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Supplementary Methods 1. Protocol to measure plasma lutein-zeaxanthin and γ-tocopherol levels.

Chemicals and reagents

Ethanol and hexane, both High performance liquid chromatography (HPLC) grade, were acquired to J.T.Baker (Center Valley, U.S.A.). Methanol Liquid chromatography–mass spectrometry (LC-MS) grade, methyl tert-butyl ether (MTBE) > 99.0%, butylated hydroxytoluene (BHT) ≥ 99%, and the internal standards γ-Tocopherol-(ring-5,7-dimethyl-d6) 98 atom % D, 98% and canthaxanthin 94% were purchased to Sigma-Aldrich (St. Louis, U.S.A.). Certified reference solution of (+)-γ−Tocopherol was acquired to Cerilliant (Round Rock, Texas), while Lutein 96% and zeaxanthin 97% where purchased to Carotenature (Ostermundigen, Switzerland).

Instrumental conditions

A 1290 Ultra-HPLC Series Liquid Chromatograph coupled to a 6490 triple quadrupole mass spectrometer (QqQ/MS) (Agilent Technologies, Palo Alto, U.S.A.) was used for lutein, zeaxanthin and tocopherols quantification. The chromatographic column was an YMC-Carotenoid S-3 µm 250x4.6 mm (YMC, Ishikawa, Japan). Mobile phases were Methanol (solvent A) and MTBE (solvent B). Flow rate was 1mL/min. Elution gradient was 0-6min 15%B isocratic, 6.5-10.5 min 40% B isocratic, 11-16 min 95 % B isocratic, 17min 15% B. A post run of 5 min was applied. Injected sample volume was of 20 µL.
APCI conditions were 120ºC and 11 L/min of drying gas temperature and flow, respectively, 20 psi of nebulizer gas pressure, 350ºC of vaporizer temperature, 4000 V of capillary voltage in both, positive and negative mode, and a corona current of 4µA and 10 µA in positive and negative mode, respectively. QqQ operated in multiple reaction monitoring (MRM) mode, applying a fragmentor voltage of 380 V and a cell accelerator voltage of 2V. Retention time, conditions of MRM acquisition and acquisition mode are showed in Table 1 for all studied compounds and corresponding internal standards.

Supplementary Table 1. Analyzed Compounds

Compound	rT (min)	Quantity of transition	CE (V)	Quality of transition	CE (V)	Quality of transition	CE (V)	Acquisition mode
δ-Tocopherol	5.182	401->135	36	401->401	0	-	-	Negative
γ-Tocopherol	5.635	415->400	16	415->121	56	415->149	36	Negative
β-Tocopherol	5.928	415->149	36	415->121	56	-	-	Negative
α-d6Tocopherol (SI)	6.416	435->169	28	435->435	0	-	-	Negative
α-Tocopherol	6.446	429->163	28	429->429	0	-	-	Negative
Lutein	9.235	551->105	60	551->145	32	551->119	28	Positive
Zeaxanthin	9.709	569->119	60	569->145	40	569->133	52	Positive
Canthaxanthin (SI)	10.108	565->133	48	565->203	20	-	-	Positive

Retention time (rT), MRM (multiple reaction monitoring) transitions, collision energy (CE) and acquisition mode for the studied compounds and the corresponding internal standards (SI).

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Sample preparation:
Sample preparation protocol was based in the Azar *et al*. work (1). An aliquot of 100 µL of freshly thawed plasma sample was mixed with 100µL of an internal standard mixture of 443 nM of canthaxanthin and 1145 nM of α-d6-tocopherol in 0.2 g/L Butil hidroxitolueno/Ethanol, and vortexed for approximately 5 seconds. A volume of 500 µL of hexane was added, and the mixture was agitated at 1350 rpm at ambient temperature, and then centrifuged at 15000 rpm for 2 min at 4ºC.
A volume of 400 µL of supernatant was dried under a N_2 gas flow. Sample was reconstituted in 100 µL of 0.2 g/L Butil hidroxitolueno/Ethanol. The final extract was vortexed, centrifuged 10 min at 15000 rpm at 4ºC, and the supernatant was analyzed by LC-QqQ.

