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Research Article

Effect of Bedtime Pistachio Consumption for 6 weeks on Weight, Lipid Profile and Glycemic Status in Overweight Persons

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Abstract: Overweight persons trend for dyslipidemia and diabetes risk, while mono- and polyunsaturated fats in pistachios (PI) may improve lipoprotein and glycemic status. This study determined if a small amount of PI consumed by obese persons at bedtime promotes beneficial changes in metabolic status. Obese subjects were randomized to 35.4 g PI self-administered at bedtime or control (CO; no PI) for 6 weeks. There was no difference in activity level or body weight between PI and CO at weeks 0, 1, 2, 4 or 6. HDL cholesterol at wk 0 and 6 in PI was 49.2 ± 3.5 and50.3 ± 3.0, and CO was 60.8 ± 3.8 and 58.0 ± 2.7 mg/dL, at week 6 PI had improved slightly relative to CO (P= 0.12). Plasma glucose at wk 0 and 6 in PI was 104.6 ± 2.8 and 99.6 ± 2.6 mg/dL, and CO was 102.0 ± 2.8 and 101.1 ± 2.6 mg/dL, at wk 6 PI had improved slightly relative to CO (P= 0.16). Plasma Insulin at wk 0 and 6 in PI was 8.5 ± 1.0 and 8.4 ± 0.9, and CO was 7.2 ± 0.7 to 8.8 ± 0.7 μU/mL, at wk 6 PI had improved slightly relative to CO (P = 0.21). In conclusion 35.4 grams pistachios/day for 6 wks is probably near the lower end of what is needed to promote beneficial metabolic changes. Future studies may wish to look at pistachio consumption in populations with specific metabolic disorders such as type 2 diabetes.

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Introduction

Obesity is a risk factor associated with type 2 diabetes, dyslipidemia, and cardiovascular disease and may be influenced by diet. Evening snacking can be a risk factor associated with development of obesity[1]. The Dawn phenomenon is a diabetic complication resulting in late night disordered glucose homeostasis[2-3]. Cholesterol synthesis is known to peak late in the evening[4]. Diets rich in mono- and polyunsaturated fatty acids can lower LDL and total cholesterol, as well as increase HDL cholesterol, which is linked to development of or protection from cardiovascular disease[5-7], while dietary saturated fats tend to increase LDL cholesterol and the risk of type 2 diabetes[8]. Therefore nutritional qualities in a snack delivered at bedtime could alter carbohydrate and lipid metabolism and the metabolic events that occur in the evening as part of the circadian rhythm.

Pistachios have a high nutrient density, and potential to give a sense of satiety leading to lower caloric consumption in following meal? [9]. Pistachios are high in monounsaturated fats, polyunsaturated fats, and fiber which potentially lead to improved cholesterol profiles. Pistachios are also known to be rich in phytosterols, such as B-sitostero[10] that have the capacity to lower LDL-cholesterol by reducing cholesterol absorption from the gut[11]. 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, xanthophyll and carotenoids. Because pistachios are low in carbohydrate and sugars (27.5g /100g and 7.6g /100g respectively) they have a very low glycemic index that is in the range of 3.8 to 9.3[12] giving pistachios utility for improved postprandial blood glucose and lipids.

Prior pistachio studies of the beneficial metabolic effects are legion with caloric intake representing 15% of caloric intake[13],16% and 27% of caloric intake[14], 20% of caloric intake[15-16], while the USDA suggested single serving size for pistachios is 1ounce (30grams) of kernels (49grams with shell) or 1ounce protein equivalent[17].Choice of a serving size that reflects an amount commonly consumed is also an important consideration when evaluating the metabolic effect of a snack, in this regard a 200 Calorie serving size is currently suggested for snack serving sizes. The time of administration canalso influence the metabolic consequence of the snack.Effects of a snack at bedtime with a low glycemic index, high protein content and favorable ratio of mono and polyunsaturated fats has not been investigated in this regard. The present study sought to determine the effect of consumption of small 1.25 ounceserving of pre-peeled pistachios (8.3% of daily caloric intake) self-administered at bedtimeon weight maintenance, lipid profile, and glycemic status.

