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ORIGINAL ARTICLE

Acute effects of pistachio consumption on glucose and insulin, satiety hormones and endothelial function in the metabolic syndrome

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BACKGROUND/OBJECTIVE: Nut consumption has been found to decrease risk of coronary heart disease and diabetes and to promote healthy body weights possibly related to their favorable macronutrient profile. We therefore assessed the effect of pistachios on postprandial glucose and insulin levels, gut hormones related to satiety and endothelial function.
SUBJECTS/METHODS: In this randomized crossover study, 20 subjects with metabolic syndrome consumed five study meals over 5–10 weeks. The meals differed in fat type and quantity, but were matched according to available carbohydrates (CHOs). Three meals had 50 g available CHO: white bread (WB50g), white bread, butter and cheese (WB + B + Ch) and white bread and pistachios (WB + P). Two meals had 12 g available CHO: white bread (WB12g) and pistachios (P).
RESULTS: Within each group of available CHO meals, postprandial glucose levels were the highest following the white bread-only meals, and glucose response was significantly attenuated when butter and cheese or pistachios were consumed ($P<0.05$). Postprandial insulin levels were highest after the WB + B + Ch meal ($P<0.05$), but did not differ between the white bread-only and pistachio meals. Both endothelial function (reactive hyperemia index) and arterial stiffness (augmentation index) significantly increased after the white bread-only meals compared with the WB + B + Ch meal (all $P<0.05$). Insulin secretagogue levels were higher when butter and cheese or pistachios were consumed than when white bread only was consumed ($P<0.05$).
CONCLUSIONS: Compared with white bread, pistachio consumption reduced postprandial glycemia, increased glucagon-like-peptide levels and may have insulin-sparing properties. These effects could be beneficial for individuals with diabetes and metabolic syndrome.

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Keywords: pistachios; nuts; glycemic index; glycemic load; metabolic syndrome; endothelial function

INTRODUCTION

Epidemiological and interventional studies have linked the consumption of nuts to a reduced risk of coronary heart disease (CHD) and diabetes and to promoting healthy body weights.[1–4] This protective effect may in part be explained by the favorable impact of nuts on serum lipids,[5–11] oxidative stress[5,12] and markers of inflammation.[13–15] However, owing to their low available carbohydrate (CHO) content and favorable fat and protein profiles, nuts may also decrease the risk of coronary heart disease and diabetes by reducing postprandial blood glucose excursions and improving endothelial function.[16–21] Recently, it has been reported that pistachios, when eaten alone, have a minimal effect on blood glucose, and when consumed with a CHO meal, they attenuate the postprandial glucose response.[22] Pistachios have also been found to reduce blood pressure and peripheral vascular responses to stress in dyslipidemic subjects.[23]

The aim of the present study was to expand on these previous findings by examining the acute effects of pistachio consumption in individuals with the metabolic syndrome. We studied the effect of pistachios on postprandial glucose and insulin levels, markers of oxidative stress, gut hormones related to satiety and endothelial function.

MATERIALS AND METHODS

Subjects

Men and women aged 40–65 years and body mass index $>30\,kg/m^2$, with the metabolic syndrome as defined by NCEP ATP III guidelines,[24] were recruited for the study. Subjects were otherwise in good health and were not taking any medications that would interfere with glucose metabolism. For the assessment of the metabolic syndrome, waist circumference and blood pressure were measured and a blood sample was taken for the measurement of triglycerides, high-density lipoprotein-cholesterol and fasting blood glucose.[24] A total of 41 subjects were screened, of whom 18 failed the screening criteria and 3 withdrew from the study before randomization. A total of 20 subjects (8 men, 12 women), mean (s.d.) age 54 (8) years and body mass index 37.5 (7.9) kg/m^2 were entered into the study. The subject number was based on findings from our previous study,[22] in which 10 healthy subjects provided sufficient power to detect a significant difference in postprandial blood glucose response with

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pistachio consumption. The subject number was increased to 20 in the current study to account for the possible increased variance in glycemic response related to the metabolic syndrome.

Protocol

Subjects were recruited through local advertisement and the clinic volunteer roster. All tests occurred in the morning after an overnight, 12 h fast. At the beginning of each test, the subject was weighed, and fasting blood samples were obtained by finger prick and venous blood draws. Fasting endothelial function was assessed. Then test meal was provided to the subjects and a timer was started at meal commencement. Additional finger prick blood samples were collected at 15, 30, 45, 60, 90, 120, 150 and 180 min, and additional venous samples were collected at 30, 60, 120 and 180 min. Postprandial endothelial function was assessed at 60 and 180 min. At the conclusion of the testing, subjects were offered a snack and then allowed to leave. Subjects did not participate in more than one test per week. The study protocol was approved by the Western Institutional Review Board. Written informed consent was obtained from all subjects before starting the study.

