Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets[1–4]

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ABSTRACT

Background: The energy content of foods is primarily determined by the Atwater factors, which may not be accurate for certain food groups. Nuts are a food group for which substantial evidence suggests that the Atwater factors may be poorly predictive.

Objective: A study was conducted to determine the energy value of almonds in the human diet and to compare the measured energy value with the value calculated from the Atwater factors.

Design: Eighteen healthy adults consumed a controlled diet or an almond-containing diet for 18 d. Three treatments were administered to subjects in a crossover design, and diets contained 1 of 3 almond doses: 0, 42, or 84 g/d. During the final 9 d of the treatment period, volunteers collected all urine and feces, and samples of diets, feces, and urine were analyzed for macronutrient and energy contents. The metabolizable energy content of the almonds was determined.

Results: The energy content of almonds in the human diet was found to be 4.6 ± 0.8 kcal/g, which is equivalent to 129 kcal/28-g serving. This is significantly less than the energy density of 6.0–6.1 kcal/g as determined by the Atwater factors, which is equivalent to an energy content of 168–170 kcal/serving. The Atwater factors, when applied to almonds, resulted in a 32% overestimation of their measured energy content.

Conclusion: This study provides evidence for the inaccuracies of the Atwater factors for certain applications and provides a rigorous method for determining empirically the energy value of individual foods within the context of a mixed diet. This trial was registered at clinicaltrials.gov as NCT01007188. *Am J Clin Nutr* 2012;96:296–301.

INTRODUCTION

The system for determining the energy value of foods was founded >100 y ago by Atwater et al (1) at the USDA Agricultural Experiment Station in Storrs, CT. More than 100 y later, the Atwater general factors are still widely applied to foods to estimate energy content. During this past century, there have been few, if any, studies reporting on the energy value of a whole food within a mixed diet that could confirm the accuracy of Atwater's coefficients.

In 1955 Merrill and Watt published a report to update the energy content of macronutrients according to the class of food in which they were found, and this report was further updated in 1973 (2). Merrill and Watt took into consideration that compounds in a given class of macronutrient can differ in heats of combustion and that macronutrients as found in different foods can differ in digestibility. They proposed a series of energy values for macronutrients as found in different food sources, and these were termed the *Atwater specific factors*. Their proposed coefficients of digestibility for protein ranged widely, from 20% to 97%, and for carbohydrate from 32% to 98%. The proposed heats of combustion for protein (minus 1.25 kcal lost as urea) ranged from 3.75 to 4.55 kcal/g, and heats of combustion for carbohydrate ranged from 2.45 to 4.20 kcal/g. In contrast, the ranges of estimated values for coefficients of digestibility and heats of combustion for fat were much narrower, at 90–95% and 9.3–9.5 kcal/g, respectively. Moreover, the majority of foods were assigned a coefficient of fat digestibility of 90% and a heat of fat combustion of 9.3 kcal/g, which suggests that there is little variation in the energy available in a gram of fat, regardless of food source. Nuts are among the many food groups assigned a coefficient of fat digestibility of 90% and a heat of fat combustion of 9.3 kcal/g based, in part, on the work of Jaffa (3). However, much evidence has surfaced that suggests that the digestibility of fat from whole nuts (and peanuts, which are actually legumes) may be much lower than that for other food sources (4–6).

The results of studies that show more fat in feces and less fat in plasma after the consumption of nuts cast doubt on the applicability of the Atwater factors for evaluating the energy value of nuts. Moreover, a prominent recommendation of the Food and Agriculture Organization Technical Workshop of Food Energy was to recognize differences in fat digestibility and to modify energy values accordingly (7). Therefore, we conducted a feeding study in which almonds, a popular nut worldwide (8, 9), were administered to healthy adults; feces and urine were collected to empirically determine the energy value of almonds as a representative food from a group for which the Atwater factors may predict an energy value that is incorrect. Most important, we present an experimental paradigm that can be used to determine

the metabolizable energy (ME) value of any single whole food within the context of a mixed diet.

