

# Walnuts Consumed by Healthy Adults Provide Less Available Energy than Predicted by the Atwater Factors<sup>1–3</sup>

David J Baer,\* Sarah K Gebauer, and Janet A Novotny

USDA, Agricultural Research Service, Beltsville Human Nutrition Research Center, Beltsville, MD

## Abstract

**Background:** Previous studies have shown that the metabolizable energy (ME) content (energy available to the body) of certain nuts is less than predicted by the Atwater factors. However, very few nuts have been investigated to date, and no information is available regarding the ME of walnuts.

**Objective:** A study was conducted to determine the ME of walnuts when consumed as part of a typical American diet.

**Methods:** Healthy adults ( $n = 18$ ; mean age = 53.1 y; body mass index = 28.8 kg/m<sup>2</sup>) participated in a randomized crossover study with 2 treatment periods (3 wk each). The study was a fully controlled dietary feeding intervention in which the same base diet was consumed during each treatment period; the base diet was unsupplemented during one feeding period and supplemented with 42 g walnuts/d during the other feeding period. Base diet foods were reduced in equal proportions during the walnut period to achieve isocaloric food intake during the 2 periods. After a 9 d diet acclimation period, subjects collected all urine and feces for ~1 wk (as marked by a Brilliant Blue fecal collection marker) for analysis of energy content. Administered diets, walnuts, and fecal and urine samples were subjected to bomb calorimetry, and the resulting data were used to calculate the ME of the walnuts.

**Results:** One 28-g serving of walnuts contained 146 kcal (5.22 kcal/g), 39 kcal/serving less than the calculated value of 185 kcal/serving (6.61 kcal/g). The ME of the walnuts was 21% less than that predicted by the Atwater factors ( $P < 0.0001$ ).

**Conclusion:** Consistent with other tree nuts, Atwater factors overestimate the metabolizable energy value of walnuts. These results could help explain the observations that consumers of nuts do not gain excessive weight and could improve the accuracy of food labeling. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01832909. *J Nutr* doi: 10.3945/jn.115.217372.

**Keywords:** metabolizable energy, walnuts, nuts, macronutrient digestibility, Atwater factors, calories

## Introduction

Nuts provide many important nutrients, including vitamins, minerals, protein, unsaturated FAs, fiber, and antioxidants (1). Nuts are rich in minerals, such as magnesium, calcium, copper, manganese, molybdenum, zinc, selenium, and potassium (2, 3). Nuts also provide a number of vitamins, including vitamin E, vitamin K, and B-vitamins (2, 3). Research has suggested that plant-based proteins from sources such as nuts may be

favorable with respect to diseases such as cancer (4, 5) and cardiovascular disease (6, 7), and the 2010 Dietary Guidelines include a recommendation to increase consumption of plant proteins, which can be obtained from nuts (8). Nuts have a favorable FA profile (9–12), and also provide a variety of phytonutrients, including lutein, phytosterols, and flavonoids (2, 3, 13–15). Based on their nutrient composition and the scientific evidence, there are 2 qualified health claims regarding nuts and the reduced risk of coronary heart disease—one claim for nuts in general (16), and a separate claim specific to walnuts (17).

Despite their high nutrient content, nuts are considered an energy-dense food, and consumption of nuts may be inhibited by the perception that they are obesogenic (18, 19). However, inclusion of nuts in the diet has generally not led to weight gain. By contrast, cross-sectional studies have demonstrated an inverse association between nut consumption and BMI (20–22). Furthermore, prospective cohort studies have also led to conclusions that nut consumption is inversely associated with weight gain (23–25).

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<sup>3</sup> Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

\*To whom correspondence should be addressed. E-mail: [David.Baer@ars.usda.gov](mailto:David.Baer@ars.usda.gov).

**TABLE 1** Compositions of the base diet and of walnuts provided to adults who consumed control and walnut diets, each for 3 wk<sup>1</sup>

	Base diet	Walnuts
Protein, <sup>2</sup> g/kg	204 ± 3	177 ± 6
Fat, g/kg	145 ± 3	699 ± 9
Total carbohydrate, g/kg	613 ± 7	104 ± 16
Ash, g/kg	37.2 ± 0.7	19.7 ± 0.1
Gross energy, kcal/kg	5190 ± 820	8600 ± 740

<sup>1</sup> Values are means ± SEMs of chemical analyses based on  $n = 4$  samples. Base diet = background diet without walnuts. Walnuts = walnuts alone (dry weight).