Study meals, palatability and satiety

Five study meals were consumed by all subjects in a randomized order (Table 1). Three meals were matched for 50 g of available CHOs, and two meals were matched for 12 g of available CHOs. The 50 g available CHO meals included: (a) white bread alone (WB50g), (b) white bread, butter and cheese (WB + B + Ch) and (c) white bread and 3 oz of pistachios (WB + P). The WB50g meal provided data on a standard CHO meal. The WB + B + Ch and WB + P meals were matched for available CHO and total fat, although they differed substantially in their content of saturated and unsaturated fatty acids. The 12 g available CHO meals included: (a) white bread only (WB12g) and (b) pistachios only (P). These meals provided data on the effects of 3 oz of pistachios alone, and a standard CHO meal that matched the available CHO found in of 3 oz of pistachios.

Palatability was rated on a 100 mm visual analog scale anchored with 'very unpalatable' at one end and 'very palatable' at the other. The higher the number, the higher was the perceived palatability of the product.

Satiety was assessed immediately before eating the study meal and at 30, 60, 120 and 180 min The satiety quotient was calculated using the formula by Green *et al.*[25]

Biochemical analysis of blood samples

Finger prick samples (2–3 drops of capillary blood) were used to analyze blood glucose. Intravenous blood samples were obtained by a trained IV nurse using the 'needle-less' system from a forearm vein. Blood samples were collected using a plastic cannula (BD Blunt Plastic Cannula, Mississauga, ON, Canada). Immediately after each venous blood sample, the catheter was flushed with 2–3 ml of normal (0.9%) saline to keep it patent. The saline was cleared from the catheter before each venous blood sample by withdrawing 1 ml of blood into a syringe, which was subsequently discarded.

Glucose analysis was performed using a YSI model 2300 STAT analyzer (YSI Incorporated Life Sciences, Yellow Springs, OH, USA). Insulin, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP) and ghrelin levels were measured using the human insulin, GLP-1, GIP and ghrelin ELISA Kits, respectively (Alpco Diagnostics, Salem, NH, USA and EMD Millipore Corporation, Billerica, MA, USA). Free fatty acids were analyzed by the Biochemistry Laboratory at St Michael's Hospital using conventional methods.

Endothelial function

Endothelial function was assessed immediately before eating the study meal and at 60 and 180 min postprandially. Endothelial function was determined by pulse wave amplitude utilizing the Endo-PAT2000 (Itamar Medical Ltd, Casearea, Israel). The Endo-PAT uses pulse amplitude tonometry (PAT) to measure changes in pulse wave amplitude before and after an ischemic cuff is inflated for 5 min on the forearm of one arm. Endothelial function is assessed by the reactive hyperemia index (RHI), with lower values indicating greater dysfunction. The Endo-PAT also provides a measure of arterial stiffness (augmentation index), with higher values indicating greater stiffness. We have shown excellent test–retest reliability for these measures under controlled conditions.[26]

Statistical analysis

Descriptive summary statistics (mean and s.e.m.) were performed for all variables at each timepoint for each test meal. In keeping with our previous work,[17,18] results were tabulated and incremental areas under the blood response curves (incremental area under the curve (iAUC)) were calculated, ignoring the area below fasting. Differences in response to test meals were analyzed by repeated-measures ANOVA (SAS Proc Mixed, SAS Institute Inc, Cary, NC, USA) for main effects of time and test meal and the time × meal interaction. If the time × meal interaction was significant, then paired *t*-tests were conducted for each time point using the Tukey–Kramer method for multiple comparisons. Results were considered significantly different at $P<0.05$.

RESULTS

All 20 subjects completed the five test meals with no adverse events reported. The meals were well tolerated with no significant differences in the palatability ratings between them. After adjustment for multiple comparisons, the WB + P meal significantly blunted the postprandial glucose peak height and the iAUC compared with the WB50g meal (Figure 1). The WB + B + Ch meal followed a comparable pattern as the WB + P meal. Similarly, the glucose peak height and iAUC was reduced for the P meal compared with the WB12g meal.