SUBJECTS AND METHODS

Subjects

A convenience sample of men and women was recruited to participate in a feeding study. Eligible volunteers were required to be healthy (as determined by routine blood testing and urinalysis), to be nonsmoking, and to have no history of malabsorption, gastrointestinal disorders, or bariatric surgery. This study was conducted according to the guidelines in the Declaration of Helsinki. All procedures involving human subjects were approved by the Medstar Health Research Institute Committee on Human Research (Hyattsville, MD), and all volunteers provided written informed consent before participation. Of eligible volunteers, 18 individuals (10 men, 8 women) were enrolled as participants.

Study design

The study was conducted as a randomized, crossover, controlled feeding trial composed of 3 experimental phases (**Figure 1**). Each experimental phase was 18 d in length. During each phase, volunteers were administered a controlled diet containing one of the following 3 doses of natural, whole almonds: 1) 0 g/d (control), 2) 42 g/d (1.5 oz/d), and 3) 84 g/d (3 oz/d). The base diet remained the same for each treatment phase, and the amount of food administered as the base diet was reduced according to the almond dose, with the goal of making the energy intake of each volunteer during the various treatment phases isocaloric. Each treatment sequence included 3 different dose amounts (0, 42, or 84 g/d). Three volunteers were randomly assigned to treatment sequences that included repetition of the 0-g/d dose, 3 volunteers were randomly assigned to treatment sequences that included repetition of the 84-g/d dose, and 12 volunteers received all 3 doses. This partial replication design of the 0- and 84-g/d treatments was used to provide information about intraindividual variability of the energy density of almonds and to estimate the volunteer × treatment interaction. The 42-g/d dose was selected because this is the amount required to meet the US Food and Drug Administration qualified health claim for nuts. The 84-g/d dose was selected to be twice the 42-g/d dose. Volunteers were stratified by sex and initial BMI and randomly assigned to a treatment sequence.
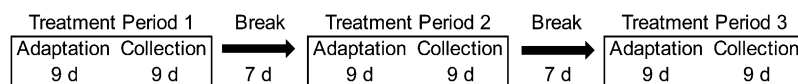
Diet

Volunteers were administered a controlled diet at weight maintenance throughout each feeding period. The diets were composed of traditional American foods incorporated into a constant 7-d menu cycle, and volunteers were instructed to consume all and only foods provided by the Beltsville Human Nutrition Research Center. Examples of foods used for 2 of the 7 d of menus included the following—1) for breakfast: French toast, syrup, margarine, egg, milk, sugar; lunch: turkey and Swiss cheese, salad (lettuce, celery, tomato, carrot, honey Dijon dressing), vegetable soup, roll, brownie; dinner: beef stroganoff, egg noodles, roll, peas, margarine, frosted cake, lemonade; and snack: pretzels; and 2) for breakfast: egg, sausage (turkey), bread, margarine, sugar, peaches (canned); lunch: taco (ground meat, refried beans, tortillas), lettuce, onions, cheddar cheese, salsa, fruit punch, grapes, brownies; dinner: turkey breast, turkey gravy, cranberry sauce, potatoes, salad (lettuce, tomato, cucumber, poppy seed dressing), pudding; and snack: graham crackers. No guidance was provided with respect to mastication of the almonds. The measured composition of the control diet and the almonds is shown in **Table 1**. Breakfast and dinner, Monday through Friday, were consumed at the Human Nutrition Research Center, and lunches and weekend meals were packed for carryout. Body weight was measured before breakfast on weekdays to identify patterns of weight loss or weight gain over periods of 7 to 10 d. If patterns of weight change were observed, portion size was adjusted for all foods [in 837-kJ (200-kcal) increments] to maintain weight. The final 9 d of each 18-d feeding period constituted the balance period (the period during which all feces and urine were collected for macronutrient and energy analysis), and no diet adjustments were made during this time. Almonds were provided with breakfast and dinner so that the treatments could be consumed under the observation of a dietitian, research associate, or investigator to verify compliance.