<sup>2</sup> Protein of base diet is calculated as nitrogen × 6.25 and protein of walnuts is calculated as nitrogen × 5.3.

Previous studies from our research group have demonstrated that the available energy from almonds and pistachios is less than that predicted by the Atwater factors (26, 27). These general factors were developed by Atwater in the early 1900s to determine the metabolizable energy (ME) value of foods. In the mid-1900s, specific factors for food groups were published. For the development of these specific factors, nuts, mature dry beans, cowpeas, peas, and legumes were combined into one group with the same specific factors, although these foods are quite diverse botanically and nutritionally. For almonds, the measured energy value is 20% less than the Atwater prediction (26), whereas the measured energy value of pistachios is 5% less than predicted (27). The range in the discrepancy between the measured and the Atwater-predicted ME content of pistachios and almonds demonstrates that broad assumptions cannot be made about the digestibility and energy value of nuts. Moreover, each nut must be evaluated individually with respect to the ME it provides.

Given the potential health benefits of consuming walnuts, including a reduced risk of cancer and cardiovascular disease, it is worthwhile to determine the energy value of walnuts in the human diet and potentially reduce barriers to their consumption. Therefore, we conducted a human feeding study to evaluate the

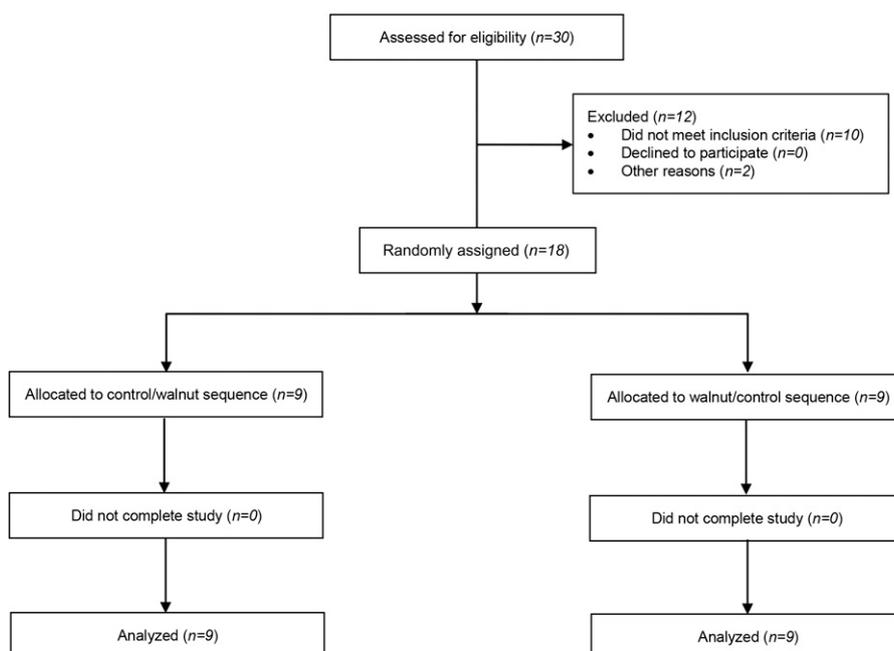
ME of walnuts when consumed as part of a typical American diet by healthy adults.

## Methods

Before the initiation of the study protocol, all study procedures were reviewed and approved by the Institutional Review Board of the Medstar Research Institute and were in accord with the Declaration of Helsinki. All participants provided written informed consent (NCT01832909).

**Study participants.** Volunteers were recruited from the Washington, DC, metropolitan area to participate in a human feeding study. Interested volunteers attended an informational meeting, at which time the study procedures and requirements were explained and informed consent was provided. Within a week of the informational meeting, interested volunteers completed a study application; completed a health history questionnaire; underwent measurement of height, weight, and blood pressure; and provided fasting blood and urine samples at screening to be analyzed for lipids, glucose, comprehensive metabolic panel, complete blood count, and urinalysis. Study participants were required to meet the following criteria: age 25–75 y, BMI 20–38 kg/m<sup>2</sup>, fasting glucose <126 mg/dL, blood pressure <160/100, fasting total blood cholesterol <280 mg/dL, and fasting TGs <300 mg/dL. Volunteers were excluded if they were smokers, were allergic to walnuts, were alcohol abusers, or had kidney disease, liver disease, gout, hyperthyroidism, untreated or unstable hypothyroidism, certain cancers, gastrointestinal disease, pancreatic disease, diabetes requiring medication, unstable body weight during the past 12 mo, or malabsorption syndrome. Women who were pregnant, lactating, or had given birth during the previous 12 mo were also excluded.