Method validation and samples quantification
For the quantitative method validation calibration curves, linearity, extraction recovery, accuracy, precision and method detection and quantification limits were studied, by analysis of a standard dilutions serial prepared in 0.2g/L Butil hidroxitolueno/Ethanol and pooled plasma samples spiked with the same standard dilutions. Calibration curves were obtained by plotting analyte/s' peak abundance ratio and the corresponding analyte/s' concentration ratio. Extraction recovery was evaluated by comparison of the spiked samples response with standard solutions calibration curve. Intraday and interday precision were determined from relative standard deviation (RSD) in the analysis of a pooled plasma sample. Limit of detection (LoD) was defined as the concentration corresponding to three times the signal/noise rate, and limit of quantification (LoQ), was defined as the lowest concentration giving a linear response. The obtained validation parameters of the method, which are showed in Table 2 for γ-tocopherol, lutein and zeaxanthin, allowed the quantification of studied compounds in the plasma samples.

Supplementary Table 2. **The method for parameters' validation**

Compound	Linearity (nM)	Calibration curve	Recovery (%)	Accuracy (%)	Intraday precision RSD%, *n*=3	Interday precision (%RSD, n=6)	LoD (nM)	LoQ (nM)
γ-Tocopherol	24-19000	Y=1.11x-0.086 (R^2=0.9992)	98.9	100.5	2.8	12.6	4.7	24
Lutein	4-3500	Y=0.046x-2E-04 (R^2=0.9998)	99.6	99.3	5.8	10.5	0.8	4
Zeaxanthin	4-3500	Y=0.025x+2E-04 (R^2=0.9989)	99.3	99.2	5.4	9.6	0.7	4

Parameters of linearity, calibration curve, recovery, accuracy, intraday and interday precision, limit of detection (LoD) and limit of quantification (LoQ) are showed for γ-tocopherol, lutein and zeaxanthin. RSD, relative standard deviation.

In the quantification of samples, standard solutions at different levels of concentration were used to obtain calibration curves, and compounds in the samples were quantified by interpoling the analyte/s peak abundance ratio in these curves. While γ-tocopherol, lutein and zeaxanthin were quantified with the calibration curve obtained with the corresponding standards, α, β and δ-tocopherol were semi-quantified by using the γ-tocopherol calibration curve. Canthaxanthin was used as internal standard for lutein and zeaxanthin, while α-d6-tocopherol was used for all the tocopherol forms.

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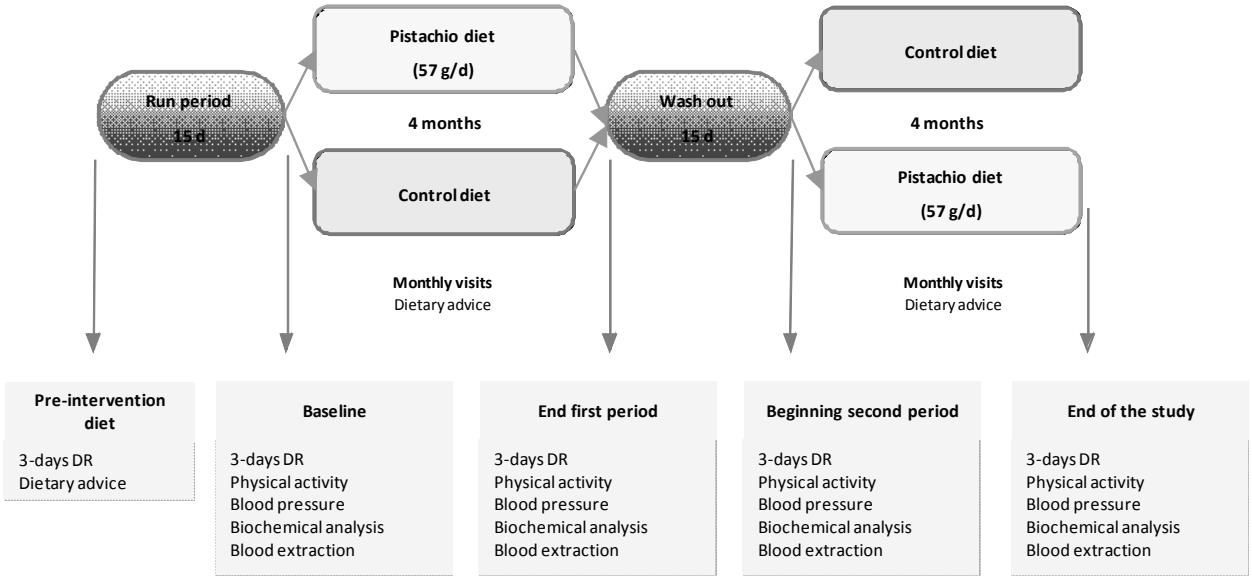
LITERATURE CITED