Methods

Study population

This study was approved by the Winona State University Institutional Review Board with subjects recruited through email, posters, and newspapers. A total of 22 obese subjects were enrolled in this 42 day study (16 female and 6 male; 57.1 ± 8.6 years of age; BMI = 31.1 ± 4.0). Study subject exclusions included smoking, use of medications for improving blood cholesterol, insulin-dependent diabetes or medication for diabetes, medications for inflammation/arthritis (i.e.corticosteroids), recent treatment for cancer or heart disease within the last 6 months, liver disease, or use of anticoagulants (such as Plavix or Coumadin). All of the subjects completed a seven day wash-out period during which they discontinued consumption of alcohol, fish oil, and all nut products prior to their first laboratory visit.

Pistachio consumption and plasma analysis

Subjects were randomized upon laboratory presentation on the first study morning to receive either no intervention (control) or an intervention consisting of 1.25oz. of pre-shelled, roasted, unsalted pistachios self-administered at bedtime for 42days. This serving contained 201 total calories representing 8.3% of their weight/age activity adjusted daily caloric intake containing 15.88g total fat (1.93g saturated, 8.39g monounsaturated, and 4.76g polyunsaturated fat), 7.73g protein, 2.74g sugars, and3.50g fiber[17]. No other nuts were permitted in either group during the duration of the study. Subjects completed a twelve hour fast before venous blood collection during which they abstained from all food or drink (except water); they also did not exercise in the twelve hour fast period. After randomization to treatments, fasting blood work was completed at days 0 and at study completion 42 days later.

Subjects were required to complete daily dietary journals and exercise records (in minutes) to ensure compliance with dietary restrictions and to estimate activity level throughout the study. Weight was measured on days 0, 7, 14, 28, and 42 when subjects returned to the lab for food diary review and to receive more pistachios.

Venous blood samples were collected at study onset (day-0) and at study completion (Day 42).

Collected plasma samples were frozen at -80^{o}C until analysis in single blind fashion on a single day using freshly thawed samples. Plasma glucose was measured with a Hitachi 912 Chemistry Analyzer using hexokinase reagent from Boehringer Mannheim (Indianapolis, Indiana, USA). Insulinwas measured with a two-site immunoenzymatic assay performed on the DxI automated immunoassay system (Beckman Instruments, Chaska, Minnesota, USA). Plasma triglycerides, total cholesterol and HDL cholesterol were also measured, and LDL cholesterol was estimated using the Friedwald equation.

Pistachio Phytochemical Analysis

Pistachios (2g) were crushed in liquid nitrogen, transferred into10ml solvent (95% n-hexane, 80% acetone + 0.1% acetic acid or 50% methanol) followed by 5 min vortex homogenization and 10min sonication. After centrifugation at 2000rpm for 20min the supernatants and pellets were collected separately. Extracts were dried in a rotary evaporator at 40ºC and redissolved in 100% methanol for analysis. A Dionex® UltiMate 3000 UPLC system coupled with Applied Biosystems API 3000™ LC/MS/MS system was used for qualitative and quantitative analysis. The Gemini® 150 x 4.6mm 5µm C18 110 Å LC column was used for liquid chromatographic separation with solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in acetonitrile under the following gradient conditions:0% B to 15% B from 0-1min; 15% B to 16% B from 1-5min; 16% B from 5-10min; 16% B to 17% B from 10-25min 17% B from 25-28min;17% B to 30% B from 28-30min; 30% B to 45% B from 30-38 min; 45% B to 80% B from 38-40min; 80% B to 0% B from 40-43min; 0% B from 43-50min with a flow rate of 1ml/min. MS analysis was carried out in heated nebulizer ion source in negative ion mode with source temperature (500°C), curtain gas (12psi), nebulizer gas (7psi), collision gas (6psi), entrance potential (-9V), collision energy (-20V), collision cell exit potential (-3V) and declustering potential (-60V). Compound structure was determined by comparison with those of standards. Compound quantification was carried out in multiple reaction monitoring scan mode based on calibration curves generated from standards. Data was acquired in Analyst software, version 1.4.2.

Statistical Analysis

All data was expressed as mean ± standard deviation.