Biological sample collection

During the balance period, the final 9 d of each treatment period, volunteers were instructed to collect all fecal material produced. Volunteers were provided coolers containing dry ice and were instructed to put fecal samples in the coolers immediately after collection. Weekday fecal samples were brought to the center during the volunteers' next visit to the center, and fecal samples produced on the weekend were brought to the center the following Monday morning. A capsule containing 15 mg Brilliant Blue dye was administered at the beginning of each fecal collection period and again 7 d later. The appearance of the Brilliant Blue marker in the feces indicated to study staff which samples should be included in the balance period and should be processed for chemical analysis. Once received at the center, fecal samples were weighed (wet weight) and placed in a freezer until they were freeze dried. Immediately after freeze drying, the samples were weighed (dry weight) and then pulverized by using a food processor to produce a homogeneous powder.

Urine was also collected for the final 9 d of each treatment period. Volunteers were provided preweighed 4-L containers with

Treatment Period 1		Break	Treatment Period 2		Break	Treatment Period 3	
Adaptation	Collection		Adaptation	Collection		Adaptation	Collection
9 d	9 d	7 d	9 d	9 d	7 d	9 d	9 d

FIGURE 1. Schematic of the crossover study design. All volunteers (n = 18) completed 3 treatment periods. Each treatment period lasted 18 d, and the initial 9 d were a period of adaptation to the diet followed by a 9-d collection period for feces and urine. Treatments consisted of 0, 42, or 84 g almonds/d, which were consumed as part of a controlled diet. To provide data on intraindividual variability, 3 volunteers were randomly assigned to treatment sequences that included repetition of the 0-g/d dose, and 3 volunteers were randomly assigned to treatment sequences that included repetition of the 84-g/d dose. Twelve volunteers received all 3 doses.

TABLE 1
Composition of base diet and almonds (dry weight)[1]

	Base diet (without almonds)	Almonds
Protein, N × 6.25 (g/100 g)	18.9	24.8
Fat (g/100 g)	13.6	53.8
Total carbohydrate (g/100 g)	64.1	18.3
Total dietary fiber (g/100 g)	8.5	10.2
Ash (g/100 g)	3.4	3.1
Energy, gross (kcal/100 g)	491	711

[1] All values are means of chemical analyses based on $n = 6$ participants.

15 g boric acid and coolers with ice. Volunteers were instructed to store all urine on ice until delivery to the center each morning, at which time they were provided with new collection containers. Urine was weighed, and subsamples were divided into aliquots and stored at −80°C until analyses were performed. The weight of the voided urine was calculated as the difference between the full container weight and the empty container weight.

For diet analysis, a complete set of foods was collected for the full 7-d rotation. Foods were mixed, then prepared for chemical analysis by homogenization in a blender with ice and water before being freeze dried.

Chemical analyses

Combustible energy contents of diets, feces, and urine were determined by adiabatic bomb calorimetry (Parr Instrument Company). Protein contents of diets, feces, and urine were calculated from nitrogen content (6.25 g protein/g nitrogen), as determined by the Dumas method (Leco Corporation). Fat contents of diets and feces were analyzed by petroleum ether extraction (Soxtec; Foss). Total dietary fiber was determined by using the Association of Official Analytical Chemists method 991.43 (Foss). Ash was determined by combustion in a muffle furnace. Total carbohydrate in samples of diet and feces (dry matter basis) was calculated by the difference from measured values for fat, protein, and ash. All analyses were performed in duplicate, and the mean of these values was used for statistical analyses.

Calculations

For each study volunteer, the ME value of almonds was calculated from the gross energy (GE) and the ME of the different diets, as follows:

$$ME_{almond}\left(\frac{kJ}{g}\right)$$
$$= \frac{[MEI_{almond\ diet}] - \left[(GEI_{almond\ diet} - GEI_{almond}) \times \left(\frac{MEI_{control\ diet}}{GEI_{control\ diet}}\right)\right]}{\Delta\ almond\ intake}$$

$$(1)$$

where GEI is the GE intake for a given diet or food item (kJ/d), MEI is the ME intake for a given diet (kJ/d), and Δ almond intake is the difference between the almond intake with the 2 diets (which was equivalent to the mass of almond incorporated into the almond-containing diet) (10). ME intake is equal to the difference between GE intake and fecal energy and urinary energy output [MEI = GEI − (fecal energy + urinary energy)].

