD compared with the PD ($P = 0.03$, PD vs. CD). Neither of the TLR genes analyzed, TLR2 and TLR4, differed statistically between treatments. The PP analysis (Supplementary Fig. 3*B*) showed similar results.

CONCLUSIONS

Several studies have evaluated the effect of nut intake on glycemia and insulin levels (21–23), but few have focused on pistachio intake (14,27) and none have been conducted in the prediabetic stage. This is the first study to evaluate the chronic effect of nut intake on glucose metabolism, insulin resistance, inflammation, and related-metabolic risk markers, at both the systemic and molecular levels in prediabetic subjects. The results of this crossover, randomized, controlled clinical trial provide evidence that chronic consumption of pistachio decreases glucose and insulin levels, thus improving insulin resistance and other inflammatory and metabolic risk markers.

A beneficial dose-response effect of pistachios has been observed on postprandial glycemia and insulinemia when pistachios are consumed with carbohydrate foods (24,25). Likewise, significant improvements have been reported in fasting blood glucose after healthy young men consumed 20% of their daily energy intake as pistachios for 4 weeks (14). Moreover, a 24-week randomized clinical trial conducted on subjects with metabolic syndrome demonstrated a significant improvement in fasting glucose but not insulin levels (27). In contrast, a 12-week weight reduction diet that included pistachios had no effect on glucose or insulin when compared with an isocaloric diet containing pretzels (32). The results of our study demonstrate that pistachio intake has a chronic lowering effect on fasting glucose and a beneficial effect on insulin resistance measured indirectly by HOMA-IR. The effect of pistachios on insulin metabolism could be partly explained by an increase in GLP-1 levels during pistachio consumption. GLP-1 and GIP are gastric hormones that stimulate pancreatic insulin secretion and suppress glucagon secretion in a

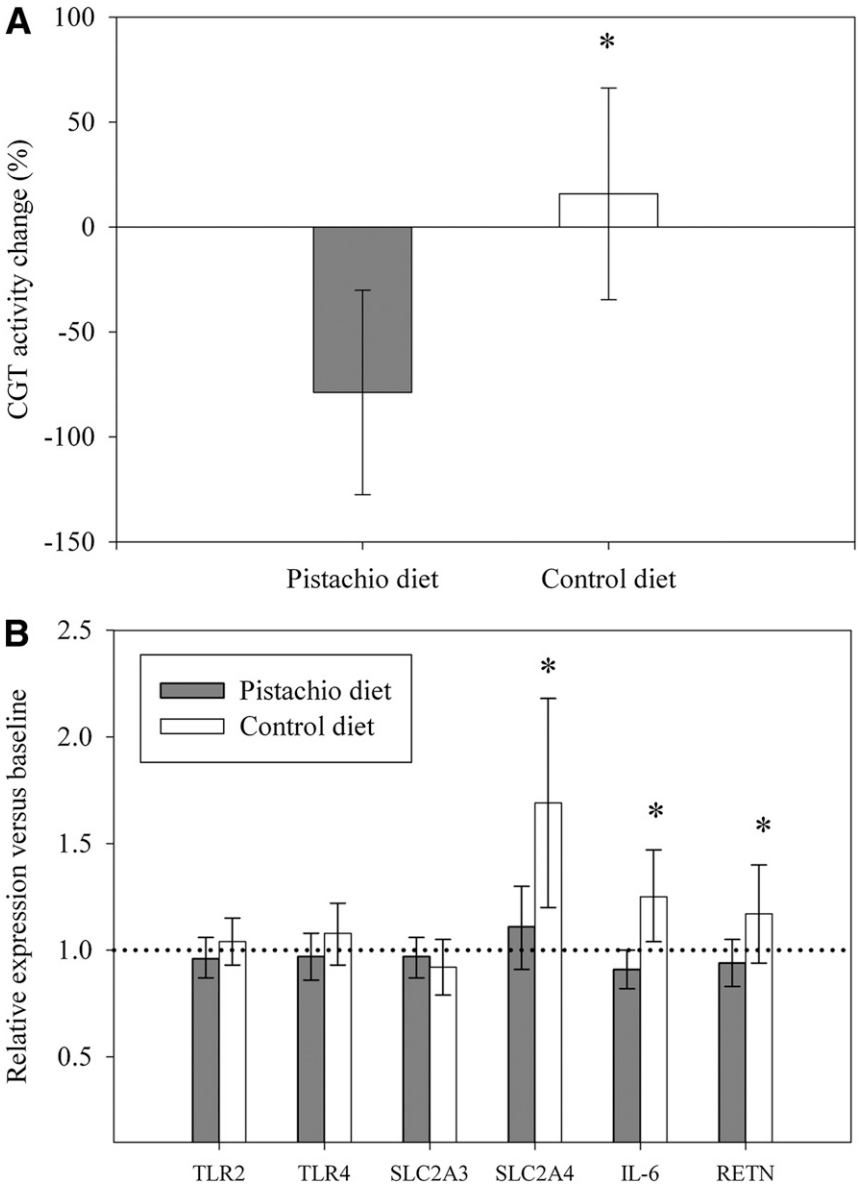

Figure 1—Changes in peripheral leukocyte gene expression and cellular glucose uptake. *A*: Percentage of change in cellular glucose transport (CGT) activity through both intervention diets. *B*: Expression changes across intervention diets. *$P < 0.05$, significant differences in changes between dietary interventions.

glucose-dependent manner (33). The acute upregulatory effect of pistachio intake on the secretion of GLP-1 was reported by Kendall et al. (25) in individuals with metabolic syndrome. In the current study, we extend their results and show that pistachios have a long-term stimulating effect on GLP-1 and insulin-sparing effects in patients with prediabetes.

In contrast to previous epidemiological and clinical studies reporting a cholesterol-lowering effect of nut consumption, including pistachios, mainly in hypercholesterolemic subjects but also in normolipidemic and healthy subjects (18), we failed to find any significant differences between the changes in total cholesterol and LDL-c between intervention diets. Our results are in agreement with those previously published on overweight and metabolic syndrome individuals (31,34,35) and support the hypothesis that the lipid profile of metabolic syndrome subjects is less likely to undergo changes because alteration in the cholesterol homeostasis inherent to the obese state makes them resistant to downregulating cholesterol (36).

Because they are richer in lutein, β-carotene, and γ-tocopherol than other nuts, pistachios tend to have a beneficial effect on the inflammatory and oxidative state. An improvement in the circulating levels of IL-6, CRP, TNF, and adiponectin has previously been reported in both healthy men and individuals with metabolic syndrome (14,27). A dose-dependent reduction in ox-LDL by a 4-week pistachio consumption diet has also been demonstrated (26). This trial found a significant reduction in ox-LDL during the PD in the PP approach. Although no change in circulating IL-6 levels was observed, other inflammatory and metabolic risk markers underwent a significant reduction. Reductions in fibrinogen and PF4 were measured during the PD.

Unlike circulating measures, however, lymphocyte expression of IL-6 and RETN was significantly lowered by pistachio consumption. These data clearly suggest a pistachio-mediated impact on classical inflammatory markers of glucose and insulin metabolism. Because lymphocytes play an important role in inflammatory responses, they need to be tightly regulated if health is to be maintained. Correct fuel requirements of these cells are vitally important for their growth and effector function (37). Therefore, whereas hypoglycemia decreases the viability of peripheral blood cells, hyperglycemia leads to an excessive glucose uptake, thus promoting immune hyperactivity and compromising health (38). Further, a significantly increased SLC2A4 protein expression on the surface of lymphocytes has been described in both diabetic subjects (39) and subjects with impaired glucose metabolism (40). We have demonstrated that pistachio consumption leads to a significant decrease in SLC2A4 mRNA (in parallel with a lower increase in cellular glucose uptake). This decrease can be explained by circulating insulin levels after dietary treatment. Therefore, these results suggest a potential mechanism by which pistachios could lead to a healthier systemic inflammatory profile. Taken together, our results reinforce the potential anti-inflammatory effect of pistachio nuts and their role in chronic inflammatory diseases.

Several strengths and limitations of our study deserve comment. Among its strengths are the crossover randomized

Table 3—Baseline and changes after intervention period in inflammatory, satiety, and other related markers

	PD		CD		Treatment effect
Characteristics	Baseline	Change	Baseline	Change	*P* value
Platelets ($\times 10^3/\mu L$)	225.70 (214.39, 237.02)	−3.90 (−8.07, 0.27)	224.00 (209.63, 238.37)	−5.68 (−12.93, 1.57)	0.89
Lymphocytes ($\times 10^3/\mu L$)	1.87 (1.74, 2.00)	−0.02 (−0.08, 0.04)	1.87 (1.72, 2.02)	−0.01 (−0.08, 0.06)	0.67
Fibrinogen (ng/mL)	71.18 (65.62, 76.75)	−2.24 (−5.94, 1.46)	65.13 (60.45, 69.81)	3.24 (−0.19, 6.67)	0.02
Tissue factor (pg/mL)	195.71 (143.16, 248.26)	16.33 (−10.60, 43.27)	225.57 (169.88, 281.26)	−14.46 (−40.35, 11.43)	0.16
PAI-1 (pg/mL)	158.37 (134.65, 182.10)	13.26 (−13.81, 40.33)	177.42 (136.40, 218.43)	−12.91 (−42.41, 16.59)	0.15
vWF (ng/mL)	0.61 (0.47, 0.75)	0.27 (0.00, 0.55)	0.99 (0.59, 1.39)	−0.04 (−0.53, 0.45)	0.15
PF4 (ng/mL)	0.20 (0.07, 0.32)	−0.07 (−0.13, −0.02)	0.12 (0.09, 0.15)	0.00 (−0.02, 0.02)	0.01
TXB2 (ng/mL)	2.20 (1.60, 2.80)	−0.18 (−0.55, 0.19)	2.20 (1.69, 2.71)	0.13 (−0.33, 0.58)	0.31
C-peptide (ng/mL)	1.83 (1.68, 1.98)	−0.06 (−0.18, 0.06)	1.75 (1.60, 1.91)	0.01 (−0.11, 0.14)	0.34
GIP (pg/mL)	32.55 (26.99, 38.11)	−0.04 (−4.17, 4.09)	34.19 (29.17, 39.21)	−1.31 (−5.08, 2.46)	0.61
GLP-1 (pg/mL)	46.62 (37.24, 56.00)	4.09 (1.25, 6.94)*	47.40 (37.77, 57.04)	−0.59 (−2.98, 1.80)	0.01
RETN (pg/mL)	105.70 (89.89, 121.50)	2.29 (−7.14, 11.73)	108.63 (89.55, 127.71)	4.19 (−21.01, 29.39)	0.67
IL-6 (pg/mL)	1.48 (1.18, 1.77)	−0.13 (−0.32, 0.06)	1.39 (1.08, 1.71)	0.01 (−0.27, 0.29)	0.27
IL-18 (pg/mL)	104.60 (87.06, 122.13)	−8.99 (−16.04, −1.94)*	115.88 (92.54, 139.22)	−12.14 (−23.50, −0.77)	0.64
Leptin (ng/mL)	10.83 (8.40, 13.27)	−0.40 (−1.49, 0.68)	10.60 (8.41, 12.79)	0.09 (−0.70, 0.88)	0.34
Adiponectin (ng/mL)	69.16 (54.33, 84.00)	−3.12 (−8.49, 2.25)	66.89 (52.42, 81.35)	−0.64 (−6.59, 5.31)	0.56
Ox-LDL (ng/mL)	279.07 (253.29, 304.85)	−2.64 (−16.14, 10.86)	267.45 (247.87, 287.03)	10.20 (2.51, 17.89)*	0.10
sRAGE (pg/mL)	319.12 (242.22, 396.01)	20.16 (−17.60, 57.92)	312.01 (237.75, 386.27)	10.93 (−15.37, 37.22)	0.72

ITT analysis, $n = 54$. All values are means (95% CI). Intragroup analysis was assessed by the paired Student *t* test. Basal-adjusted changes between groups were analyzed using basal-adjusted ANOVA of repeated measurements. PAI-1, plasminogen activator inhibitor-1; sRAGE, soluble receptor of advanced glycation end products. *Significant difference ($P < 0.05$) between baseline and end of a particular intervention period.

design, its medium-term duration, the presence of dietary compliance markers, and the two-level approach (systemic and cellular). However, by design, an important limitation of this trial is that it focuses on the prediabetic patient. Results may only be extrapolated to healthy subjects or subjects with T2DM with caution pending further studies.

This study's findings build on the literature by describing the health-enhancing properties of pistachios along with nuts in general, and in particular the benefits for glucose metabolism and cardiovascular health that could be attributed to their higher amount of polyunsaturated fatty acid and other bioactive compounds such as procyanidins and carotenoids (11). In the context of a healthy diet, pistachios have important glucose- and insulin-lowering effects and improve the inflammatory profile by downregulating both the expression and the circulating levels of several metabolic risk markers. Overall, the integration of pistachios into a balanced diet is proving to be a safe nutritional strategy that can help reverse the risks associated with prediabetes. These benefits may well provide novel dietary tools for managing other prevalent chronic inflammatory diseases as well. Future studies should be designed to investigate the impact of regular pistachio consumption on the development and management of T2DM and other chronic diseases.

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