There was a significant time × meal interaction for serum insulin levels ($P<0.001$). Serum insulin levels were very similar during the first hour for the WB50g, WB + B + Ch and WB + P meals. At 120

Table 1. Energy and macronutrient composition of the study meals

Test meal	*Abbrev*	*Amount (g)*	*Energy (kcal)*	*Protein (g)*	*Tot fat (g)*	*SFA*	*MUFA*	*PUFA*	*Av CHO (g)*	*DF (g)*
Control 1 (white bread)	WB 50 g	112.5	254	9.7	0.8	0.2	0.2	0.3	50	2.2
Control 2 (WB + butter + cheese)	WB + B + Ch	WB 110	248.6	9.5	0.7	0.1	0.1	0.3	48.9	2.2
		B 19	133.0	0	15.2	9.7	3.9	0.6	0	0
		Ch 80	322.7	19.8	26.4	17.0	7.6	0.7	1.1	0
		Total 209	704.3	29.3	42.3	26.8	11.7	1.6	50.0	2.2
Pistachio Test Meal (WB + 3 oz pistachios)	WB + P	WB 85	192.0	7.3	0.6	0.1	0.1	0.2	37.8	1.7
		Pist 85	513.3	21.7	41.3	5.0	21.8	12.5	12.3	6.8
		Total 170	705.4	29.1	41.9	5.1	21.9	12.7	50.1	8.4
Control 3 (white bread)	WB 12 g	WB 27.7	62.6	2.4	0.2	0.0	0.0	0.1	12.3	0.5
Pistachio Test Meal (3 oz pistachios)	P	Pist 85	513.3	21.7	41.3	5.0	21.8	12.5	12.3	6.8

Abbreviations: Av CHO, available carbohydrate; DF, dietary fiber; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Tot Fat, total fat.

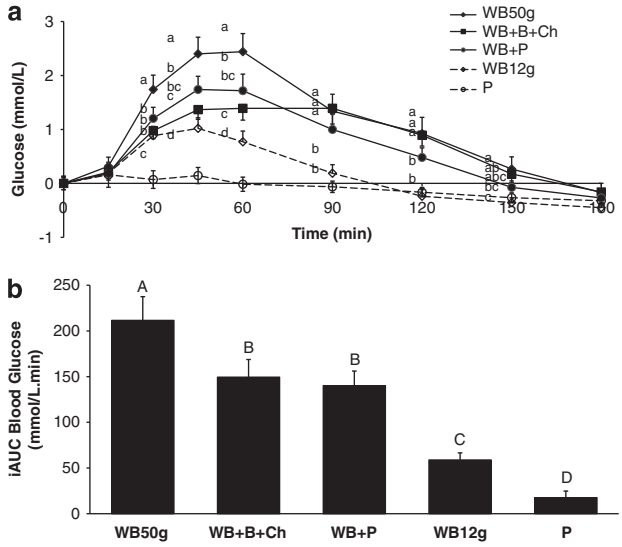

Figure 1. (**a** and **b**) Effect of study meals on postprandial blood glucose response (**a**) and incremental area under the glucose curve (**b**). Values not sharing a common superscript are significantly different ($P<0.05$).

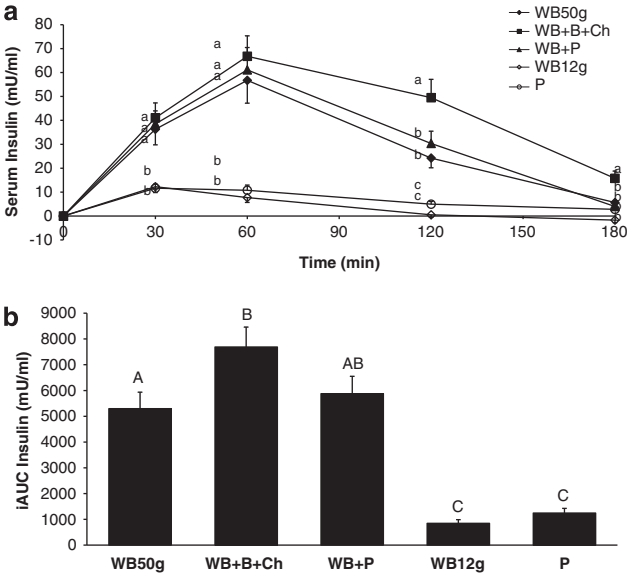

Figure 2. (**a** and **b**) Effect of study meals on postprandial serum insulin response (**a**) and incremental area under the insulin curve (**b**). Values not sharing a common superscript are significantly different ($P<0.05$).

and 180 min, serum insulin levels were significantly lower for the WB50g and WB + P meals compared with the WB + B + Ch (Figure 2a). As expected, serum insulin levels were significantly lower after the WB12g and P meals compared with the 50 g available CHO meals for all time points except for 180 min. At 180 min, insulin levels had declined to similar levels for all meals except WB + B + Ch meal, for which the levels remained significantly elevated ($P<0.0001$). The iAUC of insulin was significantly higher after the WB + B + Ch meals compared with the WB50g (Figure 2b). As expected, the insulin iAUCs of the WB50g and WB + P meals were significantly higher than the iAUCs after the WB12g and P meals ($P<0.0001$).