1. Azar M, Basu A, Jenkins AJ, Nankervis AJ, Hanssen KF, Scholz H, et al. Serum carotenoids and fat-soluble vitamins in women with type 1 diabetes and preeclampsia: a longitudinal study. Diabetes Care 2011;34:1258–64.

Supplementary Figure 1. Study design.



Intervention period and scheduled visits. 3- days DR (3-days dietary record)

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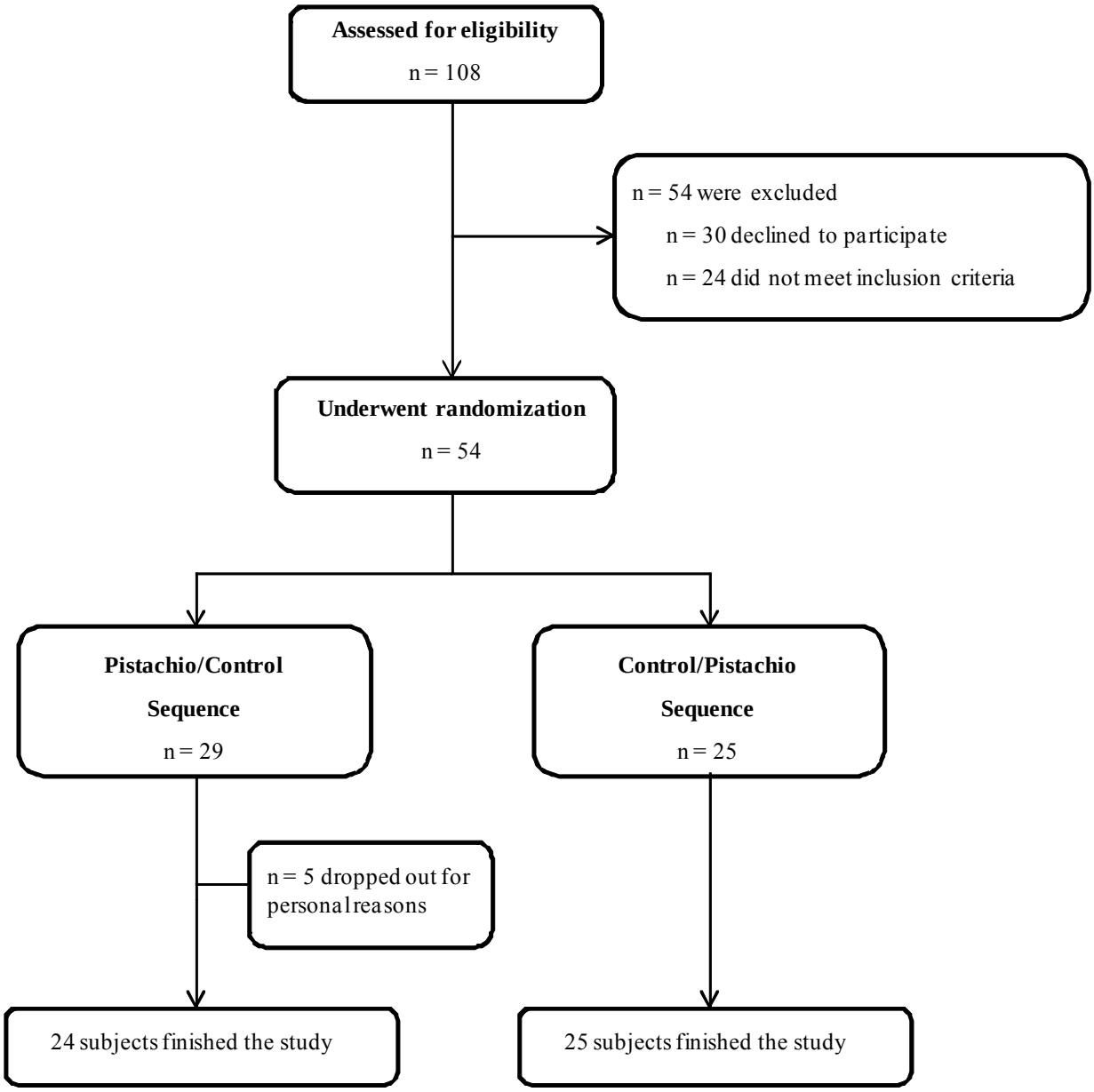
Supplementary Figure 2. Flowchart of study participants.