Nutrient and energy digestibilities were calculated as follows:

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

$$(2)$$

Statistical analysis

ME, digestibility, and data for fecal wet weight, fecal dry weight, number of bowel movements, and chemical composition of the diet and excreta were analyzed with a mixed-model ANOVA with repeated measures by using the volunteer as the random term (SAS version 9; SAS Institute Inc). The statistical model included terms for treatment and period and an interaction term for volunteer × treatment. A 2-tailed paired Student's t test was used to determine whether the measured energy density of almonds was different from the calculated energy density by use of Atwater factors.

RESULTS

Ten men and 8 women were enrolled and completed the study protocol. The study population had the following characteristics (mean ± SEM): aged 56.0 ± 8.6 y (range: 32–67 y), body weight of 79.5 ± 14.5 kg (range: 54–105 kg), and BMI (in kg/m^2) of 27.4 ± 4.2 (range: 20–34). Data from all observations from all volunteers ($n = 36$ observations) were included in statistical analyses.

The number of bowel movements per day was not different between the different dietary treatments, but fecal composition was affected by almond intake (**Table 2**). Both fecal wet weight and dry weight increased with almond consumption, as did fecal fat, carbohydrate, fiber, protein, and energy ($P < 0.0001$). The amount of total dietary fiber and protein did not increase between the 42- and 84-g/d doses of almonds.

The digestibility of macronutrients and energy from the diet as a whole was significantly affected by the addition of almonds to the diet (**Table 3**). The fat digestibility of the total diet decreased by nearly 5% when 42 g almonds were incorporated into the daily diet and by nearly 10% when 84 g almonds were incorporated into the diet daily ($P < 0.0001$). Carbohydrate, fiber, and protein digestibility decreased between the control diet and the diet containing 84 g/d ($P < 0.0001$). Total carbohydrate digestibility of the 42-g/d diet decreased compared with the control diet and was intermediate to the 84-g/d diet. However, fiber and protein digestibilities were not different between the 2 almond-containing treatments. Energy digestibility of the diet as a whole decreased by ~3% with the incorporation of 42 g almonds into the daily diet and by 5% with the incorporation of 84 g almonds into the daily diet.

The energy content of almonds in the human diet was measured twice for each subject. The mean (±SD) measured energy density of the first measure was 4.6 ± 0.7 kcal/g ($n = 18$), and the mean of the second measure was 4.6 ± 1.0 kcal/g ($n = 18$). The mean values of the replicated measures for each subject were not different ($P \geq 0.8$). The mean intraindividual CV was 6%. The mean measured energy density for the consumption of almonds at 42 g/d was 4.7 ± 0.7 kcal/g, and the mean energy density at 84 g/d was 4.3 ± 1.0 kcal/g. There was no dose effect on energy density ($P \geq 0.2$). The overall mean energy density was 4.6 ± 0.8 kcal/g or 129 kcal for a 28-g serving (the typical serving size).

TABLE 2
Number of daily bowel movements and fecal composition[1]

	Treatment			Pooled SE	Treatment-effect P value
	Control	42 g/d	84 g/d		
Bowel movements (no./d)	1.1	1.2	1.2	0.1	0.1117
Wet weight (g/d)	127.2[a]	163.0[b]	178.3[b]	13.2	<0.0001
Dry weight (g/d)	28.9[a]	40.8[b]	49.0[c]	2.4	<0.0001
Fat (g/d)	1.7[a]	6.3[b]	10.8[c]	0.8	<0.0001
Total carbohydrate (g/d)	21.6[a]	27.9[b]	31.2[c]	1.5	<0.0001
Total dietary fiber (g/d)	9.1[a]	11.5[b]	13.0[b]	0.9	<0.0001
Protein (g/d)	9.4[a]	11.3[b]	12.5[b]	0.6	<0.0001
Energy (kcal/d)	132.2[a]	217.7[b]	282.3[c]	13.4	<0.0001

[1] All values are least-square means and pooled SEs. Treatment effects were evaluated by using a mixed-model ANOVA (with fixed effects of period and treatment and a random effect of volunteer) for $n = 18$ participants in a crossover design. Mean values within a row with different superscript letters are significantly different ($P \leq 0.05$).