There was a significant time and meal interaction for serum free fatty acids ($P<0.001$). The high CHO meals (WB50g, WB + B + Ch and WB + P) generally suppressed free fatty acids to a greater extent and for a longer period than the low CHO meals (WB12g and P; Table 2).

The P meal significantly increased GIP and GLP-1 levels to a much greater extent than the WB12g meal. Similarly, WB + P and WB + B + Ch led to a significant increase in GIP and GLP-1 levels compared with WB50g. The increase in GLP-1 was not significantly different between WB + B + Ch, WB + P and P, while the increase in GIP was significantly higher with WB + B + Ch and WB + P compared with P (Table 2). Ghrelin levels appeared to be inversely related to the energy content of the meals with the highest iAUC occurring after the WB12g and the smallest iAUC occurring after the WB + P and WB + B + Ch meals (Table 2). This pattern was also observed for the subjective satiety quotient ratings where the WB12g was found to be the least satiating, even after correcting for the energy intakes of the meals.

Pistachios had no consistent effect on measures of oxidative stress. While the WB + P meal resulted in the highest levels of protein thiols (i.e. preserved protein thiols from oxidative degradation), this meal also resulted in the highest levels of conjugated dienes and thiobarbituric acid reactive substances (as markers of lipid oxidation) in the low-density lipoprotein lipid fraction (data not presented). However, there were no statistically significant differences among groups.

Endothelial function (assessed as RHI) was similar in the fasting state but varied by treatment in the postprandial state (Figure 3a). RHI increased after the WB50g meal and decreased after the WB + B + Ch meal; this differential response was statistically significant ($P=0.03$). An intermediate change in RHI was observed after the WB + P meals that did not differ from the other 50 g available CHO meals. There was no difference in RHI between the two 12 g available CHO meals. Augmentation index decreased postprandially for all of the 50 g available CHO meals, with a significantly greater reduction following the WB + B + Ch meal compared with the WB50g meal ($P=0.001$; Figure 3b). Augmentation index increased after the WB12g meal and decreased after the P meal ($P=0.005$).

DISCUSSION

The data confirm earlier studies that addition of pistachios to a CHO meal reduces postprandial glycemia.[22] However, this study is the first to test this hypothesis in meals matched for macronutrient profile. Adding pistachios to white bread (total of 50 g available CHO) produced a significantly lower glycemic response compared with the white bread alone, but did not differ from the white bread, butter and cheese meal that was matched for protein and total fat content. When consumed alone, pistachios elicited little or no increase in postprandial glucose levels. In contrast, the small white bread meal led to a robust glucose response, despite the fact that these two meals were matched for available CHO content (12 g). The higher protein and fat content of the pistachios may explain the reduced glycemic response compared with white bread alone.

It is interesting to note that insulin levels during the second hour of the test were lower after the WB + P compared with the WB + B + Ch. This may indicate an insulin-sparing effect of the pistachios, despite a similar elevation in the incretin, GLP-1, levels between the meals. This effect may be due to the acute consumption of mono- and polyunsaturated fatty acids, both of which have been shown to decrease postprandial glucose without altering insulin levels.[27] Furthermore, it is possible that the different amino-acid composition of the vegetable protein versus the animal protein of the two meals may have elicited different insulin-stimulating effects. Pistachios are richer in arginine than cheese, and although intravenous arginine has been found to trigger insulin secretion the most, oral arginine in