EPIRDEM Flow chart. Crossover design.

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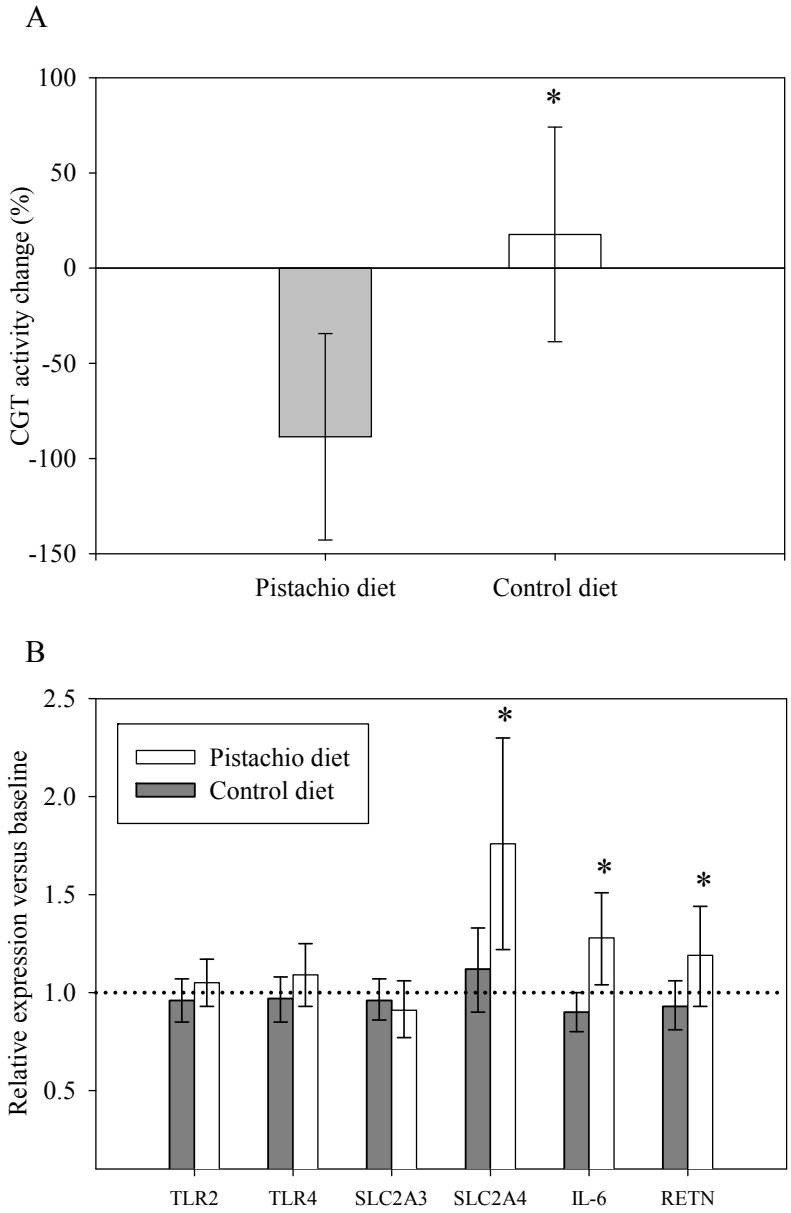
Supplementary Figure 3. Changes in peripheral leukocytes' cellular glucose uptake and gene expression.