Measured values for individual volunteers ranged from 2.2 to 6.0 kcal/g. Except for 2 of 36 observations, all measured energy values for the almonds were below the Atwater predicted value ($P \leq 0.001$). There was no effect of age or BMI (normal, overweight, or obese) on nutrient digestibility or on energy density of almonds.

DISCUSSION

In this study, we report on a dietary paradigm that allows for the measurement of the ME value of an individual food when consumed as part of a mixed diet. In contrast, most previous research that evaluated the energy value of foods during the past ≥ 100 y was restricted to measuring the ME content of the whole diet or of single-food diets consumed for weeks. The accurate determination of the energy content of foods is important for the food industry, for health professionals, and for the consumer. The US Food and Drug Administration requires that nutrition information be provided for all foods sold in the United States, with only a few exemptions. The mandatory nutrition information includes energy content per serving. According to the US Code of Federal Regulations (11), energy content may be calculated by the Atwater general factors, by the Atwater general factors minus a correction for fiber, by the Atwater specific factors, by data for specific food factors for particular foods, or by bomb calorimetry after adjustment for loss of nitrogen through urea. The most commonly used method is calculation

according to the Atwater factors. However, both sets of Atwater factors fall short, which renders the information on food labels and in databases flawed and of lesser value, at least for nuts.

Nuts are a food group for which the Atwater factors may be particularly poorly suited. A key component of the Atwater factors is the coefficient of digestibility. Numerous studies and varied evidence suggest that the coefficient of digestibility for nuts and peanuts is different from that for other foods. Levine and Silvis (4) provided volunteers with one of 3 peanut treatments: whole peanuts, peanut butter, or peanut oil. Daily fat excretion was highest for subjects consuming whole peanuts and lowest for subjects consuming peanut oil. Therefore, it can be assumed that macronutrients (and therefore energy) from whole peanuts were less available than those from peanut butter and peanut oil. Another study in 63 adults in the United States and Brazil showed that consumption of whole peanuts resulted in greater excretion of both fecal fat and fecal energy compared with consumption of a control diet (5). Two additional studies have suggested that nut digestibility may be limited for the intact nut. In one study, increased mastication of almonds resulted in less fat excretion in feces (12). In another study, feces of adults consuming almonds contained intact cotyledon cells (embryonic tissue within the seed of a plant), encapsulating lipid and other material within cell walls and rendering it unavailable for digestion (13). These studies support the premise that the coefficient of digestibility for at least fat from nuts is significantly less than that for other foods.

TABLE 3
Nutrient and energy digestibility for the diet as a whole[1]

	Treatment			Pooled SE	Treatment-effect P value
	Control	42 g/d	84 g/d		
		%		%	
Fat	97.8[a]	93.1[b]	89.9[c]	0.8	<0.0001
Total carbohydrate	94.0[a]	91.4[b]	89.5[c]	0.4	<0.0001
Total dietary fiber	80.8[a]	75.4[b]	71.9[b]	1.6	<0.0001
Protein	91.0[a]	89.0[b]	87.9[b]	0.6	<0.0001
Energy	90.5[a]	87.5[b]	85.5[c]	0.5	<0.0001

[1] Values are least-square means and pooled SEs. Treatment effects were evaluated by using a mixed-model ANOVA (with fixed effects of period and treatment and a random effect of volunteer) for $n = 18$ participants in a crossover design. Mean values within a row with different superscript letters are significantly different ($P \leq 0.05$).