Results and Discussion

Pistachio serving as percent of total caloric intake

All 22 subjects who arrived at the laboratory on day-0 completed the 42 day study. Daily food diaries were examined weekly to evaluate compliance with study requirements and discrepancies (i.e. failure to record diet, failure to eat pistachio snack or accidental consumption of peanuts) were observed on 32 of the 924 total participation days. Daily activity levels were not significantly different between groups across time. Activity, age and BMI adjusted caloric requirements were calculated (http://fnic.nal.usda.gov/fnic/interactive DRI) for each participant. Criteria for sedentary, low active, and active activity levels were met by 6, 14, and 2 participants respectively, yielding an average of 35 ± 13minutes walking/day. This was associated with an overall estimated daily requirement of 2424 ± 498 Ca/day, therefore the 1.25oz pistachio snack represented 8.3% of total caloric needs. The amount of pistachios administered in this study was about half or less than those used in the previous studies[13,14,15,16].

Pistachio effects on body weight and BMI

Weight maintenance is a primary concern among overweight persons which may prevent them from receiving health benefits associated with nuts such as pistachios[9]. Pistachio consumption in the present study was associated with neutral weight effects for the overweight persons in this study. Body weight differences at weeks 2-6 relative to week-0 were not affected by pistachio consumption (Figure 1). The 201 Calorie serving represented smaller and larger percentages of total caloric intake in the upper and lower tertiles, respectively. Therefore a tertile analysis based on BMI at study initiation was evaluatedand no statistically significant effects were observed within or between treatment tertiles (Figure 2). BMI of subjects in the control group had day- 0 and day-42 BMI values of 32.1 ± 4.5 and30.2 ± 3.4, respectively and BMI in the pistachios group had day- 0 and day-42 values of 31.8 ± 4.5 and 30.2 ± 3.3, respectively, with no statistically significant effect observed between or within groups.Several prior human studies also failed to demonstrate that pistachio consumption leads to changes in body weight[13,14,19, 20,21]. The present study demonstrates that consumer concerns about weight gain associated with the pistachio lipidand caloric content for the 1.25oz serving are unwarranted.

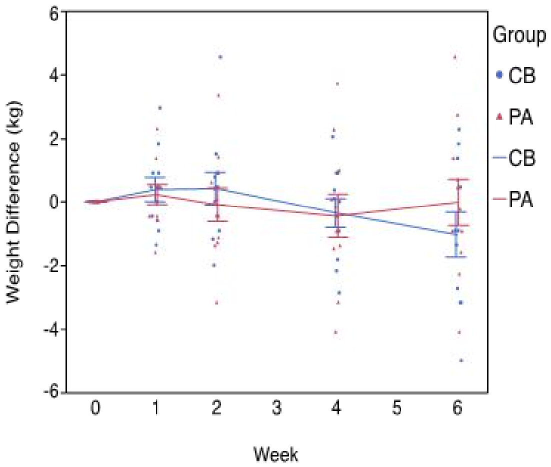

Figure 1. Pistachio consumption (1.25 oz) at bedtime had no statistically significant effect on body weight within or between groups (mean ± stdev). Please key to CB,PA

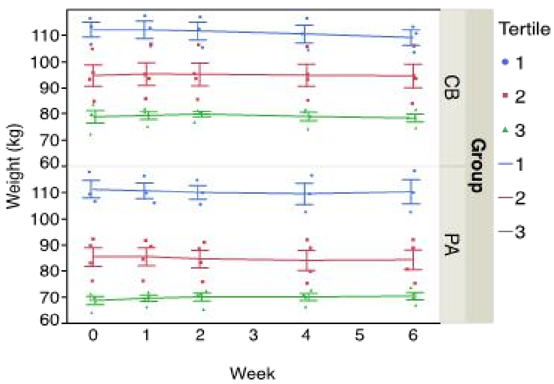

Figure 2. Pistachio consumption effect on body weight ranked as low, medium and high tertilesrelative to body mass index at week-0 was not associated with significant weight gain or loss within, between or across tertile treatment groups (mean ± stdev).

Pistachio effects on Blood Glucose, Insulin and Lipid levels

Table 1: Effect of pistachio consumption for 42 days (1.25 oz at bedtime) on plasma glucose, insulin, high density lipoprotein, low density lipoprotein, total cholesterol, and triglycerides (mean ± stdev).