Table 2. Effect of study meals on free fatty acids, GIP, GLP-1 and ghrelin

iAUC	*Control 1 (WB)*	*Control 2 (WB + butter + cheese)*	*Pistachio Test Meal (WB + 3 oz pistachios)*	*Control 3 (WB)*	*Pistachio Test Meal (3 oz pistachios)*
Free fatty acids (mEq/ml min)	0.454[a] (0.172)	0.142[a] (0.109)	0.580[a] (0.352)	1.007[a] (0.329)	1.446[a] (0.812)
GIP (pg/ml min)	17 660[a,b] (2340)	57 119[c] (6783)	52 776[c] (7761)	4015[a] (387)	27 762[b] (3580)
GLP-1 (pmol/ml min)	61[a] (18)	178[b] (24)	137[b] (21)	19[a] (8)	144[b] (22)
Ghrelin (pg/ml min)	7154[a,c] (2046)	4115[a] (1347)	4611[a] (1599)	14 801[b] (3561)	11 398[b,c] (3019)

Abbreviations: GLP-1, glucagon-like peptide-1; GIP, gastric inhibitory polypeptide; iAUC, incremental area under the curve; WB, white bread. Data are presented as mean ± (s.e.m). For each row, values not sharing a common superscript are significantly different ($P<0.05$).

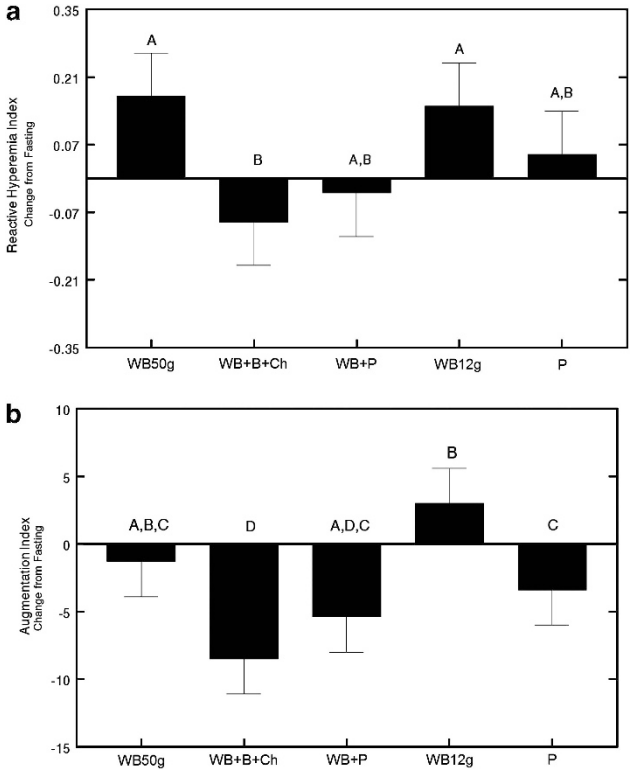

Figure 3. (**a** and **b**) Effect of study meals on postprandial endothelial function RHI (**a**) and augmentation index (**b**). Values not sharing a common superscript are significantly different ($P<0.05$).

normal physiological amounts does not stimulate insulin secretion. Rather, oral arginine attenuates the increase in blood glucose when administered with oral glucose.[28] Cheese is richer in the amino-acid proline and glutamic acids, which have both been shown to result in large insulin responses.[29,30] Lastly, the whey protein content of the WB + B + Ch may also in part explain the large insulin response. Whey protein has been shown to inhibit dipeptidyl dipeptidase IV, which can lead to an increase in the half-life of GLP-1.[31]

GLP-1 and GIP are classified as incretins, which are defined as gastric hormones that stimulate postprandial release of insulin from pancreatic β-cells. Furthermore, they act to reduce gastric emptying and inhibit the actions of glucagon. The stimulation of their release by the pistachio or the cheese-containing meal is potentially due to fat[32] and the amino-acid content or the protein load of these meals.[32,33] These observations are further supported by the finding that the 3 oz pistachio meal increased GIP and GLP-1 levels to a much greater extent than the 12 g white bread meal, without a significant difference in the insulin levels. This effect of pistachios confirms the aforementioned finding that pistachios may be insulin sparing in spite of elevated incretin levels. The present findings on the effect of the meals on incretin hormone secretion warrants further investigation in light of benefits associated with pharmaceutical GLP-1 agonists (e.g. Exenatide and Liraglutide) or dipeptidyl dipeptidase IV inhibitors (e.g. Sitagliptin) on glycemic control as mono- or adjunctive therapy in patients with type 2 diabetes.[34] Interestingly, nutritional interventions suggest that the impact of nuts extend not only beyond altering the postprandial metabolic profile but also affect calorie consumption in the next meal (the 'second meal' effect). One study showed that whole almonds and almond oil increased satiety and had a favorable effect on second meal insulin release and suppressed non-esterified fatty acid levels. In that study, GLP-1 was elevated and its return to baseline was slowed, although this trend was not significant.[35] Therefore, further investigation of long-term sustainability of GLP-1 stimulation and insulin-sparing effects of pistachios in patients with diabetes or impaired glycemic control is warranted based on the current findings.