A. Percentage of change in Cellular Glucose Transport (CGT) activity through both intervention diets; **B.** Expression changes across intervention diets. TLR2 (Toll-like receptor 2), TLR4 (Toll-like receptor 4), SLC2A3 (Solute Carrier Family 2, Facilitated Glucose Transporter Member 3), SLC2A4 (Solute Carrier Family 2, Facilitated Glucose Transporter Member 4), IL-6 (Interleukin-6), RETN (Resistin).* stands for significant differences ($P<0.05$) in changes between dietary interventions.

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Supplementary Table 1. Nutrient composition of each intervention diet

Nutrient	Control diet	Pistachio diet
Carbohydrate, (% of energy)	55	50
Protein (% of energy)	15	15
Total fat (% of energy)	30	35
Saturated fatty acids (% of energy)	7	7
Monounsaturated fatty acids (% of energy)	16	20
Polyunsaturated fatty acids (% of energy)	3	5
Fibre (g in a 2000 Kcal diet)	35.8	41.0
Cholesterol (mg in a 2000 Kcal diet)	191	166

Nutrient analyses of research diets using Spanish and USDA databases.

Supplementary Table 2. Qualitative example of a daily menu for pistachio and control diets

Meal	Daily menu
Breakfast	Skimmed milk and whole-grain cereals or Marie biscuits
Mid-morning snack	White bread sandwich with cheese (fresh or semi-cured) or ham (Serrano, turkey or baked) and olive oil. *Pistachios according to intervention group
Lunch	Pasta, rice or cereals, legumes, meat (red or white) or white fish, olive oil, fruits and white bread
Afternoon snack	Skimmed yogurt, Marie biscuit or white bread with ham (Serrano, turkey or baked) and fruits *Pistachios according to intervention group
Dinner	Pasta, rice or potatoes; white fish, white meat or eggs, vegetables or white bread and olive oil, fruits or skimmed yogurt

Representative menu for each meal of a full day.

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Supplementary Table 3. Baseline and changes after intervention period in glucose metabolism-related parameters and lipid profile

	Pistachio Diet		Control Diet		Treatment Effect
Characteristics	**Baseline**	**Change**	**Baseline**	**Change**	***P* value**
Waist circumference (cm)	94.19 (92.31, 96.07)	0.68 (-0.04, 1.40)	94.63 (92.69, 96.58)	-0.48 (-1.43, 0.46)	0.08
Weight (Kg)	77.29 (74.45, 80.13)	0.45 (-0.08, 0.98)	77.22 (74.36, 80.09)	-0.23 (-0.82, 0.35)	0.07
BMI (Kg/m^2)	28.76 (28.05, 29.48)	0.13 (-0.06, 0.34)	28.95 (28.24, 29.66)	-0.08 (-0.30, 0.14)	0.11
Systolic blood pressure (mmHg)	133.96 (129.63, 138.28)	-4.01 (-6.85, -1.18)	132.17 (128.70, 135.64)	-1.63 (-4.87, 1.62)	0.22
Diastolic blood pressure (mmHg)	79.92 (77.73, 82.11)	0.20 (-1.38, 1.79)	79.94 (77.80, 82.07)	-0.27 (-1.76, 1.22)	0.75
Fasting Plasma Glucose (mg/dL)	116.24 (112.37, 120.11)	-5.34 (-8.62, -2.07)*	109.08 (105.15, 113.02)	7.41 (5.01, 9.80)*	<0.001
Glycated HbA_{1c} (%)	5.92 (5.82, 6.02)	-0.04 (-0.13, 0.06)	5.87 (5.74, 6.00)	0.04 (-0.04, 0.11)	0.14
Glycated HbA_{1c} (mmol/mol)	41.18 (40.10, 42.27)	-0.49 (-1.50, 0.51)	40.69 (39.26, 42.13)	0.40 (-0.40, 1.20)	0.14
Fasting plasma insulin (mU/mL)	14.36 (12.65, 16.07)	-2.16 (-3.37, -0.95)*	11.64 (9.92, 13.35)	2.71 (1.13, 4.30)*	<0.001
HOMA-IR	4.22 (3.66, 4.77)	-0.73 (-1.14, -0.32)*	3.16 (2.68, 3.64)	1.05 (0.54, 1.55)*	<0.001
HOMA-BCF	98.22 (86.35, 110.09)	-3.74 (-12.40, 4.91)	97.87 (78.15, 117.59)	-0.27 (-10.32, 9.79)	0.62
Total cholesterol (mg/dL)	217.44 (208.10, 226.79)	-4.11 (-10.12, 1.89)	213.51 (204.52, 222.50)	2.33 (-4.87, 9.53)	0.15
HDL-c (mg/dL)	54.28 (50.43, 58.13)	1.45 (-1.85, 4.75)	54.35 (50.61, 58.10)	1.48 (-0.78, 3.74)	0.97
LDL-c (mg/dL)	137.93 (128.18, 147.67)	-4.51 (-10.02, 1.00)	136.27 (127.76, 144.78)	1.32 (-4.85, 7.50)	0.15
VLDL-c (mg/dL)	25.19 (22.23, 28.14)	-0.85 (-3.23, 1.53)	22.98 (20.33, 25.63)	0.40 (-1.83, 2.64)	0.38
Total cholesterol/HDL-c ratio	4.29 (3.87, 4.72)	-0.20 (-0.41, 0.00)	4.15 (3.79, 4.52)	-0.05 (-0.27, 0.17)	0.34
LDL-c/HDL-c ratio	2.77 (2.39, 3.17)	-0.16 (-0.34, 0.02)	2.69 (2.38, 2.99)	-0.05 (-0.23, 0.13)	0.35
Triglycerides (mg/dL)	125.81 (111.07, 140.56)	-4.01 (-15.85, 7.83)	115.15 (101.75, 128.54)	8.25 (-8.72, 25.22)	0.20
Lutein-Zeaxanthin (nM)	452.90 (399.86, 505.95)	245.23 (169.03, 321.43)*	482.68 (422.31, 543.06)	-18.34 (-70.09, 33.40)	<0.001
γ-tocopherol (nM)	625.49 (464.20, 786.78)	754.38 (526.67, 982.09)*	756.14 (590.85, 921.44)	-109.94 (-292.22, 72.35)	<0.001