	Week	Control	Pistachio
Glucose (mg/dL)	0	102.0 ± 2.8	104.6 ± 2.8
	6	101.1 ± 2.6	99.6± 2.6**(P=0.16)**
Insulin (µU/mL)	0	7.2 ± 0.7	8.5 ± 1.0
	6	8.8 ± 0.7	8.4 ± 0.9**(P=0.21)**
HDL Cholesterol (mg/dL)	0	60.8 ± 3.8	49.2 ± 3.5
	6	58.0 ± 2.7	50.3 ± 3.0**(P=0.12)**
LDL Cholesterol (mg/dL)	0	150.7 ± 28.8	161.9 ± 30.2
	6	144.6 ± 30.4	152.4 ± 27.9
Total Cholesterol (mg/dL)	0	211.6 ± 29.9	211.1 ± 30.7
	6	202.6 ± 31.3	202.6 ± 24.7
Triglycerides (mg/dL)	0	120.6 ± 51.7	123.7 ± 41.8
	6	111.3 ± 49.0	118.2 ± 33.7
P-value of trend towards significance within group is indicated in bold italics.			

Reductions in fasting plasma glucose, and insulinare important clinical targets for persons with type 2 diabetes and metabolic syndrome, these persons also often express obsesity as a comorbidity. In the present study bedtime consumption of 1.25oz pistachios for 42 days was associated with a trend toward beneficial reductions in fasting plasma glucose (P= 0.16) and insulin (P=0.21). Other investigators have examined the effect of pistachio consumption on glycemic and insulinemic profile[20]. demonstrated a beneficial improvement in fasting glucose when pistachios (20% of daily caloric need) were administered for 4 weeks, in contrast the studies[13,14] did not observe pistachio-dependent changes in insulin and glucose perhaps because of their larger amounts of pistachios consumed or their day-time consumption. Degree of mastication can also influence the glycemic and insulinemic response to almond[22], in the present study subjects were asked to chew thoroughly, but not given a specific number of chews prior to swallowing because the investigators has no real way to quantify masticative efficiency given the bedtime administration restriction. The present study excluded those with diabetes whose fasting glucose and insulin values might be more likely to benefit. Future therapeutic evaluations of pistachio consumption may be warranted for persons with type 2 diabetes or fasting hyperglycemia.

It is worth noting that none of these prior studies administered the phytosterol-rich pistachios at bedtime, when the nuts might have the greatest potential impact on the metabolic pathways that lead to obesity, dyslipidemia, and type 2 diabetes. On the other hand, whereas pistachios consumed alone have been shown to have a minimal effect on postprandial glycemia, the addition of pistachios (28g, 56g or 84g) to foods with a high glycemic index (pasta, parboiled rice and mashed potatoes) reduce, in an acute dose-dependent manner, the total postprandial glycemic response by 20 to 30%[12]. The beneficial impact of pistachio intake alone or in combination with high-carbohydrate foods on post-prandial glycemia has also been demonstrated (*Eur J Clin Nutr65, 696–702*). Bed-time administration of pistachios in the present study also permitted the investigators to exclude effects of satiety potential caloric intake or insulin sensitivity following snack administration.

Plant phenolic shave been suggested to have beneficial health effects related to diabetes, cardiovascular disease, inflammation, antioxidant protection and the gut microbiome. Glycosides of quercetin and catechin have been implicated in altered intestinal α-glucosidase and pancreatic α-amylase activities and glucose absorption from the gut[23,24], and potential to alter glycemic responses to pistachios. Pistachios are known to be high in anthocyanins, chlorophylls, carotenoids and phytosterols[25-26]. The γ-tocopherol content was 115μg/gin n-hexane extract, similar to that observed by[25]. Analysis of the phenolic profile of the pistachios used in this study yielded additional characterizations. The pistachio free catechin content was 0.041mg/g in the 80% aqueous acetone extract. In the 50% ethanol extract myrecetin-3-galactoside, quercetin-3-galactoside, quercetin-3-rhamnoside and free quercetin were present at 0.76μg/g, 2.08μg/g, 2.05μg/g, and trace levels respectively. This represents the first quantification of flavanolic glycosides in pistachios, although anthocyanins such as cyanidin-3-glycoside have been previously observed in pistachioskin in the 68-876 μg/g range[25] and 109-429μg/g range[26].

Conclusion

This is the first study of once daily pistachio consumption at bedtime in overweight persons. Consumption of 1.25 ounces of pistachios at bedtime by overweight persons for 42 days was not associated with statistically significant changes in body weight, BMI or activity level. Trends toward a beneficial effect were observed for HDL cholesterol, insulin, and glucose. This study was also the first to observe flavonolic glycosides in pistachios. This suggests that 1.25ounces per day at bedtime is near the minimum daily amount needed for beneficial effects without deleteriously affecting body weight.

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