In terms of satiety, ghrelin emerged as the first identified hunger hormone, where high levels of ghrelin increase food intake. In the current study, ghrelin levels appeared to be inversely related to the energy content of the meals with the highest iAUC after the small WB meal and the smallest after the WB + B + Ch and WB + P. This pattern was also seen with the subjective satiety ratings where the satiety quotient was decreased to the greatest extent after the small white bread meal in response to the hunger and desire to eat questions. Similarly, the satiety quotient was increased significantly after the small white bread meal in response to the question on how full the subjects felt, indicating a lower feeling of fullness per unit of intake. The increased GLP-1 may also account for the observed satiety trends. GLP-1 levels have been inversely associated with energy intake in lean and overweight patients, potentially due to delaying gastric emptying.[36] Long-term studies are required to determine whether these acute effects on satiety translate to long-term improvements in energy intake and satiety.

Endothelial function was significantly impaired after the WB + B + Ch meal, which was highest in saturated fat. This finding is in line with previous work that demonstrated acute endothelial dysfunction (assessed by flow-mediated dilation) after a single high saturated fat meal.[37] In contrast, endothelial function improved after the white bread-only meals, which could be due to the vasodilatory effect of insulin. Although a number of previous studies have indicated that oils high in monounsaturated fatty acids, such as olive oil, can significantly impair endothelial function when compared with other sources of omega-3 fats or when mixed with antioxidants (vitamins or salad),[38,39] the pistachio meals had no significant effect on RHI after meal.

Interestingly, arterial stiffness was reduced after the WB + B + Ch meal compared with the white bread-only meals. This was unexpected, as few previous studies have measured changes in augmentation index in the postprandial state. Nitric oxide-dependent vasodilation in peripheral resistance arterioles in the hand and forearm may be responsible for this finding. These results provide further evidence that vasodilation and arterial stiffness are differentially affected by type and quantity of dietary

fat. This interpretation is supported by a recent study which found that pistachio consumption of 3 oz per day for 4 weeks significantly attenuated stress-induced peripheral vascular constriction in dyslipidemic subjects.[23] However, endothelial function (measured as flow mediated dilation) in the longer study was not significantly affected by pistachios, possibly because testing was conducted after a 12 h fast. Thus, future studies should include nested designs in which acute and chronic effects of pistachios can be measured in the same individuals.

This study indicates that addition of pistachios to a CHO meal decreases postprandial glucose levels to a similar extent compared with other sources of fat and protein but may have insulin-sparing properties. Pistachios by themselves may stimulate an increase in GIP and GLP-1 levels. Both insulin-sparing and increased GLP-1 levels as well as a minimal effect on blood glucose are properties that could benefit those with diabetes or the metabolic syndrome.

CONFLICT OF INTEREST

CWCK has received research grants, travel funding, consultant fees, honoraria or has served on the scientific advisory board for Abbott, Advanced Food Materials Network, Almond Board of California, American Peanut Council, American Pistachio Growers, Barilla, California Strawberry Commission, Canadian Institutes of Health Research, Canola Council of Canada, Danone, General Mills, Hain Celestial, International Tree Nut Council, Kellogg, Loblaw Brands Ltd, Oldways, Orafti, Paramount Farms, Pulse Canada, Saskatchewan Pulse Growers, Solae and Unilever. ALJ is a director of Glycemic Index Laboratories, Toronto, Ontario, Canada. JC and LC are employed by Glycemic Index Laboratories. SGW and KAS have received travel funding and research funding from the American Pistachio Growers. DJAJ reported serving on the Scientific Advisory Board of Unilever, Sanitarium Company, California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg's, Quaker Oats, Procter and Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers and Canola Council of Canada; receiving honoraria for scientific advice from the Almond Board of California, International Tree Nut Council Nutrition Research and Education Foundation, Barilla, Unilever Canada, Solae, Oldways, Kellogg's, Quaker Oats, Procter and Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial and Alpro Foundation; being on the speakers panel for the Almond Board of California; receiving research grants from Loblaw Brands Ltd, Unilever, Barilla, Almond Board of California, Solae, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council and Peanut Institute; and receiving travel support to meetings from the Almond Board of California, Unilever, Alpro Foundation and International Tree Nut Council. The remaining authors declare no conflict of interest.

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