**Study design.** The study was conducted in a crossover design with two 3-wk intervention periods. There was a 1 wk compliance break between periods. Participants were divided by sex and randomly assigned with the use of a random number generator to 1 of the 2 treatment orders, and an equal number of participants completed each treatment order. During each treatment period, participants consumed a fully controlled diet, either unsupplemented (control) or supplemented with 1.5 servings (42 g) of walnut halves and pieces daily. This amount of walnuts was selected to be consistent with the qualified health claim for walnuts and coronary heart disease. After a 9-d dietary acclimation

**FIGURE 1** Participant flow diagram of a dietary intervention to determine the metabolizable energy of walnuts.

**TABLE 2** Baseline characteristics of the 10 men and 8 women who consumed control and walnut diets, each for 3 wk<sup>1</sup>

Characteristics	Values
Age, y	53.1 ± 2.2
BMI, kg/m <sup>2</sup>	28.8 ± 0.9
LDL cholesterol, mg/dL	121 ± 6.4
HDL cholesterol, mg/dL	51.4 ± 2.9
TGs, mg/dL	116 ± 12.7
Glucose, mg/dL	92.7 ± 1.7
Systolic blood pressure, mm Hg	117 ± 3.4
Diastolic blood pressure, mm Hg	71.4 ± 2.2

<sup>1</sup> Values are means ± SEMs.

period, the participants began collection of all feces and urine for a complete week, as marked by the fecal marker Brilliant Blue dye. Diets, feces, and urine were subjected to analyses of energy, protein, fat, and ash to determine the energy extracted from the walnuts. Blood was collected at baseline and at the end of each intervention period for analysis of lipids, lipoproteins, and glucose. Serum and plasma were portioned into aliquots after centrifugation (1500 g for 10 min at 4°C) and stored at -80°C.

**Controlled diet.** During each 3 wk intervention period, participants consumed a base diet consisting of typical American foods, scaled according to energy requirement such that participants remained weight stable during the study. Participants were instructed to eat all foods and only foods provided to them. The base diet provided 17% of energy from protein, 29% of energy from fat, and 54% of energy from carbohydrate. Coffee and tea were limited to 2 cups (473 mL) daily. During 1 of the 2 intervention periods, all of the food in the diet of each participant was reduced proportionately for the isocaloric inclusion (by calculated values) of 42 g (1.5 servings) of walnut halves and pieces daily. Participants consumed breakfast and dinner on weekdays at the USDA Beltsville Human Nutrition Research Center. Lunches and weekend meals were packed for carryout. On weekdays, walnuts were consumed at breakfast and dinner under the supervision of study staff. Participants completed questionnaires daily to report any dietary deviations, medication intake, unusual exercise, and general wellness. The composition of the base diet and the walnuts is given in Table 1.

**Sample Collection.** Each intervention period included a 7–10 d collection period after a 9 d dietary acclimation period. During the collection period, participants collected all urine and feces produced (26, 27). Participants were given collection supplies (dry ice, large cooler, collection bags, collection apparatus, and urine jug) and brought in samples daily when they arrived for meals. Compliance to the fecal and urine collection protocols was monitored by daily collection record forms. The collection length varied according to fecal production such that feces representing a single full week of dietary intake were collected with the use of previously described methods (26, 27). By use of these methods, we could compare the energy and macronutrient content of 1 wk of diet with 1 wk of urine and fecal excretion for evaluation of energy and macronutrient absorption.

Diet collections were conducted by preparing a full week of meal trays (for the 7 d menu rotation) of food identical to that consumed by the subjects. Foods were prepared as they would be for consumption (including cooking of eggs, toasting of breakfast breads, etc.). Foods were homogenized with ice in a blender and the mixture was freeze-dried and crushed for chemical analysis.

**Analysis.** Diet samples, feces, and urine were analyzed in duplicate for energy by adiabatic bomb calorimetry (Parr Instrument Company). Nitrogen in samples was measured by combustion (CN 2000, LECO Corporation). Protein was calculated from nitrogen content (6.25 g protein/g nitrogen for base diet, urine, and feces; 5.3 g protein/g nitrogen for walnuts). Petroleum ether extraction was used to analyze dietary fat

**TABLE 3** Number of daily bowel movements and fecal composition in adults who consumed control and walnut diets, each for 3 wk<sup>1</sup>

	Control phase	Walnut phase
Bowel movements, n/d	1.1 ± 0.1	1.1 ± 0.1
Wet weight, g/d	175 ± 12.2*	195 ± 12.2
Dry weight, g/d	29.6 ± 1.5*	38.0 ± 1.5
Fat, g/d	2.2 ± 0.6*	10.2 ± 0.6
Total carbohydrate, g/d	12.1 ± 0.7	11.7 ± 0.7
Protein, g/d	10.3 ± 0.4	11.0 ± 0.4
Energy, kcal/d	140 ± 8.9*	217 ± 8.9

<sup>1</sup> Values are least-square means ± pooled SEMs; n = 18 participants in a crossover design. \*Different from walnut phase, P < 0.0001. Treatment effects were evaluated by using a mixed-model ANOVA (with fixed effects of period and treatment and a random effect of volunteer).

and fecal fat (Foss). Ash was determined in a muffle furnace. Total carbohydrate was determined by difference. Serum lipids and lipoproteins (total cholesterol, LDL cholesterol, HDL cholesterol, and TGs) and plasma glucose were analyzed on the Vitros 5,1 FS chemistry system (Ortho-Clinical Diagnostics).

**Calculations and statistics.** The ME content of walnuts (primary outcome) and digestibility were calculated according to the methods of Novotny et al. (26) with the use of the following equation:

$$ME_{\text{Walnut}} (\text{kcal/g}) = \frac{[MEI_{\text{Walnut diet}}] - \left[ (GEI_{\text{Walnut diet}} - GEI_{\text{Walnut}}) \times \left( \frac{MEI_{\text{Control diet}}}{GEI_{\text{Control diet}}} \right) \right]}{\Delta \text{Walnut Intake}} \quad (1)$$

where MEI represents metabolizable energy intake and GEI represents gross energy intake. Nutrient digestibility and energy digestibility were calculated with the use of the following equation:

$$\text{Nutrient of energy digestibility (\%)} = \left[ \frac{\text{Intake} - \text{Excreted}}{\text{Intake}} \right] \times 100 \quad (2)$$

Differences in the number of daily bowel movements, fecal wet and dry weights, the chemical composition of the excreta, and macronutrient digestibility between the 2 diets were analyzed with a mixed-model ANOVA with repeated measures by using the subject as the random term (SAS version 9.3). The statistical model included terms for treatment and period and an interaction term for treatment × period. A paired t test was used to determine the difference between the measured ME value of walnuts and the Atwater-calculated value. The association between ME value and macronutrient digestibility was determined by Pearson product-moment correlation. Sample size was determined based on our previous studies of similar design with nuts and metabolizable energy. A sample size of 15 is required to detect a 10% difference with 80% power with a 0.7 kcal/g SD of difference (sample size was increased

**TABLE 4** Digestibility or balance of macronutrients in adults who consumed control and walnut diets, each for 3 wk<sup>1</sup>

	Control phase	Walnut phase
Dry matter, %	94.3 ± 0.2*	92.5 ± 0.2
Fat, %	97.0 ± 0.6*	89.0 ± 0.6
Total carbohydrate, %	96.3 ± 0.2	96.1 ± 0.2
Nitrogen balance, g/d	4.0 ± 3.4	3.2 ± 3.4
Energy, %	90.4 ± 0.3*	87.8 ± 0.3

<sup>1</sup> Values are least-square means ± pooled SEMs; n = 18 participants in a crossover design. \*Different from walnut phase, P < 0.0001. Treatment effects were evaluated by using a mixed-model ANOVA (with fixed effects of period and treatment and a random effect of subject).

to account for potential dropouts). Values reported are means  $\pm$  SEMs, and a  $P$  value of  $< 0.05$  was used to determine statistical significance.

## Results

All 18 participants completed the intervention (Figure 1). Ten men and 8 women participated in the study. Participant characteristics included a mean age of  $53.1 \pm 2.2$  y and a mean BMI of  $28.8 \pm 0.9$  kg/m<sup>2</sup> (Table 2).

The ME content of walnuts was found to be  $5.22 \pm 0.16$  kcal/g (146 kcal/28 g serving; 219 kcal/42 g) as compared with the Atwater-calculated amount of 6.61 kcal/g (185 kcal/28 g serving; 278 kcal/42 g). Thus, Atwater factors overestimate the ME content of walnuts by 21% ( $P < 0.0001$ ).

Fecal weight and macronutrient content were significantly higher during the walnut intervention period than during the control period. Fecal wet and dry weights were 20.3 and 8.4 g/d greater ( $P < 0.0001$ ), respectively, during walnut consumption. Fecal fat increased significantly to 10.2 g/d ( $>4$ -fold) during walnut consumption compared with the control phase ( $P < 0.0001$ ). Fecal energy content also increased significantly to 216 kcal/d during walnut consumption compared with the control phase ( $P < 0.0001$ ) (Table 3). Concomitant with these changes in fecal output, the apparent digestibility of total diet dry matter and fat and gross energy decreased significantly during walnut consumption compared with the control phase ( $P < 0.0001$ ) (Table 4).

There was a significant association between the ME value of walnuts for an individual and fat digestibility ( $r = 0.8$ ,  $P < 0.0001$ ), whereas there was no such association between the ME value and the digestibility of protein ( $r = -0.1$ ,  $P = 0.7$ ) or carbohydrate ( $r = 0.2$ ,  $P = 0.5$ ).

## Discussion

The results presented here demonstrate that walnuts, like pistachios (27) and almonds (26), provide less ME than predicted by the Atwater factors. In other words, the amount of energy in walnuts that is available to the body to use or store is lower than previously understood. Previous studies with almonds have shown that plant cell walls contribute to the reduced digestibility of nuts by limiting the accessibility of the lipid content contained in intact cells (cells that are not disrupted during mastication) (28). Comparison of the ME for walnuts in the present study with that of pistachios and almonds from our previous studies demonstrates that the digestibility of a given nut is nut-dependent. The discrepancy between actual ME and predicted ME in the 3 nuts investigated to date ranges from 5% lower than predicted (27) to 20% (26) or 21% (present study) lower than predicted. Although we do not currently know the reason for this broad range, it is likely related to the chemico-physical structure of the nuts. Mastication is also known to affect the digestibility of nuts (29); thus, it is possible that different nuts elicit different amounts of chewing, although there is no direct evidence for nut-dependent mastication to date. Additionally, differences in the fat and fiber content between nuts may contribute to the discrepancy observed between the measured and calculated ME value in walnuts, almonds, and pistachios.

Previous studies have linked nut intake with decreased body weight and/or decreased BMI (20–25). Some of the mechanisms that have been suggested to account for these findings include the content of dietary fiber delaying gastric emptying and thus increasing satiety (30); the content of protein increasing satiety (31); the content of fiber, fat, and protein increasing thermogenesis (32); or incomplete mastication leading to loss of energy via feces

(29). The present study and our previous studies on the ME of nuts (26, 27) demonstrate that the energy available from nuts is less than what has been predicted by traditional methods, which may also contribute to the beneficial effect of nuts on body weight and BMI.

Analysis of fecal composition during the walnut phase compared with the control phase revealed increased fecal weight and fat content during walnut consumption. Thus, the energy-containing macronutrient absorption of the diet was lower during the walnut phase than during the nut-free phase. Our previous studies with nuts, as well as studies by others, have demonstrated that nut consumption leads to greater excretion of fecal fat (26, 27, 29, 33), which certainly in part explains the lower energy value. Nuts processed into butters (34) or oils and flours (33) result in less fecal fat excretion than do unprocessed nuts, which suggests that the whole nut structure inhibits the digestion and absorption of nut macronutrients. How this nut structure affects the absorption of micronutrients and phytonutrients is unknown, although nut consumption is associated with increases in circulating concentrations of FAs,  $\beta$ -sitosterol,  $\alpha$ -tocopherol, and minerals (35–38), even though the reduced digestibility of nuts may influence the bioavailability of micronutrients and phytonutrients. Furthermore, in the present study, LDL cholesterol-lowering after the walnut diet phase (8.3%, data not shown) was similar to what was previously reported with walnuts (39), which suggests that a sufficient amount of FAs and phytonutrients was absorbed to elicit a cardioprotective effect. In conclusion, the ME value of walnuts consumed as part of a mixed diet is 21% less than predicted from the Atwater factors. The addition of walnuts to the diet decreases fat availability but not the availability of carbohydrate or protein. The discrepancy between the measured ME value and the value estimated by use of the Atwater factors for walnuts is similar to the discrepancy for almonds. The discrepancy was less, however, for pistachios. The reason for these differences among nuts is unclear. Nevertheless, all of these tree nuts provide less ME than the value determined by the Atwater factors, which could help to explain the observations that consumers of nuts do not gain excessive weight.

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DJB and JAN designed the research; DJB, SKG, and JAN conducted the research; DJB, SKG, and JAN analyzed the data; DJB and JAN wrote the paper; and DJB and JAN had primary responsibility for the final content. All authors read and approved the final manuscript.

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