The energy value of almonds as currently found on nutrient labels is based on the Atwater general factors, which are 4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for carbohydrate (equivalent to 17, 37, and 17 kJ/g for protein, fat, and carbohydrate, respectively). On the basis of the measured macronutrient composition of the almonds used in this study, a serving of almonds (28 g) composed of 7 g protein, 15 g fat, and 5 g carbohydrate would contain 181 kcal/serving. The US Code of Federal Regulations allows insoluble fiber to be subtracted from total carbohydrate for calculation of energy content; in this case, on the basis of the chemical composition of the almonds used in this study, the energy content of a 28-g serving would be 170 kcal (711 kJ), which is equivalent to an energy density of 6.1 kcal/g (25.4 kJ/g).

The energy value of almonds as currently found in the USDA National Nutrient Database for Standard Reference (14) is based on the Atwater specific factors for the energy contained in fat, protein, and carbohydrate in nuts, which are 3.47 kcal/g for protein, 8.37 kcal/g for fat, and 4.07 kcal/g for carbohydrate (2) and equivalent to 14.5, 35.0, and 17.0 kJ/g, respectively. By using these factors, the energy contained in a serving of the almonds used in this study would be 168 kcal (703 kJ), giving an energy density of 6.0 kcal/g (25.1 kJ/g).

The measured energy density of almonds was determined in the present study to be 4.6 kcal/g (19.2 kJ/g). This value is substantially less than the values of 6.0 and 6.1 kcal/g as calculated by the Atwater specific factors and the Atwater general factors, respectively. The empirically measured energy content of one 28-g serving of almonds is therefore 129 kcal, instead of 168–170 kcal as estimated by the Atwater factors, which overestimate the energy content of almonds by 32%. This notable decrease in energy content per serving is a stimulus that could potentially influence food choices (15, 16). It is not known whether the observed discrepancy measured in whole almonds would be consistent for other forms of almonds such as almond butter or slivered or sliced almonds. On the basis of the work of Levine, it is likely that the energy value of almond butter would not be as low as that of the whole almonds that participants consumed in this study. Globally, whole almonds are consumed in far greater proportion than are other forms.

When an 84-g serving of almonds was incorporated into the diet daily, the energy digestibility of the diet as a whole decreased by ~5%. Therefore, for individuals with energy intakes between 2000 and 3000 kcal/d, incorporation of 84 g almonds into the diet daily in exchange for highly digestible foods would result in a reduction of available energy of 100–150 kcal/d. With a weight-reduction diet, this deficit could result in more than a pound of weight loss per month. Nuts and peanuts, being relatively energy dense and high-fat foods, may be expected to contribute to weight gain. However, both epidemiologic studies and intervention studies have suggested otherwise (17–24). These studies show that despite incorporation of nuts into the diet, there were no increases in body weight or fatness. Furthermore, Wien et al (25) observed additional weight loss when almonds were incorporated into a weight-loss diet in place of carbohydrate-rich foods.

When applied to mixed diets, Atwater factors consistently overestimate the ME content of the diet. In a study to compare the Atwater-determined energy value of a mixed diet consumed by older adults to the measured energy value, it was found that the Atwater factors overestimated the ME of the food by 26% (26). In another study, the Atwater factors overestimated the energy content of a low-fat, high-fiber diet by up to 11% (27). These results are in accord with others that show inaccuracies of the Atwater calculation (28–30).

In conclusion, by measuring the actual energy content of almonds within the context of a mixed diet, we have shown that the Atwater approach for calculating their energy content produce inaccurate results, in part attributable to lower digestibility of nut macronutrients than that suggested by Atwater and Merrill and Watt. We have also presented a method by which the energy value of a single food can be measured in the context of a mixed diet. This method is a significant improvement over past methodology that required volunteers to consume a single food exclusively for an extended period of time to determine its ME value. These results also cast doubt on the ability of the Atwater factors to predict the energy value of other nuts, thus suggesting possible widespread inaccuracies in food labels. The study presented here provides rigorous evidence for the problems with certain applications of the Atwater factors and provides a means for determining the actual energy value of individual foods within the context of a mixed diet.

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