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 analyzed using adjusted ANOVA of repeated measurements. * Significant difference ($P < 0.05$) between baseline and end of a particular intervention period. BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-BCF, homeostatic model assessment of β-cell function; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very low-density lipoprotein.

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Supplementary Table 4. Baseline and changes after intervention period in inflammatory, satiety and other related markers

	Pistachio Diet		Control Diet		Treatment Effect
Characteristics	**Baseline**	**Change**	**Baseline**	**Change**	***P* value**
Platelets ($x10^3/\mu L$)	225.70 (214.39, 237.02)	-4.26 (-8.86, 0.35)	223.08 (207.52, 238.65)	-6.27 (-14.18, 1.63)	0.88
Lymphocytes ($x10^3/\mu L$)	1.87 (1.74, 2.00)	-0.02 (-0.08, 0.05)	1.87 (1.71, 2.03)	-0.01 (-0.09, 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)	16.95 (-10.85, 44.75)	231.39 (175.33, 287.45)	-14.85 (-41.56, 11.86)	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
Von Willebrand factor (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
Platelet Factor 4 (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
Thromboxane B2 (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)	0.009
Resistin (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)	0.21
Interleukine-6 (pg/mL)	1.48 (1.18, 1.77)	-0.14 (-0.33, 0.05)	1.39 (1.06, 1.72)	0.01 (-0.29, 0.31)	0.26
Interleukine-18 (pg/mL)	104.60 (87.06, 122.13)	-9.84 (-17.37, -2.32)*	116.08 (91.75, 140.42)	-13.13 (-25.07, -1.18)	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
Oxidized LDL (ng/mL)	279.07 (253.29, 304.85)	-7.22 (-21.28, 6.83)	259.62 (239.78, 279.46)	11.24 (2.82, 19.66)*	0.03
sRAGE (pg/mL)	319.12 (242.22, 396.01)	21.93 (-18.97, 62.83)	309.07 (231.74, 386.41)	11.82 (-16.68, 40.31)	0.72

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 analyzed using basal adjusted ANOVA of repeated measurements. * Significant difference ($P < 0.05$) between baseline and end of a particular intervention period. PAI-1, Plasminogen Activator Inhibitor-1; GIP, Gastric Inhibitory Polypeptide; GLP-1, Glucagon-Like Peptide-1; sRAGE, Soluble Receptor of Advanced Glycation End-